A Comparative Evaluation of Surrogate Measures of Adiposity as Indictors of Cardiometabolic Disease

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADA	American Diabetes Association
AUC	Area under the curve
β	Beta
BF%	Body fat percentage
BMI	Body mass index
BP	Blood pressure
c	Concordance statistic
C3	Complement component 3
CAD	Coronary artery disease
CI	Confidence interval
cm	Centimetre
CRP	C-reactive protein
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DEXA	Dual Energy X-ray Absorptiometry
FMC	Full medical card
FPG	Fasting plasma glucose
FPR	False positive rate
GHQ	General health questionnaire
GP	General practitioner
GPC	General practice card
HbA_{1c}	Glycated haemoglobin A_{1c}
HDL-C	High-density lipoprotein cholesterol
HL	Hosmer-Lemeshow test
HOMA-IR	Homeostasis Model Assessment Index of Insulin Resistance
HR	Hazard ratio
IDF	International Diabetes Federation
IDI	Integrated discrimination improvement
IGT	Impaired glucose tolerance
IL-6	Interleukin 6
IPAQ	International Physical Activity Questionnaire
IPH	Institute of Public Health
IPV	Independent predictor variable
kg	Kilogram
l	Litre
LDL-C	Low-density lipoprotein cholesterol
LR	Likelihood ratio
m	Metre
MetS	Metabolic syndrome
mg	Milligram
ml	Millilitre
mmHg	Millimetre of mercury
mmol	Millimole

mol	Mole
NCEP: ATP III	National Cholesterol Education Program: Adult Treatment Panel III
ng	Nanogram
NIH	National Institutes of Health
OGTT	Oral glucose tolerance test
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor-1
pg	Picogram
ROC	Receiver operating characteristic curve
RR	Relative risk
Rx	Prescription medication
SAT	Subcutaneous adipose tissue
SBP	Systolic blood pressure
SD	Standard deviation
SLÁN	Survey of Lifestyle, Attitudes and Nutrition
TILDA	The Irish Longitudinal Study on Ageing
TNF-α	Tumour necrosis factor alpha
Total-C	Total cholesterol
VAT	Visceral adipose tissue
WBC	White blood cell
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist-hip ratio
WHtR	Waist-height ratio
x^2	Chi-square

DECLARATION

This is to certify that the thesis I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

The work upon which this thesis is based was carried out in collaboration with a team of researchers and supervisors who are duly acknowledged in the text of the thesis. The Library may lend or copy this thesis upon request.

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THESIS ABSTRACT

Background and Objectives

The prevalence of obesity has escalated in many world populations, representing a major public health issue. Despite recommendations that adiposity should be routinely assessed within clinical practice, controversy exists as to how excess adiposity should be defined using anthropometry. In particular, there is uncertainty and ongoing controversy as to whether surrogate measures of central adiposity such as waist circumference (WC), waist-hip ratio or waist-height ratio (WHtR) are better indicators of obesity-related risk when compared to general adiposity as measured by body mass index (BMI).

This thesis contributes to the current evidence base regarding methods to detect patients with type 2 diabetes, and those at increased obesity-related cardiometabolic risk. In particular, it aimed to determine how useful surrogate measures of adiposity might be to identify high-risk patients within a clinical setting. The main objectives of this thesis were:

1. to examine the rationale for adiposity assessment within clinical practice, and whether methods used for disease classification and anthropometric measurement procedure are important for diagnosing cardiometabolic risk and type 2 diabetes;

2. to compare adiposity variable relationships with a range of cardiometabolic disease features, biomarkers of chronic low-grade inflammation and type 2 diabetes;

3. to explore whether central adiposity indices provide additional information regarding disease and risk status, compared to BMI;

4. to investigate the clinical utility of a composite index using both general and central adiposity measures.

Literature Review

The literature review focused on meta-analytic studies which explored adiposity variable relationships with cardiometabolic features, obesity-related diseases and mortality. A majority of studies showed that central adiposity measures were more strongly related to examined outcomes as indicated by statistical measures of association. However, with regard to the clinical utility of central adiposity assessment, the findings from the review were inconclusive.

Methods and Papers

The papers included in this thesis were derived from analysis of baseline data from the Cork and Kerry Diabetes and Heart Disease Study (Phase II), a cross-sectional study involving a random sample of 2,047 men and women aged 46-73 years, recruited from a single primary care centre. Standard diagnostic criteria were used to define cardiometabolic disease features and chronic conditions which included high blood pressure, atherogenic dyslipidaemia, insulin resistance, pre-diabetes and type 2 diabetes. Adverse inflammatory biomarker levels were classified according to percentile thresholds. Waist circumference (measured at two sites), hip circumference, pelvic width and BMI were assessed. Correlation and logistic regression analyses were used to explore metabolic, anthropometric and other health-related variable relationships. Discrimination was determined using the receiver operating characteristic curve and integrated discrimination improvement analysis.

The findings are presented in a series of five interlinked papers which relate directly to the thesis objectives. Paper 1 addressed the prevalence of type 2 diabetes within the sample, with particular reference to undiagnosed diabetes. Paper 2 examined cardiometabolic profiles in patients diagnosed with diabetes and pre-diabetes using two different diagnostic methods [glycated haemoglobin A_{1c} (HbA_{1c}) and fasting plasma glucose (FPG)]. Paper 3 compared general and central adiposity variable relationships with

cardiometabolic disease features and type 2 diabetes. In Paper 4, BMI and WC associations with biomarkers of chronic low-grade inflammation and type 2 diabetes were explored. Paper 5 investigated the utility of a composite index, using both BMI and WHtR, to assess cardiometabolic risk.

Results

Paper 1: The prevalence of type 2 diabetes within this sample was 8.5% (95% CI: 7.4%-9.8%), a rate comparable to estimates determined from recent nationally representative research within Ireland. A high percentage of diabetes cases were undiagnosed (41%), suggesting that better detection methods are needed.

Paper 2: The cardiometabolic profiles of patients diagnosed with type 2 diabetes by HbA_{1c} or FPG were broadly similar, indicating that either test is acceptable for defining this outcome. In contrast, the risk profiles of subjects classified as having pre-diabetes varied considerably according to diagnosis by either assay, with patients diagnosed by both tests displaying the least optimal profile. Adiposity, high blood pressure, atherogenic dyslipidaemia, insulin resistance and adverse cardiometabolic feature clustering were significantly related to both type 2 diabetes and pre-diabetes. Subjects with these outcomes also displayed a chronic low-grade pro-inflammatory profile as indicated by the examined biomarkers.

Paper 3: Central adiposity variables demonstrated stronger associations with adverse cardiometabolic features, metabolic feature clustering and type 2 diabetes than BMI. Central adiposity measures were also better discriminators of patients with type 2 diabetes, and they improved discrimination of diabetes by 3%-7% (men) and 5%-7% (women) compared to BMI. However, it was also noted that the utility of central adiposity measurement was significantly influenced by the procedure used for estimating WC.

Paper 4: Central adiposity defined by WC was more strongly related to a majority of the examined biomarkers of inflammation and adverse biomarker clustering. The association between chronic low-grade inflammation and type 2 diabetes was reduced in analyses which included either BMI or WC. Logistic regression models incorporating WC displayed the greatest attenuation, thus supporting the theory that measures of central adiposity are better indicators of visceral fat.

Paper 5: A combination of BMI and WHtR tertiles identified consistent and significant metabolic variable differences relative to those characterised as overweight or obese discordantly by BMI and WHtR. Significant discriminatory improvement, using joint-measurement, was also observed for detecting individual cardiometabolic disease features and adverse inflammatory biomarker levels when indices were examined as both continuous and categorical variables. In a fully adjusted regression model, only individuals within the highest tertile for both measures displayed a significant and positive association with pre-diabetes (odds ratio: 3.4, 95% CI: 1.9-6.0, P<0.001).

Conclusions

The results from this thesis suggest that surrogate measures of central adiposity provide information regarding disease status and cardiometabolic risk, independent of that provided by BMI, and that a composite index using BMI and WHtR together may help refine body fat classification.

Future research should concentrate on determining an optimal procedure for measuring WC, and whether a composite index might be useful for predicting type 2 diabetes and cardiovascular and mortality outcomes. Other novel adiposity measurement procedures should also be explored. Earlier identification of patients at increased cardiometabolic risk, and those with type 2 diabetes, could allow earlier targeted interventions to be implemented,

thus reducing the incidence of related complications, premature mortality and financial costs associated with the obesity epidemic.

CHAPTER 1

BACKGROUND, LITERATURE REVIEW, RESEARCH AIMS AND METHODS

1.0 Overview

Obesity is a chronic disorder described by the World Health Organisation (WHO) as a condition of abnormal or excessive fat accumulation to the extent that health may be impaired [1]. Over the last four decades, the percentage of people who are overweight or obese has risen dramatically across many world populations, representing a major public health issue [2-5]. The positive relationship between excess adiposity and cardiometabolic disease features such as hypertension, dyslipidaemia and insulin resistance has been repeatedly observed in both cross-sectional and prospective research [6]. Abundant evidence also supports an association between obesity and a wide range of chronic disorders including type 2 diabetes and cardiovascular disease (CVD) [7-11]. Epidemiological studies have demonstrated a clear dose-response relationship between higher levels of adiposity and cardiometabolic risk, and the consistency of this correlation across world populations reflects the strength of this relationship [12].

Research has shown that in the Republic of Ireland, 36% of adults aged 50+ are obese, with a further 43% being overweight [13]. Recent unpublished estimates from the WHO Modelling Obesity Project, presented at the European Congress on Obesity in 2015, suggest that if current trends continue, 89% of Irish men and 85% of women are likely to be either overweight or obese by 2030. If these projections are correct, Ireland will soon be the most obese nation in Europe [14]. In addition to the major health consequences associated with obesity, there are also economic implications [15]. Overweight and obesity are estimated to cost at least €1.13 billion per

annum to the Irish economy through increased health services utilisation and premature mortality [16]. The percentage of the Irish population over the age of 65 is projected to double from 11% in 2006 to 22% by 2041 [17]. Thus, the combination of an ageing population with the increasing prevalence of obesity and related chronic disorders will likely lead to considerably greater healthcare needs and financial costs [13].

1.1 Cardiometabolic Disease

Cardiometabolic disease is defined according to a number of interrelated features, associated with increased adiposity, which may contribute to the development of type 2 diabetes and atherosclerotic vascular disease [18]. These features include elevated blood pressure (BP) or hypertension and atherogenic dyslipidaemia, the presence of high triglyceride levels and reduced high-density lipoprotein cholesterol (HDL-C) concentrations. Cardiometabolic disease is also characterised by insulin resistance. This occurs when liver, skeletal and adipose tissue become less sensitive and eventually resistant to insulin, the hormone produced by β-cells in the pancreas to facilitate glucose absorption. Insulin resistance may in turn lead to dysglycaemia, as glucose is no longer being efficiently absorbed by cells and remains in the bloodstream [19].

Notably, excess adiposity is associated with a clustering of these features, a state described as the metabolic syndrome (MetS) [19]. The exact origins of MetS are not fully understood and the clinical implications of the condition

have been subject to debate [20,21]. Nevertheless, several working definitions of the MetS using combinations of these metabolic markers, in conjunction with measures of adiposity, have been devised by national and international organisations in order to facilitate the identification of individuals at increased cardiometabolic risk [21].

1.2 Type 2 Diabetes

1.2.1 Definition and diagnostic criteria

Type 2 diabetes mellitus is a chronic metabolic disease characterised by persistent hyperglycaemia. Current WHO, International Diabetes Federation (IDF) [22] or American Diabetes Association [23] definitions for diagnosing diabetes include the following: (1) a fasting plasma glucose (FPG) level $\geq$7.0 mmol/l; (2) a random blood glucose level $\geq$11.1 mmol/l and associated features; (3) a 2-hour plasma glucose level $\geq$11.1 mmol/l indicated by the oral glucose tolerance test (OGTT); (4) a glycated haemoglobin A_{1c} (HbA_{1c}) level $\geq$6.5% ($\geq$48 mmol/mol). Although controversy exists as to which test more accurately defines the condition, and prevalence estimates may vary according to procedures used [24], current diagnostic thresholds have been derived from epidemiological studies examining the prevalence and incidence of diabetes-related complications [22]. These complications may lead to a lower quality of life and reduced life expectancy.

1.2.2 Diabetes complications

Type 2 diabetes is a major cause of morbidity and mortality as chronic hyperglycaemia may lead to impairment and malfunction of the renal, ophthalmic, vascular and nervous systems [25]. If undiagnosed or untreated diabetes can lead to long-term microvascular damage. Microvascular diabetic complications include retinopathy, the leading cause of blindness in adults [26], diabetic nephropathy, the leading cause of end-stage renal disease [27] and diabetic neuropathy, the leading cause of non-traumatic lower extremity amputations [28]. In addition to microvascular damage, macrovascular complications are frequently observed. Importantly, individuals with type 2 diabetes are at increased risk of developing CVD. Research has shown that up to 80% of diabetes patients will die from cardiovascular-related events [29] and that they have a two to four-fold increased risk of coronary artery disease (CAD) and stroke when compared to diabetes-free subjects [30,31].

1.2.3 Diabetes prevalence

Diabetes has become an epidemic in developed and developing countries representing a major public health concern. Current estimates predict an excess of 400 million individuals with type 2 diabetes worldwide by 2030 [32]. In 2013, the number of people with diabetes in Europe was determined to 56 million, with an overall prevalence rate of 8.5% [33]. Until recently the prevalence of type 2 diabetes within the Republic of Ireland was largely unknown, as estimates were derived from incomplete primary care data.

However, a number of recent studies have suggested a prevalence of between 7%-10% in middle-aged adults [34]. Alarmingly, research has also indicated that a considerable proportion of diabetes cases within Ireland are undiagnosed [35] and that a high percentage of adults are at risk of developing the condition [36,37].

1.2.4 Diabetes risk factors

The diabetes epidemic has been driven by complex gene-environment interactions [38]. Approximately half of the risk of developing type 2 diabetes has been attributed to non-modifiable genetic factors [39], with the other half to environmental exposures which may contribute to excess adiposity [40], due to an increasingly sedentary lifestyle and “westernised” calorie-dense diet [41]. Other factors associated with diabetes include age, gender, ethnicity, smoking and alcohol use [42-46].

All obesity-related cardiometabolic disease markers are thought to be correlated with diabetes development. It has been observed that even before blood glucose levels are high enough for an individual to be diagnosed with the disorder, hypertension, adverse changes in lipid and lipoprotein levels, insulin resistance and dysglycaemia may occur [19]. Whether a combination of these features, as defined by MetS definitions, indicates a greater risk of developing type 2 diabetes is disputed [20,47]. However, it has been suggested that a clustering of these metabolic abnormalities may confer a

substantial additional risk, over and above the sum of the risk of each individual MetS component [19,48].

Recently the term “pre-diabetes” has also emerged as another potentially greater risk factor [47]. This umbrella term for impaired FPG, impaired glucose tolerance (IGT) [49] or HbA_{1c} levels that are higher than normal [23], represents intermediate stages of elevated glucose levels between normal glucose regulation and diabetes. A meta-analysis of prospective studies conducted in different populations estimated a relative risk of 4.7-12.0 for progression from impaired FPG and/or IGT to type 2 diabetes, with absolute annual risks between 5% and 10% [50].

1.2.5 Screening for diabetes

As type 2 diabetes has become a major public health priority, there is increasing interest in methods to identify individuals who have diabetes, and patients who are at high-risk of developing the condition. The pathway from obesity through insulin resistance, pre-diabetes to overt type 2 diabetes represents a progressive phenotype [51]. That diabetes is preventable through lifestyle changes in diet and physical activity is well accepted [52,53]. Given the long asymptomatic period preceding the disorder, earlier identification of individuals at increased risk could allow earlier targeted interventions. These might include implementation of healthy lifestyle changes or pharmacological treatments, thus attenuating development of type 2 diabetes and related micro- and macrovascular complications.

However, population screening using blood sampling to detect patients with type 2 diabetes, dysglycaemia or dyslipidaemia is time-consuming and cost prohibitive [51]. In addition, longitudinal studies have also shown that only about half of subjects with impaired FPG or IGT will progress to diabetes [54]. Increasingly, the idea of risk stratification has been seen as an important further tool for risk assessment. This may be thought of as a two-step process, whereby step one identifies a subset of individuals at increased risk, using cheap and non-invasive procedures, with step two involving blood testing [55]. Various diabetes risk assessment scores, using self-administered questionnaires, have been developed in numerous populations, either for self-assessment or for use within clinical practice [51,56].

1.2.6 Adiposity as a diabetes risk factor

Obesity is considered to be the primary modifiable risk factor related to cardiometabolic disease and diabetes development [19,57]. Accordingly, non-invasive diabetes risk scores typically include a measurement of adiposity, most commonly body mass index (BMI), an anthropometric measure of general adiposity. However, despite strong observed associations between BMI and morbidity [6], it is now well established that body fat distribution is a further indicator of health status, beyond the total body mass assessed by BMI [58,59]. Recent research has high-lighted the inherent problems of measuring body fat using BMI, as subjects with increased adiposity may exhibit favourable outcomes in some studies

[60,61]. Conversely, other studies have demonstrated increased cardiometabolic risk among non-obese and normal weight individuals [62-64].

Important behind the idea of any risk assessment tool is that it is able to detect high-risk subjects [65]. However, it is equally important not to overextend the risk criteria to low-intermediate risk patients [66]. This concept is aptly demonstrated in the relationship between adiposity and diabetes. Although prevalence rates for overweight/obesity and type 2 diabetes have increased considerably in world populations, a high percentage of individuals with increased adiposity will not have diabetes [67-70] or ever develop the condition [71,72]. It is also relevant in the context of the middle-aged population within Ireland, where a majority of subjects classified by BMI are overweight or obese [13].

1.3 Visceral Adiposity

Increasing evidence suggests that central adiposity (sometimes termed central obesity or abdominal obesity) is a greater metabolic risk factor when compared to the general adiposity (or general obesity) assessed by BMI [19,73]. Visceral adipose tissue (VAT) is defined as the adipose tissue found deep within the body cavity surrounding the internal organs in the intrathoracic, intraabdominal and intrapelvic areas [74]. Visceral adiposity is thought to play an important function in the development of cardiometabolic disease, with subcutaneous adipose tissue (SAT) playing a lesser role.

Accumulation of excess VAT is related to the development of MetS, insulin resistance and type 2 diabetes [75-77]. Although the exact mechanism of association between VAT and cardiometabolic disease is still poorly understood, various theories have been proposed to explain this connection.

According to the portal-visceral hypothesis, VAT releases nonesterified fatty acids that overload the liver and skeletal muscle with lipids, causing metabolic dysfunction within these organs [78]. Alternatively, cytokines and select proteins released by VAT may promote a low-grade inflammatory response in adipose and vascular tissue [79], leading to insulin resistance and β-cell and microvascular dysfunction. Thus the pathophysiology of cardiometabolic disease, type 2 diabetes and atherosclerotic CVD events may have a common inflammatory origin [80]. A third premise suggests that genes which predispose preferential deposition of fat in VAT depots independently cause cadiometabolic disease [81]. In this scenario, VAT may simply be a marker of a dysmetabolic profile rather than a causal factor. Intraabdominal fat accumulation may be an indicator of the inability of SAT (adipose tissue deposited beneath the skin's surface) to act as "energy sink", which might result in the accumulation of fat in undesirable locations in the liver, skeletal muscle, heart and pancreatic β-cells [73]. Nonetheless, surgical removal of VAT in animal models has documented significant improvements in both hepatic and peripheral insulin sensitivity and glucose tolerance [82].

As noted by Klein et al., these hypotheses are not mutually exclusive. It is also possible that other unknown mechanisms may contribute [83]. Some

studies have suggested SAT to be equally important as a determinant of insulin resistance [84-86]. However, surgical removal of SAT in animal models failed to demonstrate an effect on glucose tolerance [82]. Additionally, liposuction of SAT in human subjects showed no improvement in insulin sensitivity [87]. It also had no noticeable effect on other cardiometabolic disease markers. Collectively, these findings support the theory that SAT plays a less important role in the aetiology of cardiometabolic disease than VAT [88].

1.4 Direct Measurement of Adiposity

A variety of measurement procedures have been proposed to assess VAT levels in order to enumerate individual susceptibility to cardiometabolic disease. Direct imaging techniques such as computed tomography, magnetic resonance imaging, and dual energy X-ray absorptiometry (DEXA) are used and allow direct quantification of body composition. Other non-imaging methods include hydrodensitometry, bioelectrical impedance, air displacement plethysmography and photonic scanning. However, many of these procedures require expensive apparatus and specialised personnel, while certain methods may carry an added risk of radiation exposure. As a result, anthropometry is more frequently utilised as a surrogate measure of body composition in research and clinical settings.

1.5 Surrogate Measures of Adiposity

1.5.1 Body mass index

Body mass index is the traditional diagnostic tool used in overweight and obesity classification most commonly employed within epidemiological research and healthcare practice. Calculated by dividing a subject's weight by the square of their height, BMI correlates with cardiometabolic disease features, morbidity and mortality [6,89-93]. As a commonly used measure of general adiposity, BMI is understood by clinicians and public health workers, is simple to assess, and allows non-gender or ethnic-specific risk thresholds to be used. The WHO classifies a BMI of 25-29.9 as overweight, 30-34.9 as obese class I, 35-39.9 as obese class II, and one equal to or above 40 as obese class III [94]. While research has suggested that risk of type 2 diabetes and CVD development may be higher in certain populations at a cut-off lower than 25, a WHO expert consultation committee recently concluded that current classifications should remain [95].

Although straightforward to calculate, measurement of BMI does require the use of a calibrated electronic weighing scale and a stadiometer, which may not always be available in a clinical or field setting. Studies examining self-reported BMI have reported discrepancies [96,97]. More importantly, as BMI is a weight-for-height measure, it is unable to distinguish between fat and lean mass. Findings have suggested that approximately half of obese subjects are metabolically healthy when classified using DEXA-derived body fat percentage, compared to approximately one-third by BMI [98]. Furthermore, a recent meta-analysis [61] found that class I obesity was not

associated with higher all-cause mortality and that overweight was related to significantly lower all-cause mortality, a relationship noted in other studies and described as the “obesity paradox” [99]. In light of this research, it has been suggested that BMI may misclassify adiposity in certain individuals.

1.5.2 Central adiposity

1.5.2.1 Waist circumference

Waist circumference (WC) measurement has been recommended as a more direct method for central adiposity and VAT assessment. Determined by measuring the circumference of the waist using a flexible tape, studies suggest it to be more strongly related to cardiometabolic disease and mortality than BMI [58,100,101]. Waist circumference appraisal has also been adopted by the IDF as a mandatory component for diagnosing the MetS [19], and is also the only adiposity variable used in four alternative MetS definitions [21]. However, partly due to a lack of agreement on a universal measurement protocol, its clinical usefulness and superiority over BMI for evaluating cardiometabolic health has been questioned [12,102]. The WHO and IDF recommend WC assessment exactly midway between the lowest rib and iliac crest [12,19], while the United States National Institutes of Health suggest measurement at the superior border of the iliac crest [103]. Various other sites have been proposed and used, such as umbilical level, lowest rib and the narrowest point between the last rib and iliac crest [102,104-106]. Although a recent report [107] concluded that the procedure used for estimating WC had minimal effect on morbidity or mortality

outcomes, this is still uncertain [102]. Waist circumference cut-points for determining metabolic risk are used [12] but are region and gender-specific, due to ethnic and sex differences in body composition. This necessitates the use of separate risk cut-offs in different populations [108-112].

1.5.2.2 Waist-hip ratio

Waist-hip ratio (WHR) is calculated by dividing WC by hip circumference and is thought to represent an aspect of body composition, related to cardiometabolic risk, not reflected in BMI or WC measurement. This index is also associated with cardiometabolic disease and mortality [113-116], and is the only central adiposity measure included in the WHO working definition of MetS [21]. Critics of WHR claim that as a ratio, it is complicated to interpret within a clinical setting [117]. Nevertheless, it should be noted that BMI is also a ratio and is easily used. A ratio allows universal population risk thresholds to be utilised, although this has not been fully explored [112,118], and current WHO recommendations do specify different WHR cut-offs for men and women [12]. The WHR also requires an additional anthropometric measurement, which may affect the reliability of this index, although hip circumference is more easily assessed than WC. Of greater concern is that WHR may remain unchanged in an individual even when body fat levels rise, as WC and hip circumference may increase or decrease proportionally [117].

1.5.2.3 Waist-height ratio

A more recently proposed central adiposity measure, the waist-height ratio (WHtR) is calculated by dividing WC by height. Similar to WHR, this variable is thought to more accurately reflect body fat distribution, and several studies suggest that it is a better discriminator of obesity-related conditions when compared to BMI, WC and WHR [119-122]. Unlike WHR, the WHtR only varies with an increase in body composition, as adult height remains relatively constant over time. Proponents of WHtR have also advised that the inclusion of height in an adiposity variable is desirable [123], as height is inversely associated with cardiometabolic disease and mortality [124].

As a ratio, WHtR may also allow the use of non-gender or ethnic-specific risk cut-points, which might make it additionally attractive from a clinical and public health perspective [125,126]. However, as calculation of this index also requires accurate height measurement, this may affect its practical usefulness. Moreover, some studies have suggested WHtR to be minimally superior, or even inferior, to WC as an indicator of cardiometabolic risk, and have questioned the measurement of height in addition to WC [127,128].

1.5.3 Novel indices

Periodically, novel indices are constructed using transformations of general or central adiposity measures. Among these are Rohrer's Index [129], the Conicity Index [130], the Abdominal Volume Index [131], A Body Shape Index [132] and several equations for determining body fat percentage

(BF%) using sagittal diameter [133] or skin-fold thickness measurements [134,135]. Recently, two novel adiposity indices for estimating BF% were proposed; Bergman et al. [136] determined a DEXA-validated measure using hip circumference and height, while Gómez-Ambrosi et al. [137] designed an equation utilising BMI, age and gender, and conducted a comparison study with other anthropometric measures and BF% estimated using air displacement plethysmography. However, as many of these novel indices use calculations which are complex, and perhaps difficult to interpret, their clinical utility and general usability must be questioned. Furthermore, there is a lack of research validating their usefulness. It is for these reasons that this PhD thesis exclusively examined the four most commonly assessed surrogate measures of general and central adiposity: BMI, WC, WHR and WHtR.

1.6 Literature Review

Over the last 20 years a considerable number of cross-sectional and prospective studies have attempted to quantify relationships between surrogate measures of adiposity and cardiometabolic disease, morbidity and mortality. However, results have been conflicting and inconclusive, and controversy still exists as to which index better indicates obesity-related metabolic risk. Increasingly, meta-analysis has gained recognition as a useful way of pooling results from numerous cohorts in order to average effect sizes across different studies. The benefits include increasing effective sample sizes and neutralising the influence of confounding factors, thus

allowing for a more precise evaluation of a risk, intervention, treatment or test.

1.6.1 Methods

1.6.1.1 Selection of studies

This meta-review investigated BMI, WC, WHR and WHtR relationships with cardiometabolic features, obesity-related chronic diseases and mortality. Published meta-analyses relating to these topics from the year 2007 onwards were searched using PubMed, Science Direct, Web of Knowledge, Academic Search Complete, JSTOR and Google Scholar databases. Search terms included a combination of keywords: body mass index or BMI, waist circumference or WC, waist-hip ratio, WHR or waist-to-hip ratio, waist-height ratio, WHtR, waist-to-height ratio, waist-to-stature ratio or WSR, meta-analysis and systematic review and meta-analysis. There were no language restrictions as long as abstracts were published in English.

The following were included: (1) meta-analyses which compared any two of the four indices of general or central adiposity (either BMI, WC, WHR or WHtR) using male, female or mixed adults of any ethnic group or age; (2) research using prospective or cross-sectional data; (3) studies examining cardiometabolic features or morbidity and mortality outcomes, including systolic or diastolic BP (SBP, DBP), triglycerides, HDL-C, low-density lipoprotein cholesterol (LDL-C), total cholesterol (Total-C) or FPG concentrations, elevated BP and hypertension, dyslipidaemia, MetS, type 2

diabetes, CVD (including CAD) or mortality. Children or adolescents were not included in any of the examined studies.

1.6.1.2 Details of included studies

Thirteen meta-analytic studies met inclusion criteria. Details and results are presented in Table 1. Five [127,138-141] studies included research which examined BMI, WC, WHR and WHtR, five [142-146] investigated three of the four indices, one [147] included studies examining WC and WHR, one [148] compared BMI with WHtR and one [149] contrasted BMI with central adiposity as defined by either WC measurement or the WHR. Seven [127,138,139,142,143,146,148] studies examined incident or prevalent type 2 diabetes as an outcome, six [138,139,141-143,148] explored elevated BP or hypertension, three [138,143,148] dyslipidaemia, two [143,148] investigated MetS, three [143,147,148] either incident or prevalent CVD events, four [144,145,148,149] examined either all-cause or CVD mortality and one [140] compared adiposity variable correlations with metabolic features. Ten [127,138,140-144,146,148,149] studies included samples from multiple ethnic groups, one [145] included Europeans only and one [139] examined Asian subjects. Sampled populations for one meta-analysis [147] were not stated, although reviewed studies were listed. All meta-studies included subjects of both genders.

1.6.2 Results

The main findings from these studies are stratified by the measures of association or discrimination reported [relative risk (RR), hazard ratio (HR), odds ratio (OR), area under the curve (AUC) or other] and are discussed in the context of cardiometabolic disease, morbidity and mortality outcomes.

1.6.2.1 Studies reporting relative risks or hazard ratios

Seven studies presented results as RRs or HRs. Both effect measures assess the risk of an event occurring by comparing the proportion of subjects (with or without an exposure) that develop an outcome [150].

Three studies exclusively used prospective data and examined index relationships using defined thresholds. In a meta-regression analysis, using a random effects model restricted to nine cohorts which provided categorical boundaries for BMI, WC and WHR, Carmienke et al. [144] found the risk of all-cause mortality for BMI to be 27% comparing obese class II to normal weight. This contrasted with a 32% increased probability for WC and a 13% increased risk for WHR using gender-specific categorical cut-points compared to a normal reference value. Conversely, using overall pooled results from 15 studies comparing sex-specific extreme quantiles, de Koning et al. [147] suggested WHR to be more strongly related to CVD events (1.95, 95% CI: 1.55-2.44) than WC (1.63, 95% CI: 1.31-2.04) in both men and women, although this difference was not statistically significant. Interestingly, in an individual participant meta-analysis using gender-specific tertiles,

Coutinho et al. [149] determined the risk of CAD mortality in subjects with central adiposity (defined by either WC or WHR) to be 70%, whereas BMI was inversely associated with mortality: 0.64, 95% CI: 0.59-0.69.

In research employing both prospective and cross-sectional data, Savva et al. [148] also found central adiposity defined by WHtR to be a greater risk factor for CVD and all-cause mortality, type 2 diabetes and MetS, compared to BMI, in pooled estimates from 34 studies which defined both measures using optimal thresholds. Nevertheless, substantial heterogeneity between included studies was observed. It should be equally noted that the use of categorical cut-offs based on arbitrary cut-points, as used by Savva and Carmienke, poses problems regarding the validity of comparisons between adiposity measures. Also, as alluded to by Huxley et al. [151], a limitation of the de Koning study was that BMI was not included as a comparison index and that the analysis was not restricted to studies which examined both WC and WHR.

Three studies employing longitudinal data used standardised Z-scores in analysis. Standardising values allows a uniform comparison of index relationships, and RRs or HRs represent the risk associated with a standard deviation (SD) increase in each measure. In a meta-analysis comparing data from 82,864 European subjects, Czernichow et al. [145] reported measures of central adiposity to be consistently and positively related to all-cause and CVD mortality. The risk of all-cause mortality was higher for WHR (12%) compared to WC (5%) while CVD mortality risk was the same for both

measures (15%). In a multivariable-adjusted model, a one SD increase in BMI appeared to confer protection against all-cause mortality (0.95, 95% CI: 0.91-0.99), and showed no association with cardiovascular-related death (1.05, 95% CI: 0.98-1.14). In another study which used pooled RRs to examine index relationships with type 2 diabetes, Kodama et al. [127] found WC (63%) and WHtR (62%) to have a modest, but significantly stronger association compared to BMI (55%) and WHR (52%) in both men and women. Conversely, Vazquez et al. [146] observed similar risk of developing type 2 diabetes for BMI, WC, and WHR (87%-88%) using overall pooled effects from 32 cohorts.

1.6.2.2 Studies reporting odds ratios or other statistic

Three studies reported effect measures as ORs which represent the ratio of the odds of an event occurring in one group to the odds of it occurring in another. Although similar in interpretation to RRs, ORs generally overestimate associations between variables but are an approximation of the RR when the rare disease assumption holds [150].

In an individual participant meta-analysis which stratified effect measures by ethnicity, Huxley et al. [142] found a 0.5 SD increment increase in BMI to be associated with a 20%-30% increased odds of having type 2 diabetes in Asian subjects. The corresponding odds using WC or WHR were 40%. However, ORs for hypertension were comparable between BMI, WC and WHR. Mohan [141] likewise noted similar strengths of association for BMI,

WC and WHtR regarding hypertension. A 0.5 SD increase was associated with a 40% versus 30% increased odds in Asian and non-Asians respectively. Nyamdorj et al. [139] also observed both general and central adiposity measures to be equally related with hypertension, while WHtR was more strongly associated with diabetes in men and women. Finally, research conducted by van Dijk et al. [140], which calculated the Pearson product-moment correlation (an appraisal of the linear dependence of two variables), indicated WC to be more strongly correlated with each of the examined metabolic features in both genders, with the exception of HDL-C and LDL-C in men.

1.6.2.3 Studies reporting measures of discrimination

Five studies included results from receiver operating characteristic curve (ROC) analysis. The ROC curve is a graphical representation of the relationship between sensitivity and specificity. The AUC provides a scale from 0.5 to 1.0 (with 0.5 representing random chance and 1.0 indicating perfect discrimination) which allows the discriminatory abilities of different adiposity variables to be compared [150].

In a meta-analysis of 31 prospective or cross-sectional studies which examined WHtR and either BMI or WC, Ashwell et al. [143] demonstrated WHtR to be a better discriminator than both BMI and WC for detecting type 2 diabetes, hypertension, dyslipidaemia, MetS and CVD. Pooled results showed that WC improved discrimination of all outcomes by 3% ($P<0.05$),

compared to BMI, with WHtR showing an average AUC that was 4%-5% ($P<0.01$) larger than BMI. Results stratified by gender and cardiometabolic outcomes indicated similar relationships. Comparable findings were reported by Mohan [141], using cross-sectional data from the Obesity in Asia Collaboration. Central adiposity variables were found to be better discriminators of hypertension. Although the authors concluded that differences in AUC values were minimal, the WHtR displayed the highest discriminatory capacity compared to BMI in both male (AUC for WHtR=0.67 versus AUC for BMI=0.63) and female (AUC for WHtR=0.71 versus AUC for BMI=0.66) subjects.

In contrast, Nyamdorj et al. [139] found BMI to be a better discriminator of hypertension but not type 2 diabetes. In this study, which included over 20,000 subjects, AUC values for WHtR in relation to prevalent diabetes were greater than BMI in both genders, although not statistically different. Similar results were again confirmed in a pooled analysis of 10 studies comparing BMI, WC, WHR and WHtR discrimination of incident and prevalent type 2 diabetes, hypertension and dyslipidaemia. Lee et al. [138] reported that central adiposity measures were better discriminators than BMI, with WHtR showing a greater AUC for each outcome in both genders. However, statistical differences between WHtR and BMI were noticed only in men for type 2 diabetes (AUC for WHtR=0.726 versus AUC for BMI=0.672, $P<0.01$) and hypertension (AUC for WHtR=0.684 versus AUC for BMI=0.641, $P=0.04$). The authors also observed that a combination of BMI with any of the three abdominal measures did not improve discrimination of

cardiovascular risk factors. Of note is that within each of these meta-studies, index discrimination, using either BMI or central adiposity, was greater in women.

Conversely, Czernichow et al. [145] found no clinically relevant difference between BMI, WC and WHR when comparing discrimination of all-cause or CVD mortality. Integrated discrimination improvement analysis, which measures the percentage of increased discrimination when a variable is added to a prediction model, identified a modest (<1%) but significant change when WHR was substituted for BMI. Discriminatory improvement for a model with WC, and models including any two of the examined adiposity measures, was also marginal.

1.6.2.4 Recent research

Although this review concentrated on meta-analytic studies, recent research employing large cohorts should also be considered in the context of surrogate measures of adiposity and cardiometabolic disease. In a cross-sectional study, utilising data from 7,447 Spanish men and women aged 55-80, Guasch-Ferré et al. [152] concluded that measures of central adiposity displayed greater discrimination of type 2 diabetes, impaired FPG, dyslipidaemia and MetS. The AUC values for WC and WHtR were significantly higher than AUCs for BMI with respect to each outcome except hypertension. Results were not stratified by gender, as no interactions between sex and examined outcomes were observed.

In another Spanish study, using prospective data from 37,733 subjects, Huerta et al. [153] found both general and central adiposity to be independently associated with diabetes. The WHtR index displayed the highest AUC values in both men (AUC=0.687) and women (AUC=0.776) compared to BMI (AUC=0.676 for men and AUC=0.759 for women) although discriminatory differences were small. The HRs of having type 2 diabetes were greater for central adiposity indices in women only, with BMI indicating the strongest association with diabetes in men. Similar results were demonstrated by the InterAct Consortium, a pan-European cohort with 340,234 participants which examined incident diabetes. In this study, Langenberg et al. [154] also reported BMI and WC to be independently associated with diabetes. The association between WC and type 2 diabetes was especially strong in women, leading the authors to recommend central adiposity assessment as an effective strategy for risk stratification.

Gender heterogeneity was additionally noted by Wannamethee et al. [155] in a seven year prospective study which compared BMI, WC and WHR abilities to predict diabetes development in 6,923 older men and women. The ROC analysis revealed similar AUCs for BMI and WC in males (AUC=0.726 and AUC=0.713 respectively), with WHR showing the least predictive ability (AUC=0.656). In females, WC was a significantly better discriminator (AUC=0.780) compared to both BMI (AUC=0.733) and WHR (AUC=0.728, $P<0.01$ for both). Conversely, in an analysis using pooled data from four German population-based longitudinal cohorts (N=10,258), Hartwig et al. [156] found WHtR to be equally predictive of type 2 diabetes in both genders

(AUC=0.75) compared to BMI (AUC=0.72 for men and AUC=0.71 for women). However, in a cross-sectional study of 12,294 adults, Mooney et al. [157] reported similar discriminatory capabilities for all indices regarding cardiometabolic risk factors in both men and women. Although central adiposity variables were superior discriminators of impaired FPG, BMI was a better discriminator of hypertension.

1.6.3 Discussion

Of the nine meta-analyses included in this review that reported effect measures as either RRs, HRs or ORs (and which included indices of both general and central adiposity), six [127,139,142,145,148,149] showed that central adiposity, defined by either WC, WHR or WHtR, was more strongly associated with a majority of the examined obesity-related conditions or mortality. Three [141,144,146] concluded that general and central adiposity indices displayed similar risk patterns. Subsequently, these results might suggest that central adiposity is a better indicator of cardiometabolic risk and chronic disease than BMI. However, these findings are ambiguous. Although on average, central adiposity measures displayed stronger relationships when compared to BMI, similar strengths of association were observed in many of the included studies.

Equally important to consider is that measures of association do not necessarily indicate an ability to discriminate an outcome of interest. Of the five meta-studies which reported results from ROC analysis, only one [143]

demonstrated that central adiposity variables were significantly better discriminators than BMI, while one [138] reported that they were statistically superior in men only. Two [139,141] determined that AUCs were larger for central indices, but not significantly so, or that differences were minimal, and one concluded that there was no clinically relevant difference in discriminatory capabilities between either BMI, WC or WHR [145].

As discussed by Pepe et al. [158], although a strong association is a necessary condition when comparing measures in terms of ability to discriminate, it is not sufficient, as even variables with strong associations may not adequately discriminate between subjects with or without an outcome. Moreover, while two of the examined meta-studies suggested that AUC values were significantly greater for central indices regarding specific outcomes, and the WHtR index was a noticeably better discriminator in several, the AUC is a summary statistic regarding the overall discriminatory performance of a marker and lacks clinical relevance. In addition, observed discriminatory differences between BMI and central adiposity indices were modest.

Other factors should also be considered when drawing conclusions from this review. Meta-analysis, while an effective tool within epidemiological research, is not without its limitations. Several studies included numerous comparisons between one or more adiposity measures and outcomes, and less for others, thus giving some variables an inordinate weight in analysis [151]. Furthermore, as WC measurement has not been standardised

internationally, optimal measurement protocols for assessing metabolic risk may be different between included studies, thus influencing observed associations. The results from this meta-review also suggest heterogeneous relationships between adiposity measures and cardiometabolic disease relating to gender [143], and studies which do not stratify or use appropriate statistical methods may over or underestimate effect sizes and discriminatory differences. Also importantly, cardiometabolic outcomes may be classified differently within studies, with several using optimal procedures and different tests to define conditions (e.g. the OGTT for type 2 diabetes compared with FPG or the HbA_{1c} assay) and some using only self-reported diagnosis [23,159,160]. Other aspects such as age or ethnicity may also influence results [58,161].

1.6.4 Conclusions and rationale for further research

Studies comparing BMI with central adiposity suggest the latter provides additional information (beyond that which is measured by BMI), as relationships between BMI and cardiometabolic disease are attenuated in regression models after the inclusion of WC [58,100], thus indicating that central adiposity explains a greater variance of obesity-related risk, or that both adiposity variables provide independent information [59,92,144,153,154]. In addition, research has suggested that centrally obese subjects classified as normal weight by BMI represent a particularly high-risk group [149,162-164]. However, just as critics of central adiposity assessment – who claim it as unnecessary, inaccurate and time-consuming

– neglect to state how much of an increase in discriminatory accuracy would be clinically relevant, so it is also true that proponents of WC, WHR or WHtR measurement often fail to clarify how much added information these indices might provide over BMI or other variables currently utilised in cardiometabolic risk algorithms.

These concerns were examined by Klein et al. [83], who determined that measurement of WC in clinical practice would not be trivial, as providing such an assessment competes for the limited time available during patient appraisal and requires specific training to ensure reliable data are obtained. Nevertheless, WC measurement was recommended as a method to identify a potentially non-trivial number of patients at increased risk who might not be detected using conventional methods. Central adiposity indices might be effective tools for identifying metabolically unhealthy, normal weight or non-obese subjects who could benefit from an intervention or lifestyle therapy, but who would not otherwise be considered for treatment if adiposity were measured using BMI. This idea was further explored in a WHO report [12] which suggested that central adiposity variables, used in conjunction with BMI, might contribute to the development of a composite index to discriminate high-risk patients.

However, despite potential uses for central adiposity measures, a majority of research continues to demonstrate a significant and strong relationship between BMI and obesity-related conditions. This suggests its continued relevance for defining and assessing metabolic risk within epidemiology and

clinical practice. While results from this review might imply that central adiposity is a greater risk factor for cardiometabolic disease, type 2 diabetes and mortality, with regard to the clinical utility of central adiposity assessment, these findings are inconclusive.

By exploring issues raised by this review, this thesis adds to the current knowledge base regarding the clinical relevance and potential usefulness of surrogate measures of adiposity as tools to identify high-risk patients. Specifically, it examines the rationale for routine assessment of adiposity within healthcare practice, and whether methods used for disease classification and anthropometric measurement procedure are important for diagnosing cardiometabolic risk and type 2 diabetes. It also compares adiposity variable relationships with a range of cardiometabolic disease features, biomarkers of chronic low-grade inflammation and type 2 diabetes. Finally, it explores whether central adiposity indices provide additional information regarding disease and risk status, beyond that which is normally assessed by BMI, and whether a composite index using both general and central adiposity measures might be clinically useful.

Table 1—*Details and results from meta-analytic studies.*

Reference	No. of Studies	Population and No. of Subjects	Indices Examined	Main Outcome Measure	Analysis Type	Results	Comments
Ashwell et al. (2012) [143]	31	4 Europe 2 South America 2 Australasia 6 Asia 2 Middle-East 1 Caribbean 14 other 123,231 men 182,620 women	BMI WC WHtR	Incident and prevalent type 2 diabetes, hypertension, dyslipidaemia, MetS and CVD	Pooled ROC analysis	Pooled AUCs for all outcomes were: 0.667, 0.650-0.684 (BMI), 0.694, 0.678-0.709 (WC) and 0.704, 0.689-0.718 (WHtR) in men and 0.681, 0.658-0.704 (BMI), 0.714, 0.698-0.731 (WC) and 0.725, 0.709-0.741 (WHtR) in women	WHtR was a better discriminator than BMI for all five specific health outcomes Statistical comparisons of central adiposity with BMI indicated that both WC and WHtR were significantly better at discriminating type 2 diabetes Compared with BMI, WC improved discrimination of adverse outcomes by 3% ($P<0.05$) and WHtR improved discrimination by 4%-5% ($P<0.01$) Discriminatory ability was greater in women
Carmienke et al. (2013) [144]	18	6 Europe 10 North America 2 Australasia 693,739 men and women	BMI WC WHR	All-cause mortality	Pooled RR using categorical variables	RRs for all-cause mortality were: 1.27 (1.21-1.33), 1.32 (1.22-1.43) and 1.13 (1.11-1.59) for [1]BMI, [2]WC and [2]WHR respectively [1]Obese class II compared to normal weight [2]Gender-specific categorical cut-point compared to normal reference	Meta-regression analysis was restricted to nine cohorts that provided RRs and 95% CIs and which defined category boundaries for adiposity measures All measures showed similar risk patterns for upper quartiles in comparison to reference quartiles Patterns of general and central adiposity remained significantly associated with mortality when adjusted for both

Table 1 continued

Reference	No. of Studies	Population and No. of Subjects	Indices Examined	Main Outcome Measure	Analysis Type	Results	Comments
Coutinho et al. (2011) [149]	5	2 Europe 2 North America 1 Asia 15,923 (59% men)	BMI and central adiposity defined by either WC or WHR	CAD mortality	Individual participant meta-analysis HR by index tertiles	Overall gender-specific pooled RRs comparing upper to lower index tertiles were: 0.64 (0.59-0.69) for BMI and 1.70 (1.58-1.83) for central adiposity defined by either WC or WHR	Central adiposity was associated with mortality whereas BMI was inversely associated with mortality Central adiposity was also associated with higher mortality in a subset of subjects with normal BMI
Czernichow et al. (2011) [145]	9	Europe 82,864 men and women	BMI WC WHR	All-cause and CVD mortality	Individual participant meta-analysis HR comparing upper quintiles to lower quintiles and for a 1 SD increase in each index ROC and integrated discrimination improvement analysis	For all-cause mortality, multivariable adjusted HRs for a 1 SD increase were: 0.95 (0.91-0.99), 1.05 (1.00-1.09) and 1.12 (1.06-1.18) for BMI, WC and WHR respectively For CVD mortality, multivariable adjusted HRs for a 1 SD increase were: 1.05 (0.98-1.14), 1.15 (1.05-1.25) and 1.15 (1.04-1.27) for BMI WC and WHR respectively	Measures of central adiposity were more strongly associated with CVD mortality BMI was related to CVD mortality in age and gender adjusted models only There was a modest (<1%) enhancement in discriminative capability using WHR compared to BMI The advantage of using WC was also marginal Models combining two adiposity indices did not provide improvement in the prediction of mortality
de Koning et al. (2007) [147]	15	Not stated 258,114 (35.7% men)	WC WHR	Incident CVD events	Pooled RR comparing highest to lowest quantiles of WC and WHR	Overall risk estimate comparing extreme gender-specific quantiles for each measure were: 1.63 (1.31-2.04) for WC and 1.95 (1.55-2.44) for WHR	The results suggested that WHR was more strongly associated with CVD than WC although differences were not statistically significant

Table 1 continued

Reference	No. of Studies	Population and No. of Subjects	Indices Examined	Main Outcome Measure	Analysis Type	Results	Comments
Huxley et al. (2008) [142]	21	Asian (73%) Caucasian (27%) >263,000 men and women	BMI WC WHR	Prevalent type 2 diabetes and hypertension	Individual participant meta-analysis OR for a 0.5 SD increase in each index	A 0.5 SD increment increase in BMI was associated with a 20%-30% increased odds of type 2 diabetes in Asian subjects A 0.5 SD increment increase in WC or WHR was associated with a 40% increased odds of type 2 diabetes in Asian subjects	ORs of having hypertension were similar for all measures of general and central adiposity Ethnic heterogeneity was observed in obesity/morbidity associations
Kodama et al. (2012) [127]	15	8 Western 7 Non-Western 120,012 men and women	BMI WC WHR WHtR	Incident type 2 diabetes	Pooled RR for a 1 SD increase in each index	Pooled RRs for a 1 SD increase (men and women combined) were: 1.55 (1.43-1.69), 1.63 (1.49-1.79), 1.52 (1.40-1.66) and 1.62 (1.48-1.78) for BMI, WC, WHR and WHtR respectively	WC and WHtR showed a modest but significantly stronger association with type 2 diabetes compared to BMI or WHR, but measuring height in addition to WC appeared to have little additional benefit
Lee et al. (2008) [138]	10	1 Europe 7 Asian 1 Caribbean 1 Iran 88,514 (54% women)	BMI WC WHR WHtR	Incident and prevalent type 2 diabetes, hypertension and dyslipidaemia	Pooled ROC analysis	Pooled AUCs for type 2 diabetes were: 0.672, 0.646-0.697 (BMI), 0.701, 0.670-0.732 (WC), 0.721, 0.664-0.778 (WHR) and 0.726, 0.698-0.754 (WHtR) in men and 0.693, 0.629-0.757 (BMI), 0.744, 0.695-0.794 (WC), 0.748, 0.687-0.810 (WHR) and 0.756, 0.700-0.811 (WHtR) in women	WHtR was the best discriminator of type 2 diabetes, hypertension and dyslipidaemia in both genders Statistical differences between BMI and WHtR were noticed only in men for type 2 diabetes and hypertension Higher pooled AUCs were observed in females compared to males suggesting that discrimination is more precise in women The authors concluded that evidence supports the superiority of measures of central adiposity over BMI for detecting CVD risk factors

Table 1 continued

Reference	No. of Studies	Population and No. of Subjects	Indices Examined	Main Outcome Measure	Analysis Type	Results	Comments
Mohan (2008) [141]	19	Asian (62.8%) Caucasian (36.7%) Pacific Islanders (0.5%) >173,709 (53% women)	BMI WC WHR WHtR	Prevalent hypertension	Individual participant meta-analysis Adjusted linear regression between indices and SBP/DBP stratified by ethnicity OR for a 0.5 SD increase in each index ROC analysis stratified by ethnicity and overall pooled results	Similar strengths of association with hypertension were noted for BMI, WC and WHtR: 40% versus 30% in Asian and non-Asians respectively Pooled AUCs for hypertension were: 0.63, 0.62-0.66 (BMI), 0.66, 0.64-0.67 (WC), 0.65, 0.63-0.67 (WHR) and 0.67, 0.66-0.69 (WHtR) in men and 0.66, 0.64-0.68 (BMI), 0.69, 0.63-0.72 (WC), 0.68, 0.65-0.70 (WHR) and 0.71, 0.68-0.73 (WHtR) in women	Measures of central adiposity tended to be better discriminators of hypertension in both genders Overall, WHtR had the highest discriminatory capability, although the authors concluded that no anthropometric variable was systematically better than the others for discriminating hypertension Heterogeneity in associations and discriminatory capacity were observed between different ethnic populations
Nyamdorj et al. (2008) [139]	16	Asia 9,095 men 11,732 women	BMI WC WHR WHtR	Prevalent type 2 diabetes and hypertension	Individual participant meta-analysis OR for a 1 SD increase in each index ROC analysis	Pooled AUCs for type 2 diabetes were: 0.725, 0.706-0.743 (BMI), 0.729, 0.711-0.747 (WC), 0.729, 0.711-0.747 (WHR), and 0.735, 0.717-0.753 (WHtR) in men and 0.742, 0.726-0.756 (BMI), 0.749, 0.734-0.765 (WC), 0.742, 0.727-0.758 (WHR) and 0.748, 0.733-0.764 (WHtR) in women	WHtR displayed a stronger association with diabetes compared to BMI but all indices were equally strongly associated with hypertension AUCs were slightly higher for diabetes using WHtR (both genders) and for WC (in women), and greater for BMI regarding hypertension, in both genders

Table 1 continued

Reference	No. of Studies	Population and No. of Subjects	Indices Examined	Main Outcome Measure	Analysis Type	Results	Comments
Savva et al. (2013) [148]	34	Asian and non-Asians 512,809 men and women	BMI WHtR	Incident and prevalent type 2 diabetes, elevated BP, dyslipidaemia, MetS, CVD, all-cause and CVD mortality	Pooled estimate of the ratio of RRs (rRR [=RRBMI/ RRWHtR]) using optimal BMI and WHtR cut-offs	WHtR was found to have a stronger association than BMI with type 2 diabetes (RR: 0.71, 0.59–0.84) and MetS (RR: 0.92, 0.89–0.96) in cross-sectional studies In prospective studies, WHtR appeared to be more strongly associated with several outcomes including incident CVD, all-cause and CVD mortality	The usefulness of WHtR appears to be better in Asian than in non-Asian populations There was substantial heterogeneity between included studies
van Dijk et al. (2012) [140]	20	11 Europe 7 North America 1 Turkey 1 Australasia 21,139 men 24,139 women	BMI WC WHR WHtR	Metabolic features: FPG, SBP, DBP, HDL-C, LDL-C, Total-C and triglycerides	Pooled mean Pearson correlation coefficients between each index and metabolic features	Mean Pearson correlation coefficients for FPG were: 0.188 ± 0.019 (BMI), 0.227 ± 0.030 (WC), 0.213 ± 0.029 (WHR) and 0.136 ± 0.013 (WHtR) in men and 0.243 ± 0.024 (BMI), 0.289 ± 0.038 (WC), 0.261 ± 0.035 (WHR) and 0.171 ± 0.014 (WHtR) in women	WC displayed the strongest correlation with metabolic features in both men and women, except for HDL-C and LDL-C in men When comparing BMI to WC, the latter showed significantly better correlation with metabolic features, except for DBP in women and HDL-C and Total-C in men
Vazquez et al. (2007) [146]	32	9 Europe 12 North America 4 Asia 7 Other 31,702 men and women	BMI WC WHR	Incident type 2 diabetes	Pooled RR for a 1 SD increase in each index	Pooled RRs for a 1 SD increase were: 1.87 (1.67-2.10), 1.87 (1.58-2.20) and 1.88 (1.61-2.19) for BMI WC and WHR respectively	Similar associations with type 2 diabetes were noted for all adiposity measures

1.7 Thesis Outline

1.7.1 Aims and objectives

The overall aim of this PhD research was to determine how useful surrogate measures of adiposity might be to identify high-risk patients within a clinical setting. Specific aims and objectives of the research and corresponding chapters are presented in Figure 1.

Four specific research aims were identified. These were:

1. to examine the rationale for adiposity measurement within clinical practice;
2. to compare cardiometabolic feature relationships with type 2 diabetes and pre-diabetes diagnosed by different tests;
3. to investigate mechanisms of association between measures of adiposity and type 2 diabetes;
4. to assess adiposity variable discriminatory capability.

The specific objectives were:

1. to examine why adiposity measurement might be clinically useful within Ireland to identify high-risk patients, such as those with undiagnosed diabetes;
2. to determine how type 2 diabetes and cardiometabolic risk should be diagnosed within clinical practice and epidemiological research;
3. to determine which metabolic variables are related to type 2 diabetes and diabetes development with reference to both established components of the MetS and novel inflammatory biomarkers;

4. to explore adiposity variable relationships (including individual variables and derived composite indices) with features of cardiometabolic disease and type 2 diabetes;
5. to examine adiposity index discrimination of cardiometabolic risk and type 2 diabetes using different anthropometric measurements;
6. to investigate whether a composite index using measures of both general and central adiposity might be clinically useful.

1.7.2 Research outputs

Chapter 2 (Paper 1) examines the prevalence of undiagnosed and diagnosed type 2 diabetes within our sample. This study compares features between these two groups in order to determine why certain individuals remain undetected. It also evaluates variables which might be useful in screening programmes within Ireland to identify undiagnosed cases, and suggests a rationale for adiposity measurement within clinical practice. The findings from this research were published in the journal PLOS ONE in November 2013 [165].

Chapter 3 (Paper 2) compares cardiometabolic feature relationships with type 2 diabetes and pre-diabetes diagnosed by different procedures (HbA_{1c} and FPG). Although these conditions represent the two major cardiometabolic outcomes within our sample, they are “soft” outcomes defined on the basis of a positive test. Glycated haemoglobin A_{1c} measurement has been recommended for diagnosis of type 2 diabetes, and

as a procedure to detect subjects at a high-risk state of developing diabetes. However, controversy exists regarding its use within clinical practice and as a method for defining outcomes within epidemiology. In particular, research has suggested discordance between HbA_{1c} and the FPG test, which was more commonly employed as a diagnostic tool in Ireland before 2010. The objectives of this paper were to validate HbA_{1c} measurement within our sample against FPG as a method for classifying diabetes and cardiometabolic disease risk, and also to observe metabolic variable relationships with type 2 diabetes and pre-diabetes. The results from this study were published in the journal PLOS ONE in August 2015 [166].

Chapter 4 (Paper 3) examines adiposity variable relationships with diabetes-related metabolic features and type 2 diabetes. It investigates whether WC measurement protocol influences discriminatory accuracy. It also compares BMI with central obesity measures to determine whether general or central adiposity is a better indicator of cardiometabolic risk and type 2 diabetes. The findings from this research were published in the journal PLOS ONE in June 2015 [167].

Chapter 5 (Paper 4) explores general and central adiposity relationships with biomarkers of inflammation. Chronic low-grade inflammation has been suggested as a possible mechanism linking adiposity with type 2 diabetes. This paper compares BMI and WC associations with markers of low-grade inflammation and type 2 diabetes in order to ascertain whether general or

central adiposity is a greater risk factor for diabetes-related systemic inflammation.

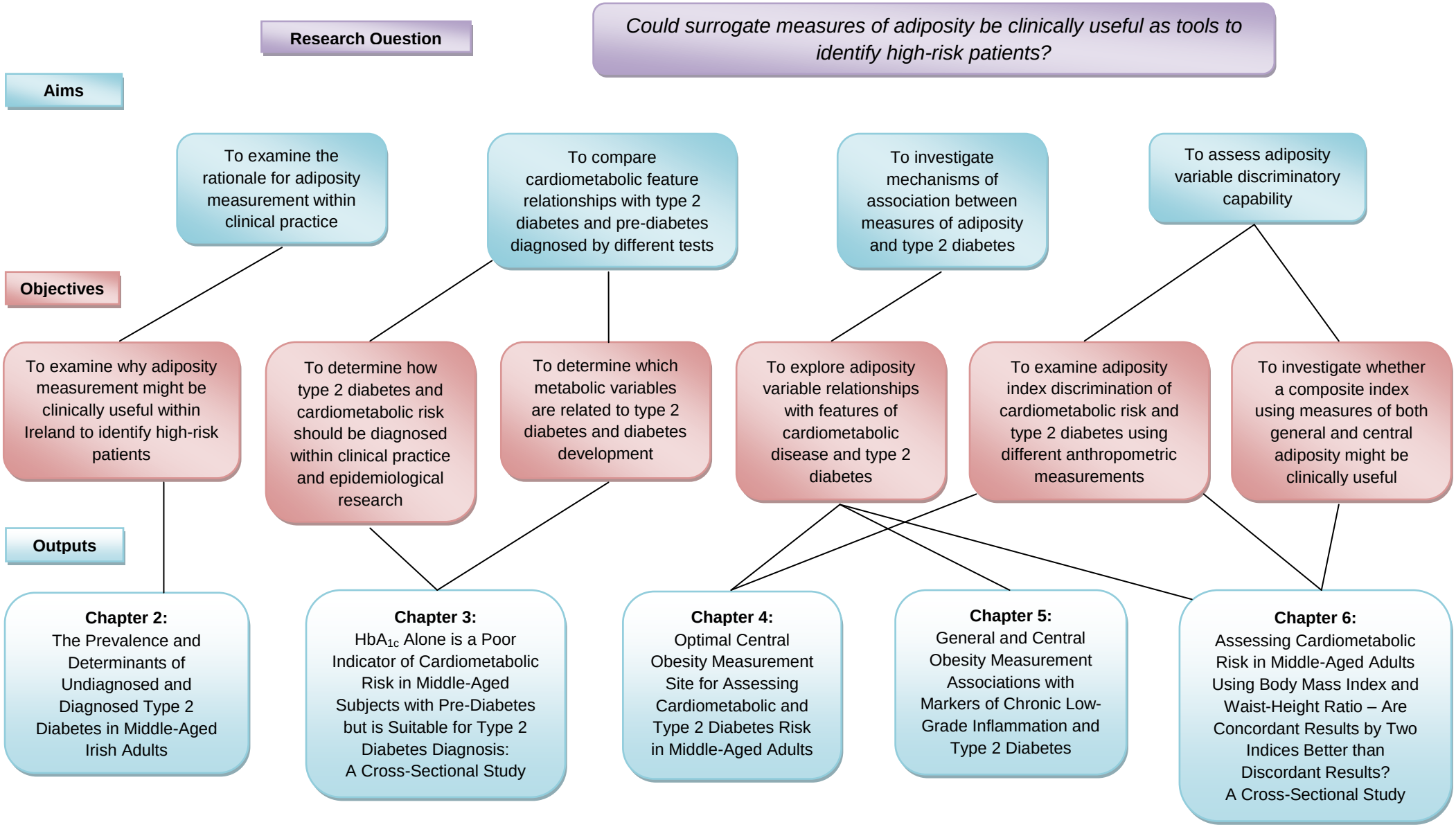
Chapter 6 (Paper 5) assesses the utility of a composite index for adiposity measurement. It examines if joint use of BMI and WHtR might refine body fat classification, and whether a combination of both adiposity measures could help stratify high and low-risk patients. The results from this study were published in the BMC journal Diabetology & Metabolic Syndrome in September 2015 [168].

1.7.3 Author's contribution

I was the lead author of the research papers presented in Chapters 3, 4, 5 and 6. This involved the formulation of the research question for each chapter, conducting literature searching, data analysis and the drafting of each manuscript.

The research question for Chapter 2 was originally examined as part of Master's thesis project on which I was a tutor. I was involved in the formulation of the research question. In addition, the data analysis, writing and revisions of the submitted manuscript were performed by me. These contributions are acknowledged in the author contributions section of the published paper.

Figure 1—*Overview of aims, objectives and research outputs.*



1.8 Methods

1.8.1 The Cork and Kerry Diabetes and Heart Disease Study

This thesis makes use of data from the Cork and Kerry Diabetes and Heart Disease Study – Phase II (The Mitchelstown Cohort). In 2010, as part of the Health Research Board Centre for Health and Diet Research, we received funding to recruit a cohort of subjects in order to provide an updated profile of glucose tolerance status, cardiometabolic health and related factors in a middle-aged Irish population. This study utilised field survey procedures and equipment similar to those used in the original 1998 Cork and Kerry Diabetes and Heart Disease Study [169].

1.8.2 Ethical approval and sampling procedure

A cross-sectional random sample of 2,047 middle-aged men and women was recruited from patients attending a single large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic includes nine general practitioners and the practice serves a catchment area of approximately 20,000, with a mix of urban and rural residents. Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork.

The name, address, gender and date of birth were provided for all registered attending patients in the clinic, and stratified sampling was employed to recruit equal numbers of men and women in the 46-73 year age group. In total, 3,807 potential participants were selected from the practice list.

Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%).

The status of non-responders included individuals refusing to participate (59.4%) and those who did not reply (40.6%). Male subjects accounted for 53.7% of non-responders while 43.5% (versus 42.8% of responders) were >60 years of age. All non-responders were followed up with a phone call where possible and otherwise with a single postal reminder. Signed informed consent was obtained from each participant before commencing the assessment.

1.8.3 Study delivery and quality control

Fasting blood samples were taken to determine lipid and lipoprotein concentrations, glycaemic status, full blood counts and a biochemical profile. Clinical measurements taken by trained researchers included BP readings and anthropometric measurements. Anthropometric variables assessed included weight, height, WC measured at two sites (midway and lowest rib), hip circumference and pelvic width. Details regarding anthropometric measurement protocols are included in Appendix 5. Survey instruments and variable definitions are addressed in each of the relevant chapters to follow.

Participants were required to complete a General Health Questionnaire (GHQ) [170]. Physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ) [171]. Both the GHQ and IPAQ have been proven to be valid and reliable and were used in the 1998 study [169] and the SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition [172].

All procedures were carried out with reference to guidelines outlined in a standard operating procedures manual and all results were recorded on a clinical report form. Data from the report form, GHQ and IPAQ were scanned using Teleform^{TM} and information was verified against the hard copy. Data were subsequently exported to MS-Excel and were again checked against the hard copy. When data entry was complete for each variable, 10% of the sample was randomly checked for errors.

CHAPTER 2

THE PREVALENCE AND DETERMINANTS OF UNDIAGNOSED AND DIAGNOSED TYPE 2 DIABETES IN MIDDLE-AGED IRISH ADULTS

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2.0 Abstract

Background and Objectives

The prevalence of type 2 diabetes within the Republic of Ireland is poorly defined, although a recent report suggested 135,000 cases in adults aged 45+, with approximately one-third of these undiagnosed. This study aims to assess the prevalence of undiagnosed and diagnosed diabetes in middle-aged Irish adults and compare features between these two groups in order to investigate why certain individuals remain undetected.

Materials and Methods

This was a cross-sectional study involving a random sample of 2,047 men and women aged 46-73 years. Univariate logistic regression was used to explore socio-economic, metabolic and other health-related variable associations with undiagnosed and diagnosed diabetes. A final multivariable analysis was used to determine odds ratios of having undiagnosed compared to diagnosed diabetes, adjusting for age, gender and significant covariates determined from univariate models.

Results

The total prevalence of diabetes was 8.5% (95% CI: 7.4%-9.8%); 72 subjects (3.5%) had undiagnosed diabetes (95% CI: 2.8%-4.4%) and 102 subjects (5.0%) had diagnosed diabetes (95% CI: 4.1%-6.0%). Adiposity, dyslipidaemia and having a family history of diabetes were positively

associated with both undiagnosed and diagnosed type 2 diabetes. When compared to diagnosed subjects, study participants with undiagnosed diabetes were significantly more likely to have higher levels of adiposity and low levels of physical activity, and they were less likely to be on treatment for diabetes-related conditions or to have private medical insurance.

Conclusions

The prevalence of type 2 diabetes within the Cork and Kerry Diabetes and Heart Disease Study is comparable to a recent estimate from the SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition, a study which was nationally representative of the general population. A high percentage of diabetes cases were undiagnosed (41%), emphasising the need for more effective detection strategies and equitable access to primary healthcare.

2.1 Introduction

Type 2 diabetes mellitus, a chronic disease which causes significant morbidity and mortality, was the ninth leading cause of death worldwide in 2008 [173]. Diabetes is associated with obesity, dyslipidaemia and hypertension, and is characterised by chronic hyperglycaemia due to insufficient insulin release, impaired insulin action, or a combination of both [23]. Importantly, the persistent hyperglycaemia that is associated with diabetes may cause serious health complications such as cardiovascular disease (CVD) and impairment and malfunction of the renal, ophthalmic, vascular and nervous systems [25]. These complications pose significant financial burdens on healthcare services; research conducted in 2006, which examined economic consequences related to type 2 diabetes, estimated that almost 10% of total healthcare expenditure was spent on diabetes care in the Republic of Ireland alone [174].

The prevalence of type 2 diabetes is increasing globally, representing a key public health issue [175]. There is a lack of research relating to diabetes in Ireland, although recent studies have indicated that the condition may be reaching epidemic proportions [176,177]. In 1998, the prevalence of diabetes amongst subjects in a primary care based sample was estimated to be 3.9% [169]. A recent report from the Irish Institute of Public Health (IPH) [178] based on findings from the SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition [172], suggested a prevalence of 8.9% in adults aged 45+. This estimate consisted of 94,000 subjects with clinically diagnosed type 2 diabetes and 41,000 with undiagnosed diabetes. While the efficacy and cost-

effectiveness of routine screening for diabetes in primary care has not been established [179-181], there is an ongoing need for contemporary data on the prevalence of type 2 diabetes, in population and primary care settings, in order to guide policy in this area. This could help formulate strategies that further develop effective diabetes prevention, detection and management, as individuals with undiagnosed diabetes are at a high-risk of diabetic complications [182].

The aim of this study was to estimate the prevalence of undiagnosed and diagnosed type 2 diabetes in a random sample of men and women aged 46-73 years, drawn from a primary care setting similar to that studied in 1998 [169], using the same field survey procedures and methods. In particular, we compared features between these two groups in order to determine why certain individuals remain undetected.

2.2 Materials and Methods

2.2.1 Study design

The study design is described in detail in Chapter 1. In summary, the Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000, with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending

patients in the 46-73 year age group. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). Details regarding the study design, sampling procedures and methods of data collection have been reported previously [183].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

2.2.2 Clinical and laboratory procedures

The weight and height of each subject were measured to the nearest 0.1 kg and 0.1 cm respectively by trained researchers. Study participants were asked to remove heavy outer clothing and footwear. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was measured using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK). Three measurements of systolic and

diastolic blood pressure (SBP and DBP respectively) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's blood pressure.

After an overnight fast, participants were invited to attend the clinic for the sampling of blood between 8 and 10 A.M. Triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica Gmbh, Hamburg, Germany). Fasting plasma glucose (FPG) concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland). Glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 (Tosoh HLD-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium).

A self-administered General Health Questionnaire (GHQ) [170] was used to collect supplementary information which included prescription (Rx) medication use, demographic characteristics, medical cover, family diabetes history, past medical history of CVD and smoking/alcohol behaviours. Physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ) [171].

2.2.3 Metabolic and anthropometric variables

Lipid, lipoprotein and FPG levels were classified according to National Cholesterol Education Program: Adult Treatment Panel III guidelines [184]. Abnormal metabolic risks were defined as high triglycerides $\geq$1.7 mmol/l, low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females) and impaired FPG $\geq$5.6 mmol/l. Dyslipidaemia was determined according to both elevated triglyceride and low HDL-C levels. Hypertension was defined according to World Health Organisation guidelines as SBP $\geq$140 mmHg and/or DBP $\geq$90 mmHg [185]. Body mass index (BMI) was calculated by dividing a subject's weight by the square of their height and was categorised as <25 = *normal weight*, 25-29.9 = *overweight* and $\geq$30 = *obese* [94].

2.2.4 Morbidity

Type 2 diabetes [23] was defined as HbA_{1c} $\geq$6.5% ($\geq$48 mmol/mol), (N=146). Undiagnosed diabetes was determined if subjects had positive HbA_{1c} tests but did not report a medical diagnosis of diabetes or oral medication use for the condition (N=72). Diagnosed diabetes was classified according to positive test results and a self-reported physician diagnosis or diabetes medication use (N=74), or by diagnosis or medication use alone (N=28, total diagnosed=102). The presence of CVD was obtained from the GHQ by asking study participants if they had been diagnosed with one of the following seven conditions: *heart attack* (including coronary thrombosis or myocardial infarction), *heart failure*, *angina*, *aortic aneurysm, hardening of*

the arteries, *stroke* or any other *heart trouble*. Subjects indicating a diagnosis of any of these disorders were classified as having CVD.

2.2.5 Covariates

Other covariates utilised from the GHQ included age, gender, use of Rx anti-hypertensive and cholesterol-lowering medications, family diabetes history, education, social class, medical cover, smoking status and alcohol use. Age was included either as a dichotomous (<60/≥60 years of age) or continuous variable in univariate or multivariable regression models. Education was divided into four categories: *primary, secondary, diploma* and *bachelor or higher*. Social class was defined according to the European Socio-economic Classification method (ESeC) [186], and collapsed into three groups: *high income, middle income and low income*. The health service variables – *private insurance, no insurance* and means-tested, state-assisted *general practice visit card* (GPC) and *full medical card* (FMC) – were transformed into a dummy variable: *private insurance, state insurance, no insurance*. Subjects reporting more than one insurance type were assigned to the higher insurance category. Self-reported physical activity within the previous six months, measured using the IPAQ questionnaire, was divided into three categories: *high, moderate* and *no physical exercise*. Alcohol use was assessed by asking study participants how often they consumed alcohol on a monthly or weekly basis, and was classified as follows: “never or less than once a month” = *non-drinker*, “2-4 times monthly” = *occasional drinker* and “twice or more weekly” = *regular drinker*. Subjects were considered to have

ever smoked if they smoked cigarettes during the recruitment phase, had smoked within the last 10 years or had smoked more than 100 cigarettes in their lifetime, and non-smokers if they had smoked less than this or had never smoked.

2.2.6 Statistical analysis

The descriptive characteristics of the study population were examined according to diabetes status. Gender differences in type 2 diabetes prevalence were compared using chi-square tests. Health condition, health behaviour, health insurance, socio-economic and metabolic variable associations with undiagnosed or diagnosed diabetes were explored through multiple univariate binary logistic regressions. Diagnosed subjects were excluded from models examining undiagnosed type 2 diabetes, while models investigating relationships between features and diagnosed diabetes excluded undiagnosed cases. Distinctions between undiagnosed and diagnosed diabetes were explored in univariate analyses excluding non-diabetic patients.

To further compare feature/morbidity relationships and strengths of association with either undiagnosed or diagnosed diabetes, multivariable logistic regressions were performed. To select independent predictor variables (IPV) to be included in analysis, IPVs that had a P value of less than 0.2 in univariate models were included in stepwise forward and backwards entry elimination multivariable analysis, with model stability

assessed using the likelihood ratio (LR). Variables indicating a significant relationship (P<0.05) with either condition were then entered sequentially, by order of magnitude of the chi-square association, into two independent logistic regressions, adjusting for gender and age as a dichotomous (<60/≥60) variable. Using the same procedures, a final multivariable model comparing undiagnosed to diagnosed diabetes was determined, adjusting for gender and age as a continuous measure.

The discriminatory properties of clinically relevant IPVs indentified in analysis were evaluated. Models including these variables were assessed for their ability to detect undiagnosed or diagnosed diabetes using the *c* statistic. The *c* statistic is identical to the area under the receiver operating characteristic curve (ROC), with values ranging from 0.5 (no better than chance) to 1.0 (indicating perfect discrimination) [150].

Primary data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) for Windows. Confidence intervals for prevalence proportions were calculated using the VasserStats statistical website [187]. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance. Glycated haemoglobin A_{1c} test results and diagnostic status information were available for 1,995 (97.5%) and 1,999 (97.7%) subjects respectively. Analysis indicated a similar percentage of missing data according to either undiagnosed or diagnosed diabetes classifications. Missing data were thus assumed to be ignorable and missing at random.

2.3 Results

2.3.1 Descriptive characteristics

The baseline characteristics of the study population for participants with undiagnosed, diagnosed and no diabetes are shown in Table 2. The total prevalence of type 2 diabetes was 8.5% (95% CI: 7.4%-9.8%); 102 (5.0%) subjects had diagnosed diabetes (95% CI: 4.1%-6.0%) and 72 (3.5%) had undiagnosed diabetes (95% CI: 2.8%-4.4%), representing 41% of all diabetes cases. The prevalence of diabetes was higher in male subjects 11% (N=112) compared to females 6% (N=62, P<0.001), and a greater proportion of males had both undiagnosed and diagnosed diabetes. When compared to non-diabetic subjects, a high percentage of patients with type 2 diabetes were overweight or obese, used Rx anti-hypertensive and cholesterol-lowering medications, had CVD and a family history of diabetes, finished education at primary level and reported having low levels of physical activity within the previous six months. Variations in health insurance were also noted, with a higher percentage of diabetes cases having state-assisted healthcare.

2.3.2 Risk feature associations with type 2 diabetes

In univariate analysis (Appendix 1, Supporting Table 1), overweight and obesity, CVD, family diabetes history, elevated triglycerides, low HDL-C and dyslipidaemia were significantly associated with both undiagnosed and diagnosed diabetes. Relationships between reduced physical activity levels and type 2 diabetes were noticeably strong, with seven-fold and approximate

two-fold increased odds for undiagnosed and diagnosed diabetes respectively. With regard to health services related factors, there was a two-fold increased likelihood of undiagnosed diabetes in patients on treatment for hypertension versus a five-fold increased odds for diagnosed diabetes. Similarly, the odds of having undiagnosed diabetes were approximately two-fold higher in patients on treatment with cholesterol-lowering therapy versus an approximate four-fold increased odds for diagnosed diabetes. The probability of both undiagnosed and diagnosed type 2 diabetes was significantly reduced in patients with private medical insurance, whilst the odds of having undiagnosed diabetes were significantly increased in subjects with no medical insurance (OR: 3.0, 95% CI: 1.6-5.6).

Multivariable analysis (Table 3) revealed overweight and obesity, use of cholesterol-lowering medication, family diabetes history and dyslipidaemia to be associated with both undiagnosed and diagnosed type 2 diabetes. Low-level physical activity (OR: 5.8, 95% CI: 2.7-12.5) and health service variables remained significant determinants of undiagnosed diabetes, with odds that were approximately two-fold higher in subjects with state-assisted healthcare and for participants without medical insurance. Characteristics associated with diagnosed diabetes included CVD, Rx anti-hypertensive therapy and alcohol use. In addition, male subjects were statistically more likely to have diagnosed diabetes compared to females (OR: 2.5, 95% CI: 1.5-4.1).

Table 4 shows univariate odds ratios of having undiagnosed compared to diagnosed type 2 diabetes. Within this subsample of diabetes cases, significant effects were observed for Rx medication use, family diabetes history, triglyceride levels and dyslipidaemia. Both health insurance and physical activity IPVs demonstrated strong associations with undiagnosed diabetes, with approximate four-fold increased odds in subjects without healthcare insurance and in those reporting low levels of physical activity. Individuals with undiagnosed diabetes were also more likely to have higher levels of adiposity. Overall, metabolic characteristics were less optimal in undiagnosed cases, and a greater proportion had uncontrolled hypertension.

Results from a multivariable analysis comparing undiagnosed to diagnosed type 2 diabetes are presented in Table 5. Significant associations were noted for BMI (continuous) and physical inactivity. Undiagnosed patients were also significantly less likely to be on treatment for hypertension or to have a family history of diabetes relative to subjects with diagnosed diabetes.

2.3.3 ROC analysis

Figures 2 and 3 show ROC curves for models to discriminate undiagnosed or diagnosed type 2 diabetes (compared to no diabetes). Models which included both health insurance and physical activity IPVs displayed a higher discriminatory capacity to detect undiagnosed subjects (*c*=0.74, 95% CI: 0.67-0.80) compared to diagnosed diabetes (*c*=0.61, 95% CI: 0.54-0.67). A model including health insurance, physical activity and BMI (continuous)

demonstrated further improved discrimination (*c*=0.81, 95% CI: 0.76-0.87 for undiagnosed diabetes versus *c*=0.70, 95% CI: 0.65-0.75 for diagnosed patients).

2.4 Discussion

The results from previous research investigating the prevalence of type 2 diabetes in middle-aged adults within Ireland are conflicting. In 1998, a study conducted by Perry et al. [169] suggested an overall prevalence of 3.9%, 30% of whom were undiagnosed, whereas research in 2003, examining diabetes in primary care [188], estimated a population prevalence of 9.2%, with undiagnosed patients representing 23.5% of all cases. Prevalence disparities between these studies are possibly explained by differences in age groups assessed or by methods used for diabetes detection.

The higher percentage of undiagnosed cases identified within our sample may be due to use of the HbA_{1c} assay as compared to the FPG test that was more commonly employed in the Republic of Ireland before 2010. Research conducted in the United States and Germany, which compared FPG and oral glucose tolerance test procedures, reported that overall prevalence would have been lower if FPG had been used alone [189,190]. We also observed that 14 (19%) undiagnosed patients (who were positively identified according to HbA_{1c} concentrations) had FPG levels that were less than 5.6 mmol/l, and would have been classified as non-diabetic if this test had been used to diagnose diabetes in the present study. This finding is consistent with

previous research high-lighting discrepancies between HbA_{1c} and FPG [191,192].

A recent study from the United States suggested that regular use of HbA_{1c} as a diagnostic procedure would not significantly alter type 2 diabetes prevalence, and that diabetes categorisation would remain unchanged in 97.7% of subjects [193]. However, evidence to support this claim is still equivocal. Numerous studies have shown poor concordance between HbA_{1c} and FPG [191], in particular regarding pre-diabetes classification [194-196]. In addition, factors such as age or ethnicity are thought to influence results [191,192,197]. Nevertheless, as discussed by Bonora et al., comparisons between diagnostic methods for type 2 diabetes are ambiguous, as a true gold standard test is unavailable [24]. It should be noted that although we classified type 2 diabetes using HbA_{1c} in this study, the discrepancies we observed between HbA_{1c} and FPG led us to further evaluate the appropriateness of this diagnostic test. The implications of using HbA_{1c} alone for diagnosing diabetes and cardiometabolic risk are examined in Chapter 3.

The Irish IPH report [178], based on the nationally representative SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition [172] (which also used the HbA_{1c} test), estimated the prevalence of type 2 diabetes in adults 45+ to be 8.9%, which is similar to the result suggested by our study. In the IPH report, undiagnosed diabetes prevalence was determined to be 2.7% (30% of all diabetes cases). Of note, however, is that the IPH research determined the prevalence of undiagnosed and diagnosed diabetes in adults aged 55-64 to

be 4.6% and 6.3% respectively, which are comparable to outcomes attained from this study population (3.6% and 5.6%) for the same age group.

Also of interest, is that results from the SLÁN data are consistent with our finding that the prevalence of type 2 diabetes in Ireland's middle-aged population is higher in men. Although this gender difference may be a consequence of selection bias due to non-response, similarity in outcomes between the 2007 SLÁN survey and the Cork and Kerry Diabetes and Heart Disease Study imply that observed prevalence estimates are valid. It is possible that the lower prevalence of diabetes in women may be as a result of random opportunistic screening due to higher GP consultation rates observed in females [198]. An alternative explanation may be the higher percentage of overweight and obese individuals within this male population (males: 85.8% versus females: 70.6%, P for difference <0.001), a relationship noted in previous research examining obesity within Ireland [198,199].

As numerous studies have indicated, adverse cardiometabolic disease features such as high triglycerides and low HDL-C were significantly and positively associated with both undiagnosed and diagnosed diabetes [19,57,190,200]. It was observed that a greater proportion of undiagnosed patients had uncontrolled hypertension, increased triglyceride concentrations and dyslipidaemia, perhaps reflecting access to treatment, as a higher percentage of diagnosed subjects used Rx anti-hypertensive and cholesterol-lowering medications. Undiagnosed individuals were also less

likely to have a family history of diabetes and CVD, or to engage in regular physical activity compared to diagnosed subjects. Nevertheless, unfavourable lipid/lipoprotein profiles, family diabetes history, low-level physical activity and CVD were all positively associated with both undiagnosed and diagnosed diabetes. The inverse relationship between diabetes and regular alcohol use was also of interest as correlations between alcohol use and MetS have been reported previously [201]. Markedly, 96.6% (N=168) of study participants with both undiagnosed and diagnosed type 2 diabetes were either overweight or obese, confirming results from previous research which suggest that obesity is a primary and significant risk factor related to diabetes development [202]. Screening for type 2 diabetes may be more efficient within these subgroups, particularly individuals with a combination of these features.

Within Ireland, residents accessing public healthcare are divided into two categories: (1) those who hold a medical card (either a FMC or GPC) and thus qualify for means-tested, state-assisted healthcare insurance. A FMC entitles individuals to free GP services, Rx medications, public hospital services, dental, optical and aural services, community care and personal social services. A GPC entitles individuals to free GP care; (2) non-card holders, who are entitled to free public hospital services but who must pay for GP care and may also have to pay in-patient and out-patient hospital charges. In addition to the public health system there is also a large private healthcare market [203].

Results from the present study suggest that within this population, subjects with private medical insurance are less likely to have type 2 diabetes. This may indicate that these individuals have greater financial resources and access to healthcare, or an increased awareness of diabetes risk factors. This awareness could be due to higher educational levels, as it was also noted that study participants who had only completed education to a primary level were more likely to have the condition. Although social class (defined by the ESeC) [186] was not a diabetes risk factor, it is possible that the lower prevalence of diabetes amongst subjects with private medical insurance was due to socio-economic inequalities, as study participants in receipt of state-assisted medical insurance were notably at a higher risk. These findings suggest that diabetes cases occur disproportionately amongst individuals who are economically deprived and who have lower educational levels, and this concurs with previous research which found significant correlations between social deprivation and type 2 diabetes [204].

Importantly, our findings also imply that health service inequalities are significant determinants of diagnostic status, as a greater proportion of undiagnosed cases indicated having no state-assisted or private healthcare insurance. This is consistent with outcomes observed in previous studies which have examined relationships between healthcare inequities and diabetes [182,205]. Univariate analysis suggested three-fold and four-fold increased odds of having undiagnosed type 2 diabetes in subjects without medical insurance when compared to individuals with no diabetes (Appendix 1, Supporting Table 1) or diagnosed diabetes (Table 4) respectively. This

association was also noted in a multivariable logistic regression comparing undiagnosed to non-diabetic individuals (Table 3) but was not observed in multivariable analysis restricted to patients with type 2 diabetes (Table 5).

To investigate this discrepancy, we forced the health insurance IPV into a model and entered covariates independently to assess confounder-adjusted relationships. In a logistic regression which controlled for family diabetes history, Rx anti-hypertensives, BMI, age and gender, having no healthcare insurance remained strongly associated with undiagnosed diabetes (OR: 3.5, 95% CI: 1.2-10.4, P=0.025) although this was attenuated when the physical activity IPV was included (OR: 2.4, 95% CI: 0.7-8.9, P=0.184). This may indicate a relationship between physical activity and both health insurance and undiagnosed diabetes or that physical activity levels explain most of the variance. Equally possible is that missing data from the IPAQ questionnaire resulted in a loss of statistical power.

We further explored health insurance/physical activity relationships with undiagnosed/diagnosed type 2 diabetes using the LR. Tests for model assessment included significant covariates, age, gender and either health insurance or physical activity IPVs. Both models implied similar goodness-of-fit (LR chi-square: 33.29, P<0.001 for a model with health insurance versus LR chi-square: 32.68, P<0.001 for a model with physical activity) in full models against a constant, indicating that both variables may be clinically relevant. In addition, it was noted that models including health insurance, physical activity and adiposity IPVs displayed differences in discriminative

ability to detect undiagnosed and diagnosed patients (Figures 2 and 3). This suggests that use of these variables in type 2 diabetes screening algorithms may be useful for identifying a subset of diabetes cases.

Although assessment of physical activity levels in clinical practice (as measured using self-completed patient questionnaires) is subject to reporting bias, socio-economic status (determined using a proxy measure such as health insurance status) and adiposity levels are variables that may be objectively assessed by a clinician. In particular, as non-invasive diabetes risk scores typically include a measurement of adiposity, determining an appropriate method for assessing overweight and obesity was the primary aim of this research. Optimal methods for measuring adiposity are explored in the following chapters.

2.4.1 Strengths and limitations of the research

As one of the largest cross-sectional studies performed to date within the Republic of Ireland, the Cork and Kerry Diabetes and Heart Disease Study sample size is comparable to other related Irish studies. Selection bias was minimised as a comparable number of male and female subjects, aged 46-73 years of age, were randomly selected from a register of patients within a single primary care centre. Furthermore, non-responders had similar numbers for both males and females and likewise for age groups. Few studies have assessed the prevalence of undiagnosed or diagnosed type 2 diabetes within one broadly representative population sample or compared

features between undiagnosed and diagnosed subjects. Finally, use of HbA_{1c} measurement demonstrated prevalence rates comparable to those from a recent nationally representative study, the SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition [172].

Notwithstanding these strengths, several limitations can be identified. The use of self-reported questionnaires is subject to potential inaccuracies, recall and reporting bias [159,160]. In particular, misclassification of diabetes from self-reporting is a recognised limitation present in all surveys, and is particularly relevant in Ireland due to the absence of a unique health identifier within the Irish healthcare system [206]. This makes linkage with other records, such as disease registries or death records problematic [183]. Nonetheless, several studies have indicated a reasonable or high degree of concordance between type 2 diabetes prevalence and self-reporting [160,207-209]. Additionally, within this sample there was a high level of agreement between self-reported physician diagnosis of diabetes and Rx diabetes medication use (Kappa: 0.854).

Equally of concern is that prevalence estimates were derived from a single primary care based sample which may not be representative of the source population. However, previous research suggests that approximately 98% of Irish adults are registered with a GP and that, even in the absence of a universal patient registration system, it is possible to perform population-based epidemiological studies that are representative using our methods [210]. Further studies are needed to definitively confirm this conclusion. If

correct, it may indicate that findings from the Cork and Kerry Diabetes and Heart Disease Study are generalisable to the Irish middle-aged adult population. Also, as this research makes use of cross-sectional data, interpretations of these findings are compromised by the inability to infer causal relationships. Nevertheless, the relationships described have been extensively replicated in other prospective cohort studies. Finally, with regard to statistical procedures employed in analysis, the possibility of model over-fitting or type II errors cannot be discounted, and results should be considered preliminary and exploratory, as future studies with larger sample sizes and greater statistical power might find other relationships [211].

2.5 Conclusions

The prevalence of type 2 diabetes within the Republic of Ireland is consistent with trends worldwide [175,212], and is primarily driven by the increasing obesity epidemic [13,175,213]. Despite policies and continued investment in services which promote awareness and knowledge of a disease that is largely preventable, the prevalence of diabetes in Ireland may be rising [178].

Socio-economic and health service inequalities are significant risk factors for having undiagnosed diabetes. The results from this study indicate that subjects with state-subsidised healthcare insurance, and those without private or state-assisted medical cover, are more likely to be undetected. These findings suggest that individuals from lower socio-economic backgrounds should be targeted. Observed low levels of physical activity,

adiposity assessment and recognition of untreated cardiometabolic conditions may also improve identification of diabetes cases within clinical practice. Finally, as a successful programme to detect patients with type 2 diabetes may depend on regular General Practice attendance, a strategic approach that identifies subjects without access to primary healthcare services, and which furthers efforts to promote affordable and equitable healthcare, is needed to prevent predictable sequelae for affected individuals.

Table 2—*Characteristics of the study population.*

Feature	No diabetes N=1873 (91.5%)	Undiagnosed diabetes N=72 (3.5%)	Diagnosed diabetes N=102 (5.0%)
Health conditions			
Male	893 (47.8)	43 (59.7)	69 (67.6)
Age	59 (54-64)	60 (56-65)	62 (57-65)
Age ≥60	875 (46.9)	38 (52.8)	65 (63.7)
On Rx for hypertension	486 (26.0)	32 (44.4)	66 (64.7)
On Rx for cholesterol	609 (32.6)	35 (48.6)	67 (65.7)
BMI (kg/m^2)	28.3 ± 4.6	33.1 ± 6.3	31.2 ± 4.4
BMI category:			
<25	439 (23.6)	4 (5.6)	2 (2.0)
25-29.9	857 (46.0)	24 (33.3)	43 (42.2)
≥30	566 (30.4)	44 (61.1)	57 (55.9)
Family diabetes history	315 (16.9)	21 (29.2)	54 (52.9)
CVD	167 (8.9)	16 (22.2)	29 (28.4)
Socio-economic			
Education:			
Bachelor or higher	175 (10.0)	4 (5.9)	5 (5.3)
Diploma	239 (13.7)	6 (8.8)	6 (6.3)
Secondary	863 (49.5)	31 (45.6)	40 (42.1)
Primary only	466 (26.7)	27 (39.7)	44 (46.3)
Social class:			
High income	244 (18.2)	6 (11.5)	11 (13.3)
Middle income	396 (29.5)	18 (34.6)	25 (30.1)
Low income	704 (52.4)	28 (53.8)	47 (56.6)
Medical cover			
Health insurance:			
Private insurance	1196 (64.0)	27 (37.5)	51 (50.0)
State insurance	437 (23.4)	29 (40.3)	44 (43.1)
No insurance	236 (12.6)	16 (22.2)	7 (6.9)
Health behaviours			
Physical activity:			
High	795 (48.4)	10 (17.5)	31 (34.8)
Moderate	536 (32.6)	19 (33.3)	35 (39.3)
No physical exercise	313 (19.0)	28 (49.1)	23 (25.8)
Smoker	889 (47.6)	38 (52.8)	60 (58.8)
Alcohol use:			
Non-drinker	800 (44.7)	38 (55.1)	54 (53.5)
Occasional drinker	367 (20.5)	12 (17.4)	27 (26.7)
Regular drinker	623 (34.8)	19 (27.5)	20 (19.8)
Metabolic			
Triglycerides (mmol/l)	1.19 (0.9-1.6)	1.80 (1.3-2.4)	1.36 (1.0-2.0)
Triglycerides ≥1.7	417 (23.0)	37 (52.9)	36 (37.5)
HDL-C (mmol/l)	1.48 ± 0.4	1.22 ± 0.3	1.18 ± 0.3
Low HDL-C[1]	267 (14.7)	32 (45.7)	45 (45.0)
Dyslipidaemia[2]	122 (6.7)	24 (34.3)	21 (21.0)
SBP (mmHg)	129.25 ± 16.7	134.18 ± 19.3	132.94 ± 16.4
DBP (mmHg)	80.20 ± 9.7	80.12 ± 10.9	78.79 ± 9.5
Hypertension[3]	552 (29.7)	28 (39.4)	28 (27.5)
FPG (mmol/l)	4.90 (4.6-5.3)	6.60 (5.6-7.5)	7.50 (5.7-9.4)
FPG ≥5.6	238 (13.1)	58 (80.6)	80 (80.8)

Mean and ± SD are shown for continuous and % are shown for categorical variables.
Age, triglycerides and FPG are shown as a median (interquartile range). Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.
[1]HDL-C <1.03 (males) or <1.29 (females).
[2]Dyslipidaemia: triglycerides ≥1.7 and HDL-C <1.03 (males) or <1.29 (females).
[3]Hypertension: SBP ≥140 and/or DBP ≥90.

Table 3—*Odds ratios (95% CI) of having undiagnosed or diagnosed type 2 diabetes compared to no diabetes – multivariable logistic regression adjusting for age, gender and all significant covariates.*

Feature	Odds ratio	95% CI
Undiagnosed diabetes compared to no diabetes[1]		
Male	1.4	(0.8-2.5)
Age ≥60	1.0	(0.6-1.9)
On Rx for cholesterol	2.2	(1.2-3.9)
BMI category:		
<25	1	
25-29.9	4.5	(1.0-19.5)
≥30	6.8	(1.6-29.4)
Family diabetes history	1.9	(1.0-3.6)
Health insurance:		
Private insurance	1	
State insurance	2.2	(1.2-4.2)
No insurance	2.3	(1.0-5.2)
Physical activity:		
High	1	
Moderate	1.9	(0.8-4.2)
No physical exercise	5.8	(2.7-12.5)
Dyslipidaemia[3]	4.3	(2.3-8.3)
Diagnosed diabetes compared to no diabetes[2]		
Male	2.5	(1.5-4.1)
Age ≥60	1.4	(0.9-2.3)
On Rx for hypertension	2.7	(1.7-4.4)
On Rx for cholesterol	2.0	(1.2-3.3)
BMI category:		
<25	1	
25-29.9	8.2	(1.9-34.6)
≥30	9.4	(2.2-40.3)
Family diabetes history	5.9	(3.7-9.4)
CVD	2.0	(1.1-3.5)
Alcohol use:		
Non-drinker	1	
Occasional drinker	1.3	(0.7-2.2)
Regular drinker	0.4	(0.2-0.7)
Dyslipidaemia[3]	1.9	(1.0-3.5)

[1]Model excludes subjects with diagnosed diabetes. Final model covariates entered in order: dyslipidaemia, BMI category, physical activity, health insurance, on Rx for cholesterol, family diabetes history, age and gender.

[2]Model excludes subjects with undiagnosed diabetes. Final model covariates entered in order: family diabetes history, on Rx for hypertension, BMI category, on Rx for cholesterol, CVD, dyslipidaemia, alcohol use, age and gender.

[3]Dyslipidaemia: triglycerides ≥1.7 and HDL-C <1.03 (males) or <1.29 (females).

Table 4—*Univariate odds ratios (95% CI) of having undiagnosed compared to diagnosed type 2 diabetes.*

Feature	Undiagnosed diabetes N=72 (41.4%)	Diagnosed diabetes N=102 (58.6%)	Odds ratio	95% CI
Health conditions				
Female	29 (40.3)	33 (32.4)	1	
Male	43 (59.7)	69 (67.6)	0.7	(0.4-1.3)
Age <60 years	34 (47.2)	37 (36.3)	1	
Age ≥60 years	38 (52.8)	65 (63.7)	0.6	(0.3-1.2)
Not on Rx for hypertension	40 (55.6)	36 (35.3)	1	
On Rx for hypertension	32 (44.4)	66 (64.7)	0.4	(0.2-0.8)
Not on Rx for cholesterol	37 (51.4)	35 (34.3)	1	
On Rx for cholesterol	35 (48.6)	67 (65.7)	0.5	(0.3-0.9)
BMI (kg/m^2)	33.1 ± 6.3	31.2 ± 4.4	1.1	(1.0-1.1)
BMI category:				
<25	4 (5.6)	2 (2.0)	1	
25-29.9	24 (33.3)	43 (42.2)	0.3	(0.1-1.7)
≥30	44 (61.1)	57 (55.9)	0.4	(0.1-2.2)
No family diabetes history	51 (70.8)	48 (47.1)	1	
Family diabetes history	21 (29.2)	54 (52.9)	0.4	(0.2-0.7)
No CVD	56 (77.8	73 (71.6)	1	
CVD	16 (22.2)	29 (28.4)	0.7	(0.4-1.5)
Socio-economic				
Education:				
Bachelor or higher	4 (5.9)	5 (5.3)	1	
Diploma	6 (8.8)	6 (6.3)	1.3	(0.2-7.1)
Secondary	31 (45.6)	40 (42.1)	1.0	(0.2-3.9)
Primary only	27 (39.7)	44 (46.3)	0.8	(0.2-3.1)
Social class:				
High income	6 (11.5)	11 (13.3)	1	
Middle income	18 (34.6)	25 (30.1)	1.3	(0.4-4.2)
Low income	28 (53.8)	47 (56.6)	1.1	(0.4-3.3)
Medical cover				
Health insurance:				
Private insurance	27 (37.5)	51 (50.0)	1	
State insurance	29 (40.3)	44 (43.1)	1.2	(0.6-2.4)
No insurance	16 (22.2)	7 (6.9)	4.3	(1.6-11.8)
Health behaviours				
Physical activity:				
High	10 (17.5)	31 (34.8)	1	
Moderate	19 (33.3)	35 (39.3)	1.7	(0.7-4.2)
No physical exercise	28 (49.1)	23 (25.8)	3.8	(1.5-9.3)
Non-smoker	34 (47.2)	42 (41.2)	1	
Smoker	38 (52.8)	60 (58.8)	0.8	(0.4-1.4)
Alcohol use:				
Non-drinker	38 (55.1)	54 (53.5)	1	
Occasional drinker	12 (17.4)	27 (26.7)	0.6	(0.3-1.4)
Regular drinker	19 (27.5)	20 (19.8)	1.4	(0.6-2.9)
Metabolic				
Triglycerides (mmol/l)	1.80 (1.3-2.4)	1.36 (1.0-2.0)	1.5	(1.1-2.0)
Triglycerides <1.7	33 (47.1)	60 (62.5)	1	
Triglycerides ≥1.7	37 (52.9)	36 (37.5)	1.9	(1.0-3.5)
HDL-C (mmol/l)	1.22 ± 0.3	1.18 ± 0.3	1.7	(0.6-4.7)
Optimal HDL-C	38 (54.3)	55 (55.0)	1	
Low HDL-C[1]	32 (45.7)	45 (45.0)	1.0	(0.6-1.9)

Table 4 continued

Feature	Undiagnosed diabetes N=72 (41.4%)	Diagnosed diabetes N=102 (58.6%)	Odds ratio	95% CI
Metabolic				
No dyslipidaemia	46 (65.7)	79 (79.0)	1	
Dyslipidaemia[2]	24 (34.3)	21 (21.0)	2.0	(1.0-3.9)
SBP (mmHg)	134.18 ± 19.3	132.94 ± 16.4	1.0	(0.99-1.0)
DBP (mmHg)	80.12 ± 10.9	78.79 ± 9.5	1.0	(0.98-1.0)
No hypertension	43 (60.6)	74 (72.5)	1	
Hypertension[3]	28 (39.4)	28 (27.5)	1.7	(0.9-3.3)

Mean and ± SD are shown for continuous variables. Triglycerides are shown as a median (interquartile range). Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.

[1]HDL-C <1.03 (males) or <1.29 (females).

[2]Dyslipidaemia: triglycerides ≥1.7 and HDL-C <1.03 (males) or <1.29 (females).

[3]Hypertension: SBP ≥140 and/or DBP ≥90.

Table 5—*Odds ratios (95% CI) of having undiagnosed compared to diagnosed type 2 diabetes – multivariable logistic regression adjusting for all significant covariates.*

Feature	Model 1		Model 2[1]	
	Odds ratio	95% CI	Odds ratio	95% CI
BMI (kg/m^2)	1.1	(1.0-1.2)	1.1	(1.0-1.2)
On Rx for hypertension	0.3	(0.2-0.7)	0.3	(0.1-0.7)
Family diabetes history	0.4	(0.2-0.8)	0.4	(0.2-0.8)
Physical activity:				
High	1		1	
Moderate	1.6	(0.6-4.3)	1.6	(0.6-4.3)
No physical exercise	3.5	(1.3-9.3)	3.4	(1.3-9.1)

Final model covariates entered in order: family diabetes history, physical activity, on Rx for hypertension and BMI.
[1]Adjusted for age and gender.

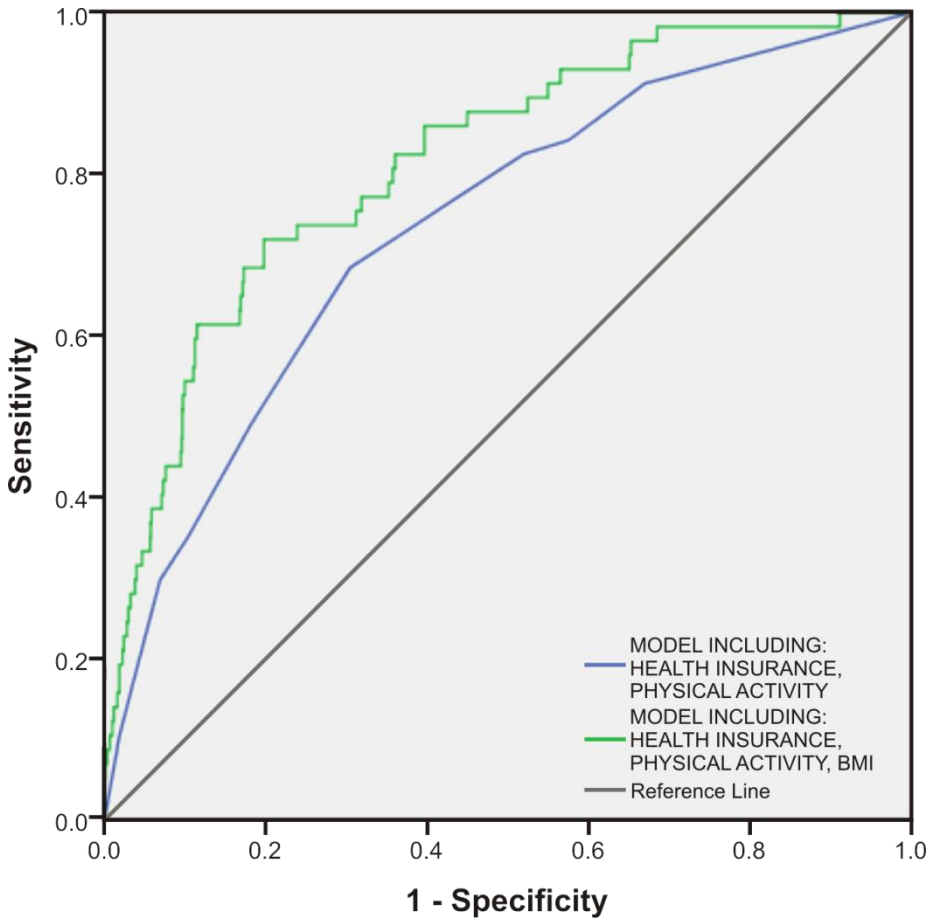

Figure 2—*Receiver operating characteristic curves for models to discriminate subjects with undiagnosed type 2 diabetes.* The *c* statistics were *c*=0.74 (95% CI: 0.67-0.80) for a model including health insurance and physical activity and *c*=0.81 (95% CI: 0.76-0.87) for a model including health insurance, physical activity and BMI.

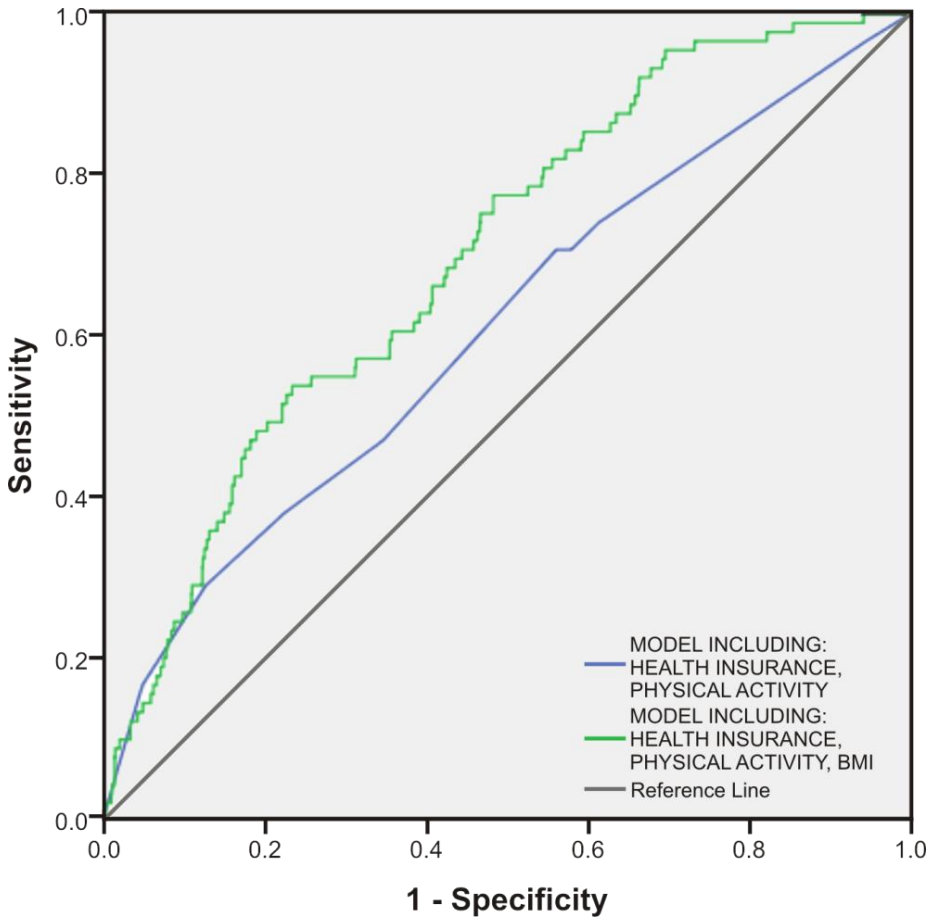

Figure 3—*Receiver operating characteristic curves for models to discriminate subjects with diagnosed type 2 diabetes.* The *c* statistics were *c*=0.61 (95% CI: 0.54-0.67) for a model including health insurance and physical activity and *c*=0.70 (95% CI: 0.65-0.75) for a model including health insurance, physical activity and BMI.

CHAPTER 3

HbA_{1c} ALONE IS A POOR INDICATOR OF CARDIOMETABOLIC RISK IN MIDDLE-AGED SUBJECTS WITH PRE-DIABETES BUT IS SUITABLE FOR TYPE 2 DIABETES DIAGNOSIS: A CROSS-SECTIONAL STUDY

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3.0 Abstract

Background and Objectives

Glycated haemoglobin A_{1c} (HbA_{1c}) measurement is recommended as an alternative to fasting plasma glucose (FPG) for the diagnosis of pre-diabetes and type 2 diabetes. However, evidence suggests discordance between HbA_{1c} and FPG. In this study we examine a range of cardiometabolic features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell counts to determine which assay more accurately identifies individuals at increased cardiometabolic risk.

Materials and Methods

This was a cross-sectional study involving a random sample of 2,047 men and women aged 46-73 years. Binary and multinomial logistic regression were employed to examine risk feature associations with pre-diabetes [either HbA_{1c} levels 5.7%-6.4% (39-46 mmol/mol) or impaired FPG levels 5.6-6.9 mmol/l] and type 2 diabetes [either HbA_{1c} levels $\geq$6.5% ($\geq$48 mmol/mol) or FPG levels $\geq$7.0 mmol/l]. Receiver operating characteristic curve analysis was used to evaluate the ability of HbA_{1c} to discriminate pre-diabetes and diabetes defined by FPG.

Results

Stronger associations with diabetes-related phenotypes were observed in pre-diabetic subjects diagnosed by FPG compared to those detected by

HbA_{1c}. Individuals with type 2 diabetes exhibited cardiometabolic profiles that were broadly similar according to diagnosis by either assay. Pre-diabetic participants classified by both assays displayed a more pro-inflammatory, pro-atherogenic, hypertensive and insulin resistant profile. Odds ratios of having three or more cardiometabolic disease features were also noticeably increased (OR: 4.0, 95% CI: 2.8-5.8) when compared to subjects diagnosed by either HbA_{1c} (OR: 1.4, 95% CI: 1.2-1.8) or FPG (OR: 3.0, 95% CI: 1.7-5.1) separately.

Conclusions

In middle-aged Caucasian-Europeans, HbA_{1c} alone is a poor indicator of cardiometabolic risk but is suitable for diagnosing diabetes. Combined use of HbA_{1c} and FPG may be of additional benefit for detecting individuals at highest odds of type 2 diabetes development.

3.1 Introduction

The prevalence of type 2 diabetes, a chronic disease which causes significant mortality, has increased considerably in world populations, representing a major public health issue [175]. Diabetes is associated with a clustering of cardiometabolic features including obesity, dyslipidaemia, hypertension, insulin resistance, chronic low-grade inflammation [23,214], and may lead to severe cardiovascular complications [215].

Pre-diabetes, a condition defined by glycaemic profiles that are higher than normal but which do not meet thresholds for diabetes, is a strong risk factor for type 2 diabetes and related complications [216]. The American Diabetes Association (ADA) classifies type 2 diabetes as a fasting plasma glucose (FPG) level $\geq$7.0 mmol/l and pre-diabetes as impaired FPG levels between 5.6-6.9 mmol/l [23]. In 2009 the International Expert Committee recommended glycated haemoglobin A_{1c} (HbA_{1c}) as an alternative marker [217], and in 2010 the ADA introduced HbA_{1c} cut-points of $\geq$6.5% ($\geq$48 mmol/mol) for diabetes diagnosis and between 5.7%-6.4% (39-46 mmol/mol) as a criterion to identify individuals at a high-risk state of developing diabetes [23]. Perceived benefits of the use of HbA_{1c} measurement, over FPG, include greater pre-analytical stability, lower biological variability and that the assay may be performed in non-fasting blood samples [24,218]. However, use of HbA_{1c} as a screening tool has been controversial, with research showing discordance between HbA_{1c} and FPG [192,196,219,220], and several studies suggesting that factors such as age or ethnicity may influence diagnostic performance [191,221,222].

The aim of this study was to compare the metabolic profiles in subjects with pre-diabetes and type 2 diabetes, using ADA-recommended HbA_{1c} and FPG diagnostic thresholds, in a random sample of 2,047 middle-aged men and women. In particular, we examined a range of diabetes risk factors, metabolic syndrome (MetS) features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell (WBC) counts to determine which assay more accurately identifies individuals at increased cardiometabolic risk.

3.2 Materials and Methods

3.2.1 Study design

The study design is described in detail in Chapter 1. In summary, the Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000, with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending patients in the 46-73 year age group. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). Details regarding the study

design, sampling procedures and methods of data collection have been reported previously [183].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

3.2.2 Clinical and laboratory procedures

Study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, family diabetes history, physician-diagnosed type 2 diabetes and prescription (Rx) medication use were gathered through a self-completed General Health Questionnaire [170]. Triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica GmbH, Hamburg, Germany). Fasting glucose concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and HbA_{1c} levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh

Europe N.V, Tessenderlo, Belgium]. Serum insulin, c-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), adiponectin, leptin, resistin and plasminogen activator inhibitor-1 (PAI-1) were assessed using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Complement component 3 (C3) was measured by immunoturbidimetric assay (RX Daytona; Randox Laboratories). White blood cell counts were determined by flow cytometry technology as part of a full blood count.

Three independent measurements of systolic and diastolic blood pressure (BP) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP. The weight and height of each participant were measured to the nearest 0.1 kg and 0.1 cm respectively. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was measured using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and body mass index (BMI) was calculated as weight divided by the square of height. A BMI $\geq$30 kg/m^2 was classified as obese [94]. Waist circumference (WC) was measured midway between the lowest rib and iliac crest on bare skin. Subjects were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. Two independent measurements of WC were taken and the mean of the two was used in analysis. Central obesity was defined as a WC level $\geq$102 cm for males and $\geq$88 cm for females [12].

3.2.3 Classification of biochemical and blood pressure measurements

Lipid, lipoprotein and BP measurements were classified according to National Cholesterol Education Program: Adult Treatment Panel III (NCEP: ATP III) guidelines [184]. Abnormal metabolic risks were defined as high triglycerides $\geq$1.7 mmol/l and low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females). Dyslipidaemia was determined according to both high triglyceride and low HDL-C levels. Elevated BP was classified as systolic BP $\geq$130 mmHg and/or diastolic BP $\geq$85 mmHg or Rx anti-hypertensive medication use. High serum insulin was defined as a level equal to or above the 75th percentile in the study sample.

Metabolic syndrome was determined according to a modified version of the NCEP: ATP III criterion, substituting serum insulin 75th percentile for impaired FPG. Three or more MetS features ($\geq$3 MetS) was characterised as any combination of the following: obesity defined by WC, high triglyceride levels, low HDL-C, elevated BP and high insulin concentrations. According to ADA guidelines, pre-diabetes was classified as elevated HbA_{1c} levels between 5.7%-6.4% (39-46 mmol/mol) or impaired FPG levels between 5.6-6.9 mmol/l. Type 2 diabetes was defined as HbA_{1c} $\geq$6.5% ($\geq$48 mmol/mol) or FPG $\geq$7.0 mmol/l [23].

As internationally agreed risk cut-points for the examined biomarkers have not been established, low-grade inflammation was determined as a level above the study population median for each biomarker (C3, CRP, IL-6, TNF-

α, leptin, resistin, PAI-1 and WBC) with the exception of adiponectin (below median level).

3.2.4 Statistical analysis

Descriptive characteristics were examined according to diagnosis of pre-diabetes and type 2 diabetes. Categorical features are presented as percentages and continuous variables are displayed as a mean (plus or minus one standard deviation) or a median and interquartile range for skewed data. Binary logistic regression was used to explore diabetes-related risk factor and inflammatory biomarker relationships with pre-diabetes (compared to normoglycaemic subjects) and type 2 diabetes (compared to individuals without diabetes) defined using HbA_{1c} and FPG diagnostic cut-points. Models examining metabolic feature associations with pre-diabetes excluded patients with type 2 diabetes indicated by either HbA_{1c} or FPG, a physician diagnosis or Rx diabetes medication use. Risk feature relationships with pre-diabetes (either HbA_{1c} alone, FPG alone or dual categorisation by both HbA_{1c} and FPG) were further evaluated using multinomial logistic regression. Subjects classified as normoglycaemic by both assays were used as the reference category.

The ability of HbA_{1c} to discriminate pre-diabetes (defined by impaired FPG) and type 2 diabetes (defined by FPG levels $\geq$7.0 mmol/l) was assessed using receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) provides a scale from 0.5 to 1.0 (with 0.5 representing random

chance and 1.0 indicating perfect discrimination) by which to compare the ability of a marker to detect a positive result [150]. The diagnostic properties of different HbA_{1c} thresholds were contrasted by determining sensitivity and false positive rates (FPR). Levels of agreement between diagnostic methods were ascertained using Cohen's kappa coefficient.

Primary data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) for Windows. Confidence intervals for prevalence proportions were calculated using the VasserStats statistical website [187]. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance. Assay results for HbA_{1c} and FPG were available for 1,995 (97.5%) and 1,994 (97.4%) subjects. Participants missing either HbA_{1c} or FPG data were excluded from multinomial and ROC analyses. Low-level missing values were found within most independent variables. Analysis indicated a similar percentage of missing data according to either HbA_{1c} or FPG pre-diabetes and diabetes classifications. Missing independent variable data were thus assumed to be ignorable and missing at random.

3.3 Results

3.3.1 Descriptive characteristics

Characteristics of the study population according to pre-diabetes and type 2 diabetes classifications are presented in Table 6. The prevalence of pre-diabetes was 47.9% (95% CI: 45.7%-50.0%) by elevated HbA_{1c} and 11.2%

(95% CI: 9.9%-12.7%) by impaired FPG. Subjects categorised as pre-diabetic using HbA_{1c} had lower BMI and WC levels, lower triglyceride and insulin concentrations, higher HDL-C levels, were less hypertensive, and a greater proportion were female when compared to individuals with pre-diabetes defined by FPG.

3.3.2 Logistic regression

In binary logistic regression analyses (Table 7), associations between commonly assessed diabetes risk features and pre-diabetes were stronger in subjects diagnosed by FPG. Odds ratios for pre-diabetes indicated by HbA_{1c} were non-significant for having a family diabetes history and elevated triglyceride levels, while there was a three-fold increased likelihood (OR: 3.0, 95% CI: 2.2-3.9) of having $\geq$3 MetS features in participants identified by FPG compared to an odds ratio of 1.6 (95% CI: 1.3-2.0) in pre-diabetes by HbA_{1c}. In contrast, metabolic risk factor relationships with type 2 diabetes were generally comparable according to diagnosis by either assay, with odds ratios of having $\geq$3 MetS features being 6.1 (95% CI: 4.2-8.8) and 6.8 (95% CI: 4.1-11.2) for subjects diagnosed by HbA_{1c} and FPG respectively. Regardless of definition, patients with pre-diabetes and type 2 diabetes displayed a chronic pro-inflammatory profile as characterised by elevated C3, IL-6, WBC levels and reduced adiponectin concentrations.

The results from multinomial regression models exploring risk factor relationships with pre-diabetes classified by HbA_{1c} alone, FPG alone, or by

both HbA_{1c} and FPG together are displayed in Table 8. Odds ratios of having high levels of adiposity, elevated BP, increased insulin concentrations and MetS were higher for participants classified by both assays, with four-fold increased odds (OR: 4.0, 95% CI: 2.8-5.8) of having ≥3 MetS features, compared to either HbA_{1c} alone (OR: 1.4, 95% CI: 1.2-1.8) or FPG alone (OR: 3.0, 95% CI: 1.7-5.1). Stronger associations with markers of low-grade inflammation were also observed in subjects identified by both criteria.

3.3.3 ROC analysis

Receiver operating characteristic curves for HbA_{1c} to detect pre-diabetes and type 2 diabetes are shown in Figures 4 and 5. The ability of HbA_{1c} to discriminate pre-diabetes characterised by impaired FPG was low (AUC=0.67, 95% CI: 0.63-0.71). The HbA_{1c} ≥5.7% (≥39 mmol/mol) pre-diabetes threshold demonstrated marginal sensitivity (72%) and a high FPR (52%). The level of agreement between both diagnostic methods was also poor (Kappa: 0.084). Discriminatory capacity for type 2 diabetes defined by FPG ≥7.0 mmol/l was high (AUC=0.94, 95% CI: 0.90-0.98). Sensitivity, FPR and Kappa for the ADA-recommended HbA_{1c} ≥6.5% (≥48 mmol/mol) cut-off were 84%, 4% and 0.60 respectively.

3.4 Discussion

In this study of 2,047 middle-aged Caucasian-European men and women we show that subjects with HbA_{1c} levels 5.7%-6.4% (39-46 mmol/mol) or FPG

levels 5.6-6.9 mmol/l may exhibit different cardiometabolic profiles. Stronger relationships with diabetes-related risk features were found using impaired FPG compared to elevated HbA_{1c} to diagnose pre-diabetes. Conversely, the metabolic profiles of patients with type 2 diabetes, defined by either HbA_{1c} $\geq$6.5% ($\geq$48 mmol/mol) or FPG $\geq$7.0 mmol/l concentrations, were broadly similar. In addition, it was noted that pre-diabetic individuals diagnosed by both tests displayed the least optimal profile when compared to subjects classified by either assay separately. These results suggest that a combination of both criteria may be useful for detecting patients at increased cardiometabolic risk.

Noticeably, within this population, a higher percentage of patients were identified as having pre-diabetes by HbA_{1c} (47.9% versus 11.2% for FPG). A higher prevalence of pre-diabetes by HbA_{1c} in a United Kingdom cohort (N=8,696) was also noted by Mostafa et al. [223], who reported a prevalence of 44.9% in participants diagnosed by HbA_{1c} compared to 16.2% in subjects detected by an oral glucose tolerance test (OGTT). Similar findings were determined using FPG as the glucose-based criterion. Our results are also consistent with those reported in a recent Chinese study (N=2,318) and from research examining a Palestinian Arab population (N=1,370). Du et al. [194] and Kharroubi et al. [224] found reasonable or moderate concordance between HbA_{1c} and FPG for type 2 diabetes, but a higher prevalence by HbA_{1c} and limited overlap for pre-diabetes using ADA-designated thresholds.

However, our results contrast with findings reported in the United States by the Insulin Resistance Atherosclerosis Study (N=855), which found a higher prevalence of pre-diabetes by FPG (31.1% versus 10.6% for HbA_{1c}) [225]. Similarly, research utilising data from the National Health and Nutrition Examination Survey (1999-2006) found the prevalence of pre-diabetes in a sample of 7,029 adults to be 28.2% and 12.6% using FPG and HbA_{1c} respectively [195]. Possible reasons for observed prevalence disparities between HbA_{1c} and FPG may include age, gender or ethnic differences in examined populations [192,221,222]. In addition, as glucose continues to be metabolized in blood cells even after sampling, discrepancies may be due to biochemical analysis intervals within different studies [24,224].

Longitudinal research has suggested that combined use of HbA_{1c} and FPG may be beneficial for identifying high-risk subjects. In two Asian studies, Inoue et al. [226] and Heianza et al. [227] demonstrated hazard ratios for type 2 diabetes to be greater for subjects classified by both assays when compared to those diagnosed by either HbA_{1c} or FPG separately. Findings from the Kansai Healthcare Study showed that joint use of both methods improved predictive ability [228]. In ROC analysis, AUCs for models including both HbA_{1c} and FPG were larger than those for HbA_{1c} (0.853 versus 0.771, $P<0.001$) or FPG (0.853 versus 0.818, $P<0.001$) alone. Recent research by Lipska et al. also revealed that addition of HbA_{1c} to a model with impaired FPG improved discrimination and calibration [229]. The results from the present study imply that the mechanism for this association is that individuals

with diabetes-related phenotypes are more accurately identified using combined criteria.

Established risk factors for type 2 diabetes include adiposity, raised triglyceride and low HDL-C levels, hypertension and insulin resistance [19]. In particular, subjects with a combination of these features have been shown to have a five-fold increased risk of developing diabetes [230]. Cardiometabolic disease and type 2 diabetes are also characterised by a low-grade but chronic inflammatory state [231,232]. This may be reflected in an increased production of pro-inflammatory cytokines and proteins (IL-6, TNF-α, leptin, resistin) and also in higher levels of acute-phase response proteins (C3, CRP), coagulation factors (PAI-1), macrophages and immune cells and lower levels of adiponectin, the anti-inflammatory adipokine [232,233].

In our study it was noted that pre-diabetic individuals categorised by both assays demonstrated a stronger association with cardiometabolic feature clustering and displayed a more pro-inflammatory, pro-atherogenic, hypertensive and insulin resistant profile. Though few prospective studies have comprehensively identified features related to pre-diabetes development, it has been suggested that risk factors for pre-diabetes mirror those for type 2 diabetes [234]. Subsequently, on the basis of the similar risk profiles noted in this study between pre-diabetes (defined using both HbA_{1c} and FPG) and type 2 diabetes (classified by either method), these findings

also indicate that combined use of both assays may be clinically useful for detecting individuals at highest odds of developing diabetes.

Although HbA_{1c} has long been used as a marker for glycaemic control, its diagnostic performance for type 2 diabetes is still questioned [235-237]. Though a more expensive assay, when compared with FPG, HbA_{1c} has advantages including convenience, greater pre-analytical stability, lower biological variability and increasing international standardisation [24,237]. Moreover, HbA_{1c} has been shown to correlate with cardiovascular disease and all-cause mortality [238]. However, as diabetes is clinically defined by elevated blood glucose, and not by glycation of proteins, there is concern that using HbA_{1c} to classify type 2 diabetes may lead to major changes in the pathophysiological paradigm that defines the condition [24]. Although a report from the United States inferred that diagnosis by HbA_{1c}, rather than FPG, would not significantly alter type 2 diabetes prevalence, and that categorisation would remain unchanged in as many as 97.7% of subjects [193], this is still uncertain [165].

Within our sample, a higher prevalence of diabetes was determined using HbA_{1c} (7.1%, 95% CI: 6.1%-8.3%) compared to FPG (4.2%, 95% CI: 3.4%-5.1%). However, a similar type 2 diabetes prevalence rate in middle-aged Irish adults, defined by HbA_{1c}, was recently reported using data from the nationally representative SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition (7.1%, 95% CI: 5.2%-9.0%) [35,165]. It was also noted that diabetic subjects identified by HbA_{1c} or FPG within the present study displayed

markedly similar cardiometabolic profiles. In addition, HbA_{1c} demonstrated excellent discrimination of type 2 diabetes diagnosed by FPG $\geq$7.0 mmol/l levels. Conversely, HbA_{1c} showed poor discriminatory capacity for pre-diabetes defined by impaired FPG.

As HbA_{1c} reflects long-term glycaemic exposure, including postprandial glucose spikes, rather than the acute dysglycaemia indicated by FPG, it is rational to assume that each assay may identify different individuals. Our results suggest that HbA_{1c} may provide greater sensitivity for diagnosing type 2 diabetes within this sample. However, the limited overlap and substantially varied cardiometabolic profiles in subjects diagnosed with pre-diabetes, by either HbA_{1c} or FPG, imply that HbA_{1c} alone may lack specificity to accurately detect individuals at risk of diabetes development. It was also noted that metabolic risk profiles in pre-diabetic subjects, classified by impaired FPG levels only, were also increased. This indicates that a percentage of at-risk individuals would be missed if HbA_{1c} was employed as a sole diagnostic criterion.

3.4.1 Strengths and limitations of the research

This study has several strengths, including a high participation rate (67%). As far as we are aware, ours is the first to compare pre-diabetes and type 2 diabetes prevalence, defined using both HbA_{1c} and FPG criteria, in a middle-aged Irish population. Additionally, few studies have compared a broad range of metabolic risk features and biomarkers with pre-diabetes and type 2

diabetes diagnosed by both assays. Our results are of potential clinical significance in terms of screening and the use of HbA_{1c} as a method for diagnosing diabetes, assessing cardiometabolic risk and for defining outcomes within epidemiological research. Accurate estimates of progression rates to type 2 diabetes are needed for efficient allocation of resources in order to optimise public health prevention strategies [239]. Importantly, these findings indicate that caution should be taken with regard to how risk is defined as inexact methods may overestimate future diabetes burden [240,241].

Notwithstanding these strengths, several limitations can be identified. These include single measurements of HbA_{1c} and FPG and that we did not have OGTT results as a comparison test. Although use of a third assay would have allowed a more thorough evaluation of HbA_{1c} and FPG, as discussed by Bonora et al. [24], comparisons between diagnostic methods for pre-diabetes and type 2 diabetes are ambiguous as a true gold standard test is unavailable. Also, cross-sectional data precludes examination of temporal relationships. Consequently, though results from our research suggest associations between variables, they do not demonstrate an ability to predict type 2 diabetes or future cardiovascular events.

Equally of concern is that our data were derived from a single primary care based sample. Although results from the Cork and Kerry Diabetes and Heart Disease Study demonstrate prevalence rates for overweight/obesity and type 2 diabetes that are comparable to those observed in other nationally

representative Irish studies [13,35,165], the possibility that this sample is not representative of the source population must be acknowledged. However, previous research suggests that approximately 98% of Irish adults are registered with a GP and that, even in the absence of a universal patient registration system, it is possible to perform population-based epidemiological studies that are representative using our methods [210]. In addition, Ireland presents a generally ethnically homogeneous population [242]. Thus, the associations we observed between cardiometabolic features and HbA_{1c} and FPG may be similar in other middle-aged Irish adults. As random sampling of subjects and the use of validated methods for data collection ensured internal sample validity, it is equally possible that the relationships described may be generalisable to a similar middle-aged Caucasian-European population. Nevertheless, future studies utilising longitudinal data in different samples will be needed to confirm these findings. In particular, it will be necessary to determine whether risk stratification, using both assays, is clinically useful as a method for predicting type 2 diabetes.

3.5 Conclusions

In summary, our results suggest that in middle-aged Caucasian-Europeans, when using ADA-recommended cut-points, HbA_{1c} alone is a poor indicator of diabetes risk, but is appropriate for type 2 diabetes diagnosis. Furthermore, combined use of HbA_{1c} and FPG identifies subjects at substantially increased cardiometabolic risk. Although the efficacy and cost-effectiveness of routine

screening for diabetes in primary care has not been established [179-181], in light of the increasing prevalence of type 2 diabetes worldwide, there is a need to identify high-risk patients. Dual screening, utilising both HbA_{1c} and FPG, may provide a more accurate method for predicting type 2 diabetes. Earlier detection of high-risk subjects could enable earlier targeted interventions or therapies, thus attenuating development of diabetes and related cardiovascular complications.

Table 6—*Characteristics of the study population according to pre-diabetes and type 2 diabetes status.*

Feature	Full cohort	Pre-diabetes[1]		Type 2 diabetes[2]	
		HbA_{1c}	FPG	HbA_{1c}	FPG
	(N=2047)	(N=980)	(N=230)	(N=146)	(N=85)
Male	1008 (49.2)	441 (45.0)	150 (65.2)	95 (65.1)	59 (69.4)
Age	59 (55-64)	60 (55-64)	61 (56-65)	60 (57-65)	61 (57-65)
Age ≥60	981 (47.9)	510 (52.0)	125 (54.3)	83 (56.8)	51 (60.0)
Diagnosed diabetes	101 (4.9)	-	-	73 (50.0)	51 (60.0)
On Rx for diabetes	78 (3.8)	-	-	60 (41.1)	41 (48.2)
On Rx for hypertension	584 (28.5)	307 (31.3)	98 (42.6)	81 (55.5)	48 (56.5)
On Rx for cholesterol	711 (34.7)	385 (39.3)	93 (40.4)	88 (60.3)	49 (57.6)
BMI (kg/m^2)	28.6 ± 4.7	28.8 ± 4.7	30.5 ± 5.2	32.2 ± 5.5	31.8 ± 5.5
BMI ≥30	668 (32.7)	345 (35.2)	109 (47.4)	85 (58.2)	49 (57.6)
WC (cm)	97.0 ± 13.2	97.1 ± 12.9	102.4 ± 12.8	107.9 ± 13.7	108.5 ± 13.9
WC (HIGH)	1119 (54.8)	562 (57.4)	150 (65.2)	119 (81.5)	66 (77.6)
Family diabetes history	390 (19.1)	176 (18.0)	46 (20.0)	62 (42.5)	41 (48.2)
Triglycerides (mmol/l)	1.22 (0.9-1.7)	1.23 (0.9-1.7)	1.41 (1.0-2.0)	1.58 (1.2-2.3)	1.68 (1.2-2.3)
Triglycerides ≥1.7	490 (24.6)	230 (23.8)	85 (37.9)	65 (45.5)	40 (48.8)
HDL-C (mmol/l)	1.45 ± 0.4	1.45 ± 0.4	1.32 ± 0.3	1.17 ± 0.3	1.17 ± 0.4
HDL-C (LOW)	353 (17.6)	165 (17.0)	59 (26.1)	66 (45.2)	35 (41.2)
Dyslipidaemia	168 (8.4)	78 (8.0)	32 (14.0)	37 (25.3)	22 (25.9)
Systolic BP (mmHg)	129.60 ± 16.8	130.10 ± 16.1	134.78 ± 15.5	134.19 ± 17.3	136.24 ± 17.4
Diastolic BP (mmHg)	80.12 ± 9.7	80.24 ± 9.6	82.25 ± 9.1	79.50 ± 10.3	80.72 ± 10.5
BP ≥130/85	1045 (51.3)	521 (53.4)	155 (67.7)	89 (61.4)	56 (66.7)
Insulin (μU/ml)	8.65 (5.3-14.1)	8.98 (4.6-11.8)	12.67 (7.4-19.5)	18.27 (10.6-31.9)	19.21 (12.1-30.9)
Insulin 75^{th} percentile	497 (25.0)	238 (24.6)	98 (43.2)	94 (65.7)	59 (70.2)
≥3 MetS features[3]	606 (29.6)	298 (30.4)	112 (48.7)	103 (70.5)	63 (74.1)
HbA_{1c} (%)	5.7 (5.5-6.0)	5.9 (5.7-6.0)	5.8 (5.6-6.1)	7.0 (6.7-8.1)	7.6 (6.8-9.0)
HbA_{1c} (mmol/mol)	39 (37-42)	41 (39-42)	40 (38-43)	53 (50-65)	60 (51-75)
FPG (mmol/l)	4.90 (4.7-5.4)	5.00 (4.7-5.3)	5.80 (5.7-6.1)	6.90 (6.0-9.0)	8.50 (7.6-10.8)

Table 6 continued

Feature	Full cohort	Pre-diabetes[1]		Type 2 diabetes[2]	
	(N=2047)	HbA_{1c} (N=980)	FPG (N=230)	HbA_{1c} (N=146)	FPG (N=85)
C3 (mg/dl)	135.92 ± 24.7	138.85 ± 24.5	141.41 ± 25.8	148.13 ± 28.6	149.20 ± 24.9
CRP (ng/ml)	1.35 (1.0-2.3)	1.43 (1.0-2.4)	1.38 (1.0-2.3)	1.79 (1.1-3.2)	1.91 (1.2-3.0)
IL-6 (pg/ml)	1.81 (1.2-2.9)	1.91 (1.3-3.0)	2.02 (1.5-3.0)	2.92 (1.7-4.8)	2.83 (1.8-4.6)
TNF-α (pg/ml)	5.97 (4.9-7.3)	6.02 (5.0-7.3)	5.94 (4.9-7.5)	6.99 (5.5-8.3)	7.09 (5.6-8.1)
Adiponectin (ng/ml)	4.75 (2.9-7.5)	4.92 (3.1-7.5)	3.63 (2.4-5.6)	2.82 (1.7-4.6)	2.73 (1.9-4.7)
Leptin (ng/ml)	1.95 (1.1-3.1)	2.09 (1.3-3.5)	2.06 (1.3-3.8)	2.28 (1.3-3.9)	2.09 (1.1-3.4)
Resistin (ng/ml)	5.07 (3.9-6.7)	4.93 (3.8-6.6)	4.89 (3.7-6.7)	6.15 (4.6-7.3)	5.53 (4.5-7.3)
PAI-1 (ng/ml)	27.38 ± 12.6	27.87 ± 12.0	29.56 ± 13.2	31.35 ± 15.9	30.03 ± 11.0
WBC (10^9/l)	6.00 ± 1.9	6.12 ± 2.1	6.33 ± 1.72	7.39 ± 2.4	7.21 ± 1.9

Mean and ± standard deviation are shown for continuous variables. Age, triglycerides, insulin, HbA_{1c}, FPG, CRP, IL-6, TNF-α, adiponectin, leptin and resistin are shown as a median (interquartile range). Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.

[1]Pre-diabetes: HbA_{1c} levels 5.7%-6.4% (39-46 mmol/mol) or FPG levels 5.6-6.9 mmol/l.

[2]Type 2 diabetes: HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l.

[3]MetS features: WC (HIGH), triglycerides ≥1.7, HDL-C (LOW), BP ≥130/85 or Rx and insulin 75th percentile.

Table 7—*Odds ratios (95% CI) of having risk features according to diagnosis of pre-diabetes and type 2 diabetes by HbA_{1c} or FPG.*

Feature	Odds ratios (95% CI)[1]							
	Pre-diabetes compared to normoglycaemia[2]				Type 2 diabetes compared to no diabetes[3]			
	HbA_{1c}	P value	FPG	P value	HbA_{1c}	P value	FPG	P value
Male	0.8 (0.6-0.9)	<0.001	2.3 (1.7-3.0)	<0.001	2.0 (1.4-2.9)	<0.001	2.5 (1.5-3.9)	<0.001
Age ≥60	1.6 (1.3-1.9)	<0.001	1.4 (1.1-1.9)	0.011	1.5 (1.1-2.2)	0.018	1.7 (1.1-2.7)	0.017
Family diabetes history	1.2 (0.9-1.5)	0.182	1.4 (1.0-2.1)	0.043	4.1 (2.9-5.9)	<0.001	5.2 (3.3-8.1)	<0.001
BMI ≥30	1.8 (1.4-2.2)	<0.001	2.2 (1.7-3.0)	<0.001	3.1 (2.2-4.3)	<0.001	2.8 (1.8-4.4)	<0.001
WC (HIGH)	1.5 (1.2-1.9)	0.001	2.0 (1.4-3.1)	0.001	5.4 (2.5-11.8)	<0.001	7.4 (2.3-23.5)	0.001
Triglycerides ≥1.7	1.2 (0.9-1.5)	0.134	2.1 (1.5-2.8)	<0.001	2.5 (1.8-3.6)	<0.001	2.8 (1.8-4.4)	<0.001
HDL-C (LOW)	1.4 (1.1-1.8)	0.018	2.3 (1.7-3.3)	<0.001	4.6 (3.2-6.6)	<0.001	3.6 (2.3-5.7)	<0.001
Dyslipidaemia	1.6 (1.1-2.4)	0.019	2.6 (1.7-4.1)	<0.001	4.3 (2.8-6.5)	<0.001	4.1 (2.4-6.9)	<0.001
BP ≥130/85 or Rx	1.4 (1.2-1.7)	<0.001	2.5 (1.8-3.5)	<0.001	3.0 (1.9-4.8)	<0.001	4.4 (2.2-8.6)	<0.001
Insulin 75th percentile	1.6 (1.3-2.0)	<0.001	3.1 (2.3-4.2)	<0.001	6.5 (4.5-9.4)	<0.001	7.2 (4.4-11.7)	<0.001
≥3 MetS features[4]	1.6 (1.3-2.0)	<0.001	3.0 (2.2-3.9)	<0.001	6.1 (4.2-8.8)	<0.001	6.8 (4.1-11.2)	<0.001
C3[5]	1.8 (1.5-2.2)	<0.001	1.4 (1.0-1.8)	0.032	3.3 (2.2-4.9)	<0.001	3.1 (1.9-5.0)	<0.001
CRP[5]	1.4 (1.1-1.7)	0.001	1.2 (0.9-1.5)	0.293	1.5 (1.1-2.2)	0.02	1.6 (1.0-2.6)	0.032
IL-6[5]	1.6 (1.3-1.9)	<0.001	1.5 (1.1-2.0)	0.005	2.8 (1.9-4.1)	<0.001	2.8 (1.7-4.6)	<0.001
TNF-α[5]	1.2 (1.0-1.4)	0.078	1.0 (0.7-1.3)	0.738	2.3 (1.6-3.3)	<0.001	2.7 (1.6-4.4)	<0.001
Adiponectin[5]	1.4 (1.1-1.7)	0.004	2.0 (1.4-2.7)	<0.001	4.0 (2.5-6.2)	<0.001	3.2 (1.8-5.6)	<0.001
Leptin[5]	1.5 (1.2-1.8)	<0.001	1.4 (1.1-1.9)	0.014	1.5 (1.0-2.1)	0.026	1.2 (0.8-1.8)	0.48
Resistin[5]	0.9 (0.8-1.1)	0.305	0.9 (0.7-1.2)	0.391	2.4 (1.7-3.5)	<0.001	1.8 (1.1-2.8)	0.012
PAI-1[5]	1.3 (1.1-1.6)	0.005	1.3 (1.0-1.7)	0.108	1.5 (1.0-2.1)	0.028	1.5 (1.0-2.4)	0.078
WBC[5]	1.7 (1.4-2.1)	<0.001	1.6 (1.2-2.1)	0.001	3.4 (2.3-5.0)	<0.001	3.3 (2.0-5.5)	<0.001

[1]Binary logistic regression. Gender adjusted for age (continuous), age ≥60 adjusted for gender, all other variables adjusted for age (continuous) and gender.
[2]Pre-diabetes: HbA_{1c} ≥5.7% (≥39 mmol/mol) or FPG ≥5.6 mmol/l, models exclude subjects with type 2 diabetes: HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l or physician diagnosis or Rx diabetes medication use.
[3]Models exclude 24 subjects that indicated a physician diagnosis or Rx diabetes medication use but who did not have positive HbA_{1c} or FPG test results.
[4]MetS features: WC (HIGH), triglycerides ≥1.7, HDL-C (LOW), BP ≥130/85 or Rx and insulin 75th percentile.
[5]Threshold: above median level in the study population except adiponectin (below median level).

Table 8—*Odds ratios (95% CI) of having risk features according to diagnosis of pre-diabetes[1] by HbA_{1c} alone, FPG alone, or by both HbA_{1c} and FPG together.*

Feature	Odds ratios (95% CI)[2]					
	HbA_{1c} alone (N=814)	P value	FPG alone (N=62)	P value	HbA_{1c} & FPG (N=162)	P value
Male	0.8 (0.6-0.9)	0.006	3.3 (1.8-5.9)	<0.001	1.6 (1.2-2.3)	0.005
Age ≥60	1.6 (1.3-1.9)	<0.001	1.4 (0.8-2.3)	0.251	2.0 (1.4-2.8)	<0.001
Family diabetes history	1.1 (0.8-1.4)	0.474	1.2 (0.6-2.4)	0.651	1.7 (1.1-2.6)	0.013
BMI ≥30	1.6 (1.3-2.0)	<0.001	1.7 (1.0-3.0)	0.051	3.4 (2.4-4.9)	<0.001
WC (HIGH)	1.4 (1.2-1.8)	<0.001	2.0 (1.2-3.4)	0.011	2.6 (1.8-3.7)	<0.001
Triglycerides ≥1.7	1.2 (0.9-1.5)	0.267	2.5 (1.4-4.3)	0.001	2.3 (1.4-4.3)	<0.001
HDL-C (LOW)	1.3 (1.0-1.7)	0.095	2.5 (1.3-4.7)	0.004	2.8 (1.8-4.3)	<0.001
Dyslipidaemia	1.6 (1.0-2.5)	0.041	3.5 (1.6-7.8)	0.002	3.5 (2.0-6.2)	<0.001
BP ≥130/85 or Rx	1.3 (1.0-1.6)	0.012	2.2 (1.2-3.9)	0.009	3.3 (2.2-5.1)	<0.001
Insulin 75th percentile	1.5 (1.2-2.0)	0.002	3.4 (2.0-5.9)	<0.001	4.1 (2.8-5.9)	<0.001
≥3 MetS features[3]	1.4 (1.2-1.8)	0.003	3.0 (1.7-5.1)	<0.001	4.0 (2.8-5.8)	<0.001
C3[4]	1.8 (1.5-2.3)	<0.001	1.4 (0.9-2.4)	0.17	2.2 (1.5-3.1)	<0.001
CRP[4]	1.4 (1.1-1.7)	0.002	1.1 (0.7-2.0)	0.640	1.5 (1.1-2.2)	0.017
IL-6[4]	1.5 (1.2-1.9)	<0.001	1.4 (0.8-2.4)	0.212	2.1 (1.5-3.0)	<0.001
TNF-α[4]	1.2 (1.0-1.5)	0.096	0.8 (0.5-1.4)	0.524	1.1 (0.8-1.6)	0.446
Adiponectin[4]	1.3 (1.0-1.6)	0.043	1.3 (0.7-2.3)	0.373	2.6 (1.8-3.9)	<0.001
Leptin[4]	1.4 (1.2-1.8)	<0.001	1.3 (0.8-2.2)	0.345	2.0 (1.4-2.9)	<0.001
Resistin[4]	1.0 (0.8-1.2)	0.626	1.3 (0.7-2.1)	0.389	0.8 (0.5-1.1)	0.139
PAI-1[4]	1.3 (1.1-1.6)	0.008	1.4 (0.8-2.4)	0.2	1.6 (1.1-2.2)	0.014
WBC[4]	1.6 (1.3-2.0)	<0.001	1.3 (0.7-2.2)	0.371	2.6 (1.8-3.7)	<0.001

[1]Pre-diabetes: HbA_{1c} ≥5.7% (≥39 mmol/mol) or FPG ≥5.6 mmol/l, models exclude subjects with type 2 diabetes: HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l or physician diagnosis or Rx diabetes medication use.

[2]Multinomial logistic regression, reference category: normoglycaemia by both HbA_{1c} and FPG. Gender adjusted for age (continuous), age ≥60 adjusted for gender, all other variables adjusted for age (continuous) and gender.

[3]MetS features: WC (HIGH), triglycerides ≥1.7, HDL-C (LOW), BP ≥130/85 or Rx and insulin 75th percentile.

[4]Threshold: above median level in the study population except adiponectin (below median level).

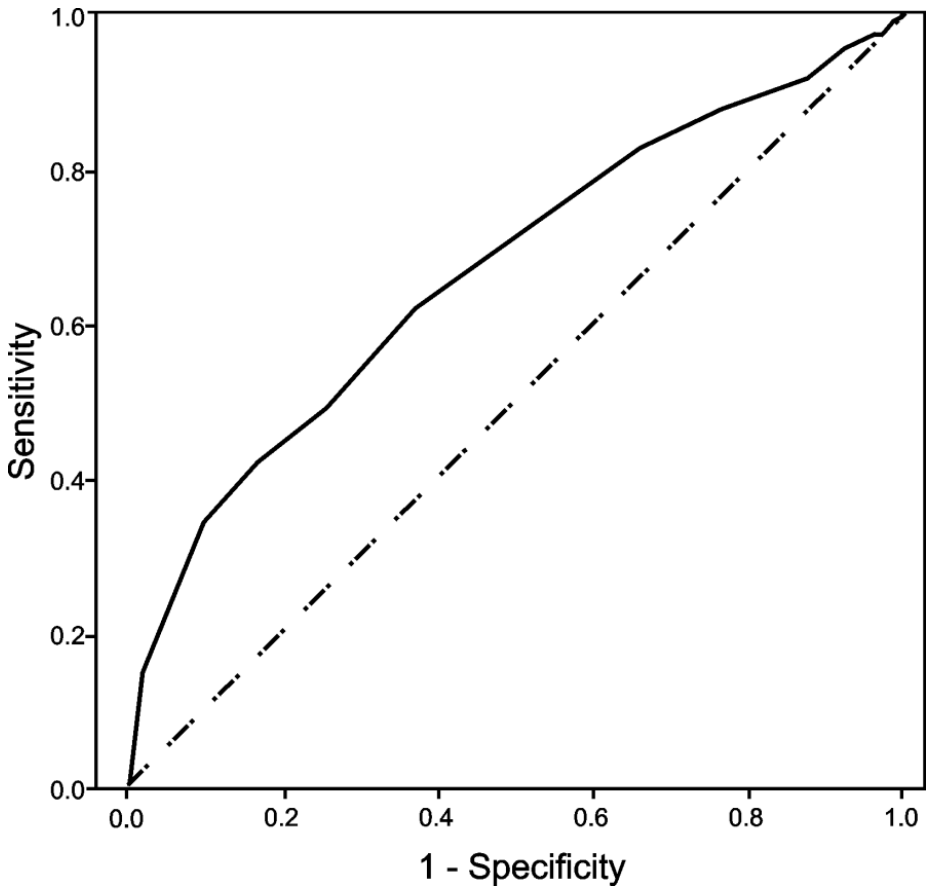

Figure 4—*Receiver operating characteristic curve for HbA_{1c} to discriminate subjects with pre-diabetes.* The figure shows an ROC curve for HbA_{1c} (continuous) to discriminate subjects with pre-diabetes (impaired FPG $\geq$5.6 mmol/l). The area under the curve value was AUC=0.67, 95% CI: 0.63-0.71.

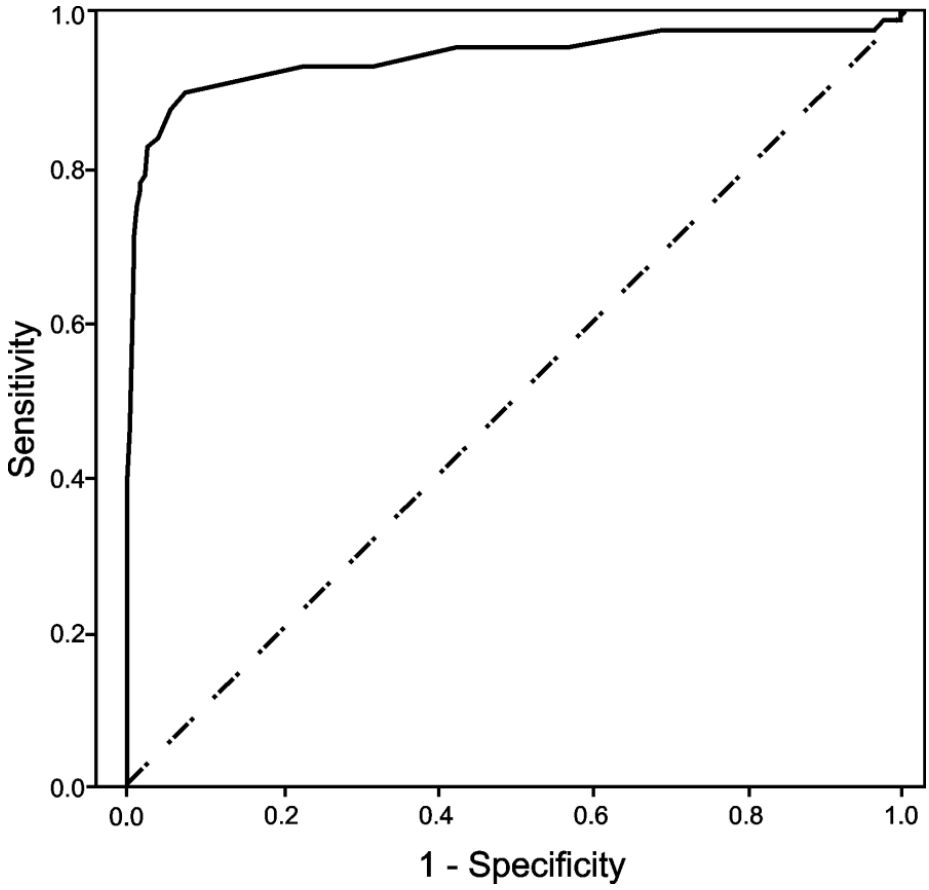

Figure 5—*Receiver operating characteristic curve for HbA_{1c} to discriminate subjects with type 2 diabetes.* The figure shows an ROC curve for HbA_{1c} (continuous) to discriminate subjects with type 2 diabetes (FPG $\geq$7.0 mmol/l). The area under the curve value was AUC=0.94, 95% CI: 0.90-0.98.

CHAPTER 4

OPTIMAL CENTRAL OBESITY MEASUREMENT SITE FOR ASSESSING CARDIOMETABOLIC AND TYPE 2 DIABETES RISK IN MIDDLE-AGED ADULTS

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4.0 Abstract

Background and Objectives

Despite recommendations that central obesity assessment should be employed as a marker of cardiometabolic health, no consensus exists regarding measurement protocol. This study examined a range of anthropometric variables and their relationships with cardiometabolic features and type 2 diabetes in order to ascertain whether measurement site influences discriminatory accuracy. In particular, we compared waist circumference (WC) measured at two sites: (1) immediately below the lowest rib (WC rib) and (2) between the lowest rib and iliac crest (WC midway), which has been recommended by the World Health Organisation and International Diabetes Federation.

Materials and Methods

This was a cross-sectional study involving a random sample of 2,002 men and women aged 46-73 years. Metabolic profiles and WC, hip circumference, pelvic width and body mass index (BMI) were determined. Correlation, logistic regression and area under the receiver operating characteristic curve analyses were used to evaluate adiposity variable relationships with metabolic risk phenotypes and type 2 diabetes.

Results

WC rib measures displayed the strongest associations with non-optimal lipid and lipoprotein levels, high blood pressure, insulin resistance, impaired fasting glucose, a clustering of metabolic risk features and type 2 diabetes, in both genders. Rib-derived indices improved discrimination of type 2 diabetes by 3%-7% compared to BMI and 2%-6% compared to WC midway (in men) and 5%-7% compared to BMI and 4%-6% compared to WC midway (in women). Index models including BMI and central obesity variables displayed a significantly higher area under the curve for WC rib (AUC=0.78, P=0.003), Rib/height ratio (AUC=0.80, P<0.001), Rib/pelvis ratio (AUC=0.79, P<0.001), but not for WC midway (AUC=0.75, P=0.127), when compared to one with BMI alone (AUC=0.74).

Conclusions

WC rib is easier to assess and our data suggest that it is a better method for determining obesity-related cardiometabolic risk than WC midway. The clinical utility of rib-derived indices, or alternative WC measurements, deserves further investigation.

4.1 Introduction

Obesity is associated with dyslipidaemia, hypertension, insulin resistance and the development of metabolic syndrome and type 2 diabetes [6], leading to a greater likelihood of premature death. However, not all obese subjects are at increased cardiometabolic risk as a proportion are considered to be metabolically healthy [232]. The prevalence of obesity has escalated in many world populations [2,5]. Thus, there is an increasing need for inexpensive and non-invasive risk assessment tools for use in clinical practice to help identify overweight and obese individuals at highest odds of developing metabolic abnormalities and type 2 diabetes.

Body mass index (BMI) has traditionally been the chosen surrogate method used to determine excess body fat, but because it is a weight-for-height measure, BMI is unable to distinguish between fat and lean mass. Recent research has indicated that general obesity categorisation based on BMI might be inadequate [61,99], and studies have shown that BMI may misclassify adiposity [243-245].

Increasing evidence suggests that central obesity is a more important cardiometabolic risk factor [79,83] and waist circumference (WC) measurement has been recommended as a method for central obesity assessment. However, partly due to a lack of agreement on a universal measurement protocol, its clinical usefulness and superiority over BMI for detecting patients at increased cardiometabolic risk has been questioned [12,246]. Various transformations of WC have also been used, such as the

waist-height ratio (WHtR) [143] and waist-hip ratio (WHR) [114]. Although extensive research has attempted to quantify relationships between different adiposity measures and morbidity [246], considerable controversy still exists as to which measurement site or index most accurately defines non-optimal body fat distribution [151].

In this study we examined a range of anthropometric variables and their relationships with metabolic risk phenotypes, including lipid and lipoprotein levels, high blood pressure (BP), insulin resistance, impaired fasting plasma glucose (FPG), a clustering of metabolic risk features and type 2 diabetes, in a random sample of 2,002 middle-aged men and women. In particular, we compared the discriminatory performance of WC measured at two locations (immediately below the lowest rib, and between the lowest rib and iliac crest), and variations of these measures, to explore the hypothesis that the measurement site for central obesity affects its accuracy as a discriminator of cardiometabolic risk.

4.2 Materials and Methods

4.2.1 Study design

The study design is described in detail in Chapter 1. In summary, the Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000,

with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending patients in the 46-73 year age group. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). Details regarding the study design, sampling procedures and methods of data collection have been reported previously [183].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

4.2.2 Clinical and laboratory procedures

Study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, physician-diagnosed type 2 diabetes and prescription (Rx) medication use were gathered through a self-completed General Health Questionnaire [170]. Triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were

measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica GmbH, Hamburg, Germany). Fasting plasma glucose concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and fasting serum insulin was calculated using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium]. Three independent measurements of systolic and diastolic BP were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP.

4.2.3 Anthropometric variables

Anthropometric measurements were taken by researchers who were thoroughly trained according to the study research protocols [183]. The weight and height of each subject were measured to the nearest 0.1 kg and 0.1 cm respectively. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was assessed using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and BMI

was calculated as weight divided by the square of height. Midway WC (WC midway) was measured between the lowest rib and iliac crest on bare skin. Participants were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. Rib WC (WC rib) was measured immediately below the lowest rib at the mid-axillary line and hip circumference was determined at the maximum perimeter of the hips. Pelvic width was calculated as the diameter between the right and left iliac crests using callipers. For each central obesity variable, the mean of two independent readings was used in analysis. Height, hip circumference and pelvic width were divided into WC midway and WC rib measurements deriving six variables: (1) *Midway/height ratio*, (2) *Midway/hip ratio*, (3) *Midway/pelvis ratio* and (4) *Rib/height ratio*, (5) *Rib/hip ratio,* (6) *Rib/pelvis ratio*.

4.2.4 Classification of biochemical and blood pressure measurements

According to American Diabetes Association guidelines, type 2 diabetes was defined as HbA_{1c} $\geq$6.5% ($\geq$48 mmol/mol) or FPG $\geq$7.0 mmol/l [23]. Individuals on insulin therapy and subjects indicating a diagnosis of diabetes (either self-reported physician diagnosis or Rx diabetes medication use), but who did not have positive HbA_{1c} or FPG test results, were excluded from analysis (N=45).

Lipid, lipoprotein, FPG and BP measurements were classified according to National Cholesterol Education Program: Adult Treatment Panel III criteria

[184]. Abnormal metabolic risks were defined as high triglyceride levels ≥1.7 mmol/l, low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females) and impaired FPG levels 5.6-6.9 mmol/l. High BP was classified as systolic BP ≥130 mmHg and/or diastolic BP ≥85 mmHg or Rx anti-hypertensive medication use. The Homeostasis Model Assessment Index of Insulin Resistance (HOMA-IR) [247] was derived from FPG and insulin concentrations as [(FPG x fasting serum insulin)/22.5], and insulin resistance was defined as a level equal to or above the 75th percentile in the study population. Having three or more cardiometabolic risk features was characterised as any combination of these variables.

4.2.5 Statistical analysis

The distribution of each metabolic characteristic was assessed using Shapiro-Wilk and Kolmogorov-Smirnov statistics. Categorical features are presented as percentages and continuous data are shown as a mean, plus or minus one standard deviation, or a median and interquartile range. Gender differences were evaluated using chi-square tests, independent *t*-tests or a Mann-Whitney U for skewed data. Relationships between anthropometric measurements and continuous cardiometabolic variables were investigated using partial correlations. Variables presenting a non-normal distribution were log transformed. All obesity measures were gender-standardised and separate and stratified binary logistic regression models were used to compare index associations with cardiometabolic risk features and type 2 diabetes, adjusting for age.

The ability of selected indices to discriminate three or more cardiometabolic risk features and type 2 diabetes was measured using receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) provides a scale from 0.5 to 1.0 (with 0.5 representing random chance and 1.0 indicating perfect discrimination) by which to appraise the capability of an adiposity measure to detect a positive result [150]. A higher AUC generally indicates greater diagnostic accuracy. Covariate-adjusted analysis [248] was performed to account for the potential confounding influence of both age and gender (full cohort) or age alone in stratified models. The AUC values were compared for statistical differences and were further evaluated by determining false positive rates at specific points on the curve corresponding to 90%, 80%, 70% and 60% sensitivities.

To further judge the ability of central adiposity to discriminate type 2 diabetes, we compared a logistic regression model containing BMI to models which included both BMI and selected central obesity measures. The accuracy of each model was assessed using the ROC curve. We additionally evaluated discrimination using integrated discrimination improvement (IDI) analysis, which indicates the magnitude of improvement in the performance of a model by adding another variable [249]. To assess goodness-of-fit, the likelihood ratio (LR) chi-square statistics were examined by comparing models with or without an additional anthropometric measure. Calibration was measured using the Hosmer-Lemeshow (HL) test.

Data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) and Stata SE Version 13 (Stata Corporation, College Station, TX, USA) for Windows. Seven subjects had missing anthropometric values. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance.

4.3 Results

4.3.1 Descriptive characteristics

Characteristics of the study population are presented in Table 9. According to BMI classification recommended by the World Health Organisation (WHO) [23], 1,550 (77.7%) participants were either overweight or obese, with 835 (85.6%) male subjects having a BMI $\geq$25 kg/m^2 compared to 715 (70.2%) females (P for difference <0.001). Mean WC and pelvic width measurements were also significantly increased in men while hip circumference levels were greater in women. Distinctions between WC midway and WC rib were observed in both genders, with average midway values being higher. With consideration to metabolic risk factors, male subjects were significantly more likely to have abnormal triglyceride levels, high BP, insulin resistance, impaired FPG, a clustering of cardiometabolic risk features and type 2 diabetes.

4.3.2 Partial correlations between anthropometric measurements and cardiometabolic variables

After adjustment for age, positive correlations for triglycerides, systolic BP, diastolic BP, HOMA-IR, HbA_{1c}, FPG, and negative correlations for HDL-C, were observed with weight, BMI and measurements of central adiposity (Table 10). Significant inverse relationships were also noted for height with triglyceride and FPG concentrations in men, while HDL-C was positively correlated with height in women. Relationships were stronger between WC rib and a majority of metabolic variables, with triglycerides, HDL-C and HOMA-IR showing the highest correlative strengths. Nevertheless, metabolic variable correlations with BMI and WC midway, although reduced, were of a similar magnitude in men.

4.3.3 Associations between adiposity measures and adverse cardiometabolic features and type 2 diabetes

The results from regression models examining adiposity variable associations with individual metabolic risk factors (Appendix 2, Supporting Figure 1), three or more cardiometabolic risk features (Figure 6) and type 2 diabetes (Figure 7) are shown. Results are adjusted for age and odds ratios represent the odds associated with a one standard deviation increase in each obesity measure. Although the strength of relationship varied according index type, WC rib or rib-derived indices displayed, without exception, stronger associations with individual cardiometabolic risk factors, metabolic feature clustering and type 2 diabetes, in both genders. In general, stronger

relationships with cardiometabolic variables were noted in women, with differences between BMI and central obesity being less pronounced in male subjects.

4.3.4 ROC analysis

In ROC analysis, both WC rib and Rib/height ratio demonstrated a significantly higher AUC to detect three or more cardiometabolic risk features compared to WC midway in male subjects (Figure 8). In females, significant differences in the AUC were observed when compared to both WC midway and BMI. For type 2 diabetes (Figure 9), WC rib measures showed a higher discriminatory capacity in both genders, with the exception of the Rib/hip ratio in men. Rib-derived indices improved discrimination by 3%-7% compared to BMI and 2%-6% compared to WC midway (in men) and 5%-7% compared to BMI and 4%-6% compared to WC midway (in women). Rib measures also displayed greater specificity across a range of sensitivities (Figure 10). At higher sensitivities classification accuracy was improved by 10% or more. However, false positive rates for the Rib/hip ratio were noticeably increased when compared to other adiposity variables in men.

4.3.5 Evaluation of index discrimination models

As presented in Table 11, we compared models which included BMI and an additional central obesity measure to discriminate type 2 diabetes. The HL test showed P values that were non-significant, suggesting that model fits

were acceptable. Additionally, the LR chi-squares were reduced in models including central adiposity variables, indicating improved goodness-of-fit. Using the IDI statistic, a significant but marginal increase in discrimination was observed for WC midway, with a small and non-significant increase in the AUC (AUC=0.75, P=0.127) (Figure 11). In contrast, models including BMI and WC rib measures displayed significantly higher AUC values (Figures 12-14) for WC rib (AUC=0.78, P=0.003), Rib/height ratio (AUC=0.80, P<0.001) and Rib/pelvis ratio (AUC=0.79, P<0.001) when compared to a model with BMI alone (AUC=0.74).

4.4 Discussion

Both the WHO and International Diabetes Federation (IDF) have suggested midway WC measurement as the preferred method for central obesity assessment [12,19]. In contrast, the United States National Institutes of Health (NIH) recommend measuring WC at the superior border of the iliac crest [103]. However, there is a lack of scientific rationale to support either of these measurement protocols [102]. Although previous studies have compared these two criteria, to the best of our knowledge, this is the first to comprehensively evaluate both rib and midway WC measurements and BMI. Our findings suggest that WC rib, rather than WC midway, is a better indicator of central obesity as it improves discrimination of type 2 diabetes within our population. One possible explanation for this relationship may be that rib-level measurement is less influenced by inter-individual variables

such as body posture or elasticity of the abdominal wall, which are partly unrelated to actual body adiposity.

The results from previous research investigating different WC measurement criteria are conflicting. A systematic review of 120 studies [107] concluded that measurement procedure had no substantial influence on WC relationships with morbidity and mortality, leading the authors to recommend the NIH protocol as it may be more readily adapted by healthcare practitioners and is more suitable for self-measurement by the general public. However, effect sizes and discriminatory differences between WC sites were not compared. In contrast, Ma et al. [250] found WC midway to be slightly better than NIH-recommended iliac measurement to predict hypertension, metabolic syndrome and diabetes. Nevertheless, WC rib was not assessed in this study. Bosy-Westphal et al. [102] also observed reduced associations between the iliac site and metabolic characteristics and visceral adipose tissue (VAT) in females. Relationships between cardiometabolic variables and WC midway and rib were similar in men, while WC rib was more strongly correlated with VAT in women.

Regardless of controversies surrounding WC measurement protocol, both advantages and disadvantages exist regarding the general application of central obesity assessment within clinical practice. Although some studies have suggested WC to be the simplest and best overall method for cardiometabolic health appraisal [127], as metabolic risk cut-points for WC

are different between genders, and vary between ethnic groups [12,251], the practical usability of WC measurement is still uncertain [246].

In keeping with other findings [115,143], our results imply that transformations of WC may improve discrimination. The use of a ratio to define central adiposity is also potentially beneficial as it might allow uniform diagnostic thresholds to be used (between ethnicities, genders or both), making it attractive from a public health perspective [125,126]. Notably, however, the WHR was a markedly poor indicator of risk in male subjects within this sample. Reduced associations for WHR were also observed by Schneider et al. [121], who theorised that as both WC and hip circumference exhibit strong relationships with cardiometabolic features, a ratio of the two may show less. Additionally, both measures may increase or decrease proportionally in an individual [117]. It could be that sex differences observed for WHR are due to gender variations in body composition, and that changes in hip circumference, relative to WC, are more pronounced in middle-aged men than in women.

Although WC rib measures demonstrated stronger relationships with metabolic variables, consistent with previous research [246], our study also revealed that index associations with a majority of the examined cardiometabolic features and type 2 diabetes were reduced in men. One possible explanation for this finding is the greater prevalence of overweight and obesity amongst males within this population, thus reducing associations between variables and discriminatory abilities. It was also noted that

discriminatory differences between central adiposity and BMI were greater when detecting type 2 diabetes compared to a clustering of metabolic features, in both genders. A reason for this may be that central obesity independently predicts type 2 diabetes, beyond commonly assessed cardiometabolic disease markers [100].

Compared with BMI, central adiposity is thought to be more strongly correlated with VAT [83]. Research has suggested that fatty acids released from VAT drain into the liver and skeletal muscle causing metabolic dysfunction within these organs [78]. Proteins secreted from VAT may also contribute to cardiometabolic disease through inflammation of adipose and vascular tissue [79]. Increased VAT levels have been shown to be associated with increased risk of dyslipidaemia, hypertension and type 2 diabetes [75,76]. Subsequently, differences in discrimination of cardiometabolic disease features and type 2 diabetes (observed within this sample) might suggest that central obesity should be independently evaluated as a diabetes risk factor, and that its inclusion as a mandatory component of the metabolic syndrome may be appropriate [19]. The idea that central obesity assessment provides additional information related to diabetes – beyond commonly measured cardiometabolic disease features, including BMI – is explored further in Chapter 5.

Nevertheless, the findings from previous studies which have compared central adiposity variables with BMI have been inconclusive [151,246]. Possible reasons for variations between studies may include different WC

measurement protocols or dissimilar methods for classifying chronic conditions. Although AUC values for central obesity measures are frequently reported to be larger when compared to BMI for discriminating type 2 diabetes [143], as the AUC lacks clinical relevance, there is argument against using it as a summary statistic of the ROC curve as similar AUC values may have different diagnostic properties [248]. Though other studies have reported metabolic risk thresholds for obesity indices based on maximum sensitivity, optimal sensitivity and specificity, the furthest distance from the chance line or the shortest distance to the y axis [12], cut-points are necessarily arbitrary, and may vary between different populations.

Central obesity measures have been proposed as stand-alone, pre-screening tools [126] for use in high-risk populations to enable clinicians to detect those who might benefit from further diagnostic or therapeutic procedures [252,253]. In this scenario it is desirable to optimise sensitivity (the percentage of people with or at risk of a condition, who would be correctly identified), in order to rule out healthy subjects. Importantly, by comparing false positive rates (the proportion of healthy individuals who would be misclassified) across a range of sensitivities for multiple indices, our results demonstrate WC rib measures to be more accurate classifiers, at higher sensitivities, compared to WC midway and BMI.

However, debate exists regarding the clinical efficacy of central adiposity assessment. To some extent this is due to a lack of evidence regarding how much of an increase in discriminatory accuracy central obesity measures

might add over traditionally assessed indicators of cardiometabolic disease [246]. Though findings from this study suggest that central adiposity indices provide additional information when compared to general adiposity measured by BMI, these results also demonstrate that the degree of improvement is significantly influenced by the procedure used for estimating WC.

While only requiring a flexible measuring tape, midway WC is difficult to obtain as it requires the identification of two bony landmarks, a computed distance between the two, and a circumference evaluation – essentially four separate measurements. Given that central obesity assessment competes for the limited time available during patient appraisal, and necessitates specific training to ensure reliable data are obtained [83], a simpler measurement protocol is desirable. WC rib is more easily determined and offers a more practical method for use within healthcare practice and epidemiological research, and would be equally suitable for self-assessment. Furthermore, Bosy-Westphal et al. [102] and Wang et al. [254] also concluded that WC rib had a higher reproducibility. As measurement error may limit the minimal detectable difference in a parameter [102], it is possible that the higher discriminatory accuracy we observed may be due to greater measurement precision.

4.4.1 Limitations of the research

Though our findings are of potential public health and clinical significance, several limitations should be considered. Given the modest number of outcomes within our sample we did not adjust for multiple factors in analyses. Our primary aim was to compare general and central obesity relationships, rather than to determine overall strengths of association. Nevertheless, the possibility that confounding features may influence adiposity variables in different ways cannot be discounted and future studies with larger samples might find different relationships. Also, cross-sectional data precludes examination of the temporal relationship between adiposity and diabetes. Thus, although our results may suggest a rationale for central adiposity assessment as a method for indentifying patients with type 2 diabetes, and for assessing cardiometabolic risk, they do not demonstrate that central obesity measures would be useful to predict type 2 diabetes or related cardiovascular outcomes.

Equally of concern is that we did not have other WC measurement sites to contrast and that our data were derived from a single primary care based sample. However, Ireland presents a generally ethnically homogeneous population [242]. In addition, random sampling of subjects and the use of validated methods for data collection ensured internal sample validity and the relationships described may be generalisable to a similar middle-aged Caucasian-European population. Nonetheless, future studies utilising longitudinal data in different samples will be needed to evaluate the validity and reliability of alternative WC measurements. In particular, it will be

necessary to determine whether employing central obesity measures for risk stratification is clinically useful and superior to currently recommended BMI classification [94].

4.5 Conclusions

In summary, our results indicate that measurement protocol for WC may be important for determining central obesity and assessing cardiometabolic health. Rib-level measures were more strongly associated with cardiometabolic risk features and improved discrimination of patients with type 2 diabetes. In light of the increasing prevalence of obesity, cardiometabolic disease and diabetes worldwide, effective methods to detect individuals with type 2 diabetes, and those at increased cardiometabolic risk, are needed [51]. The clinical utility of WC measured at the lowest rib, rib-derived indices or alternative WC measurements as potentially more accurate discriminators of metabolic risk and type 2 diabetes, compared to WHO and IDF-recommended midway WC measurement or BMI, deserves further investigation.

Table 9—*Characteristics of the study population.*

Feature	Males (N=981)	Females (N=1021)	P value
Age	59 (55-64)	59 (54-64)	0.791
Weight (kg)	87.4 ± 13.8	71.6 ± 13.6	<0.001
Height (m)	1.7 ± 0.1	1.6 ± 0.1	<0.001
BMI (kg/m^2)	29.1 ± 4.2	28.0 ± 5.2	<0.001
WC midway (cm)	102.6 ± 11.1	91.4 ± 12.7	<0.001
WC rib (cm)	99.9 ± 10.1	85.1 ± 12.2	<0.001
Hip circumference (cm)	99.0 ± 8.7	101.8 ± 10.7	<0.001
Pelvic width (cm)	33.0 ± 2.4	32.0 ± 2.7	<0.001
Triglycerides (mmol/l)	1.32 (0.9-1.9)	1.10 (0.8-1.5)	<0.001
High triglycerides[1]	313 (32.9)	164 (16.5)	<0.001
HDL-C (mmol/l)	1.28 ± 0.3	1.62 ± 0.4	<0.001
Low HDL-C[2]	166 (17.3)	169 (16.8)	0.676
Average systolic BP (mmHg)	130.83 ± 15.6	128.44 ±17.9	0.001
Average diastolic BP (mmHg)	79.94 ± 9.6	80.42 ± 9.9	0.339
High BP[3]	628 (64.3)	593 (58.3)	0.006
HOMA-IR	3.27 (1.3-3.8)	2.32 (1.0-2.7)	<0.001
Insulin resistance[4]	301 (32.0)	179 (18.2)	<0.001
FPG (mmol/l)[5]	5.00 (4.7-5.4)	4.80 (4.5-5.2)	<0.001
Impaired FPG[5,6]	150 (17.3)	80 (8.5)	<0.001
Three or more cardiometabolic risk features[5]	178 (20.0)	106 (10.9)	<0.001
Type 2 diabetes	92 (9.5)	50 (5.0)	<0.001

Mean and ± standard deviation are shown for continuous variables, P value calculated with a Student's *t*-test. Age, triglycerides, HOMA-IR and FPG are shown as a median (interquartile range) with a P value according to a Mann-Whitney U. % are shown for categorical values with x^2 for difference in proportions. Numbers and (%) may vary as some variables have missing values.

[1]Triglycerides $\geq$1.7.

[2]HDL-C <1.03 (males) or HDL-C <1.29 (females).

[3]BP $\geq$130/85 or on Rx for hypertension.

[4]HOMA-IR 75th percentile.

[5]Excluding subjects with type 2 diabetes.

[6]FPG $\geq$5.6.

Table 10—*Partial correlations[1] between anthropometric measurements and cardiometabolic variables, stratified by gender.*

Cardiometabolic Feature	Weight	Height	BMI	WC midway	WC rib	Hip circumference	Pelvic width
MALES							
Triglycerides[2]	0.249	-0.062	0.306	0.296	**0.319**	0.257	0.162
HDL-C	-0.347	0.063[3]	-0.350	-0.345	**-0.354**	-0.327	-0.295
Systolic BP	0.189	-0.002[3]	0.205	0.175	**0.218**	0.168	0.138
Diastolic BP	0.220	0.012[3]	**0.230**	0.198	0.228	0.187	0.168
HOMA-IR[2]	0.497	-0.005[3]	0.557	0.570	**0.572**	0.517	0.362
HbA_{1c}[2]	0.178	-0.044[3]	0.218	0.249	**0.261**	0.214	0.123
FPG[2]	0.187	-0.093	0.254	0.260	**0.267**	0.219	0.122
FEMALES							
Triglycerides[2]	0.306	-0.033[3]	0.326	0.342	**0.404**	0.281	0.205
HDL-C	-0.283	0.074	-0.314	-0.301	**-0.364**	-0.265	-0.172
Systolic BP	0.148	-0.030[3]	**0.163**	0.135	0.161	0.126	0.078
Diastolic BP	0.172	-0.019[3]	**0.186**	0.136	0.170	0.149	0.081
HOMA-IR[2]	0.516	-0.052[3]	0.550	0.493	**0.574**	0.462	0.288
HbA_{1c}[2]	0.202	-0.029[3]	0.220	0.208	**0.256**	0.177	0.103
FPG[2]	0.281	-0.017[3]	0.298	0.303	**0.347**	0.268	0.183

[1]Adjusted for age.
[2]Log transformed. All correlation coefficients are significant (P<0.05) except:
[3]P>0.05. The index associated with the highest correlative strength to the variable in the same row is highlighted.

Table 11—*Tests of calibration, goodness-of-fit and discrimination for index models to detect subjects with type 2 diabetes.*

Model[1]	HL x^2 (P value)	LR x^2 (P value)	AUC (95% CI)	IDI (95% CI)
BMI alone	4.39 (0.82)	919.38 (<0.001)	0.74 (0.70-0.78)	-
BMI and WC midway	2.32 (0.97)	900.78 (<0.001)	0.75 (0.71-0.79)[2]	0.0177 (0.002-0.0334)
BMI and WC rib	5.01 (0.76)	877.54 (<0.001)	0.78 (0.74-0.82)[3]	0.0283 (0.0111-0.0455)
BMI and Rib/height ratio	5.34 (0.72)	858.75 (<0.001)	0.80 (0.76-0.84)[4]	0.0364 (0.0162-0.0566)
BMI and Rib/pelvis ratio	6.58 (0.58)	860.73 (<0.001)	0.79 (0.75-0.82)[5]	0.0290 (0.0135-0.0445)

[1]All models include age and gender.
[2]P value=0.127 compared to model with BMI alone.
[3]P value=0.003 compared to model with BMI alone.
[4]P value<0.001 compared to model with BMI alone.
[5]P value<0.001 compared to model with BMI alone.

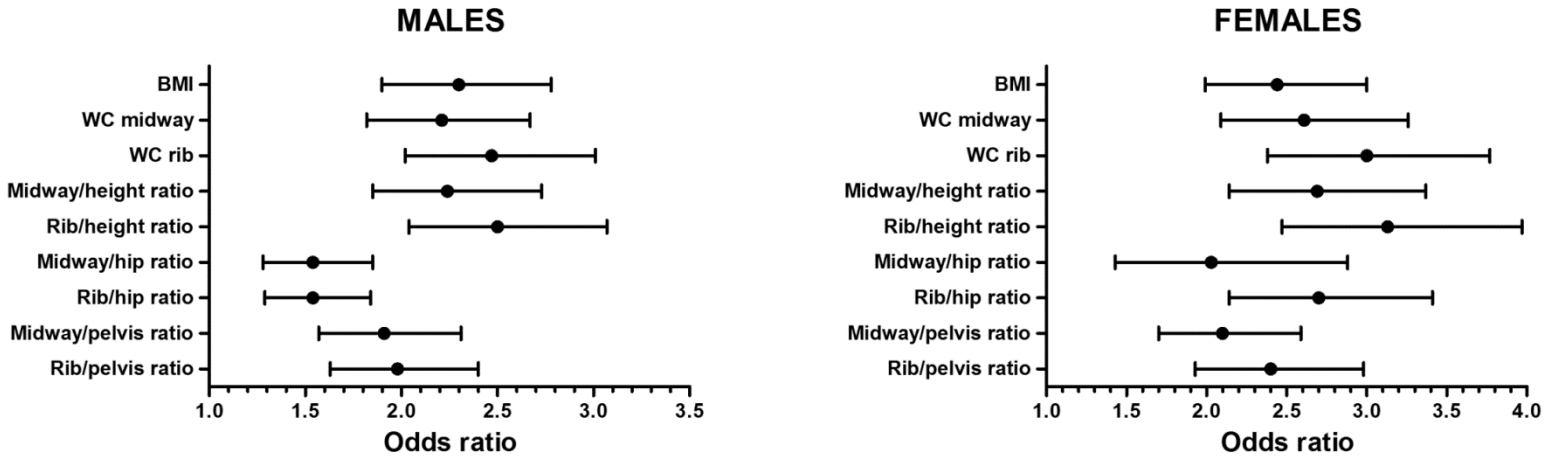

Figure 6—*Odds ratios (95% CI) of having three or more cardiometabolic risk features for a one standard deviation increase in each adiposity measure.* Results are stratified by gender and adjusted for age. All models exclude subjects with type 2 diabetes.

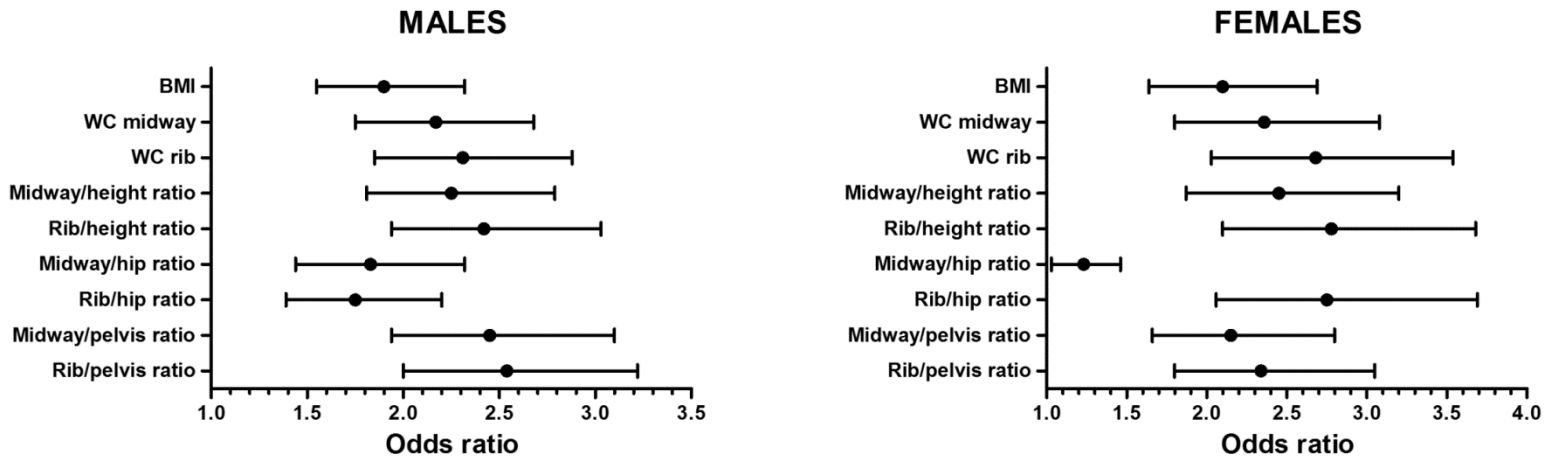

Figure 7—*Odds ratios (95% CI) of having type 2 diabetes for a one standard deviation increase in each adiposity measure.* Results are stratified by gender and adjusted for age.

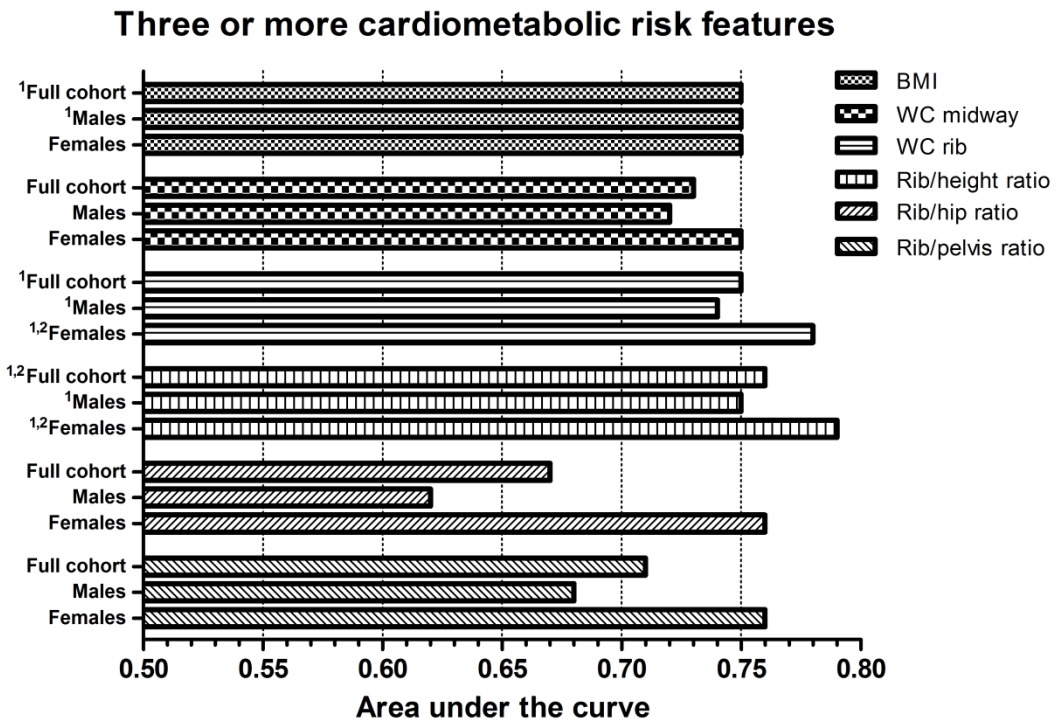

Figure 8—*Adjusted area under the receiver operating characteristic curve values for selected adiposity measures to discriminate subjects with three or more cardiometabolic risk features.* Bars represent AUC values. All models exclude subjects with type 2 diabetes. Statistical differences in the AUC values are shown in superscript Arabic numbers as: [1]P<0.05 compared to WC midway; [2]P<0.05 compared to BMI.

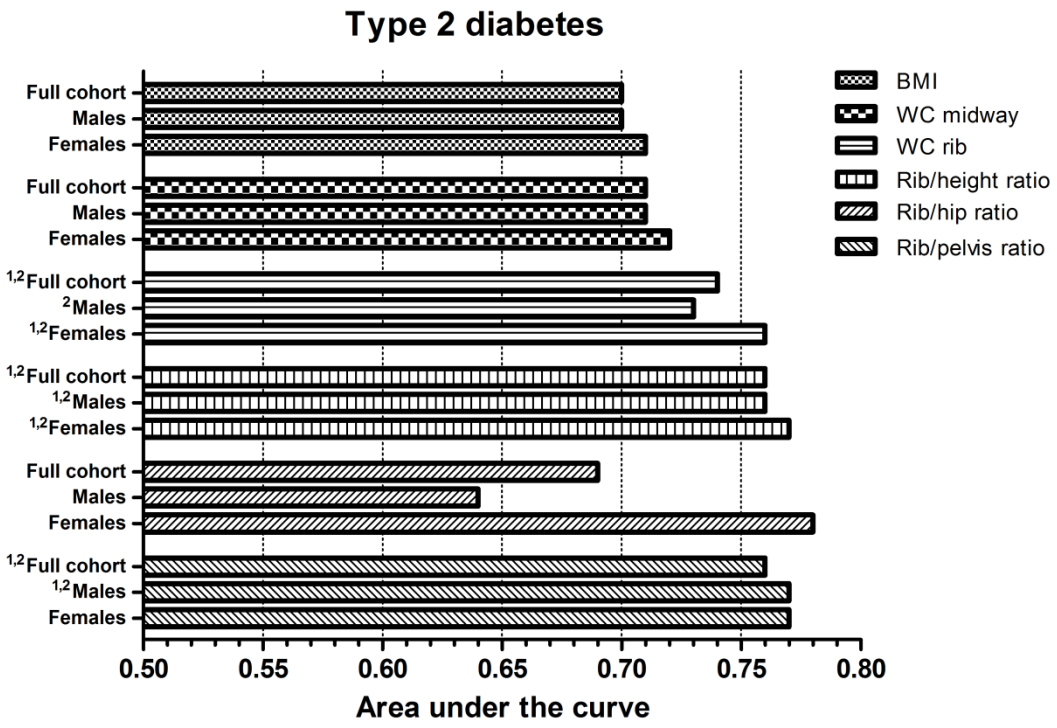

Figure 9—*Adjusted area under the receiver operating characteristic curve values for selected adiposity measures to discriminate subjects with type 2 diabetes.* Bars represent AUC values. Statistical differences in the AUC values are shown in superscript Arabic numbers as: [1]P<0.05 compared to WC midway; [2]P<0.05 compared to BMI.

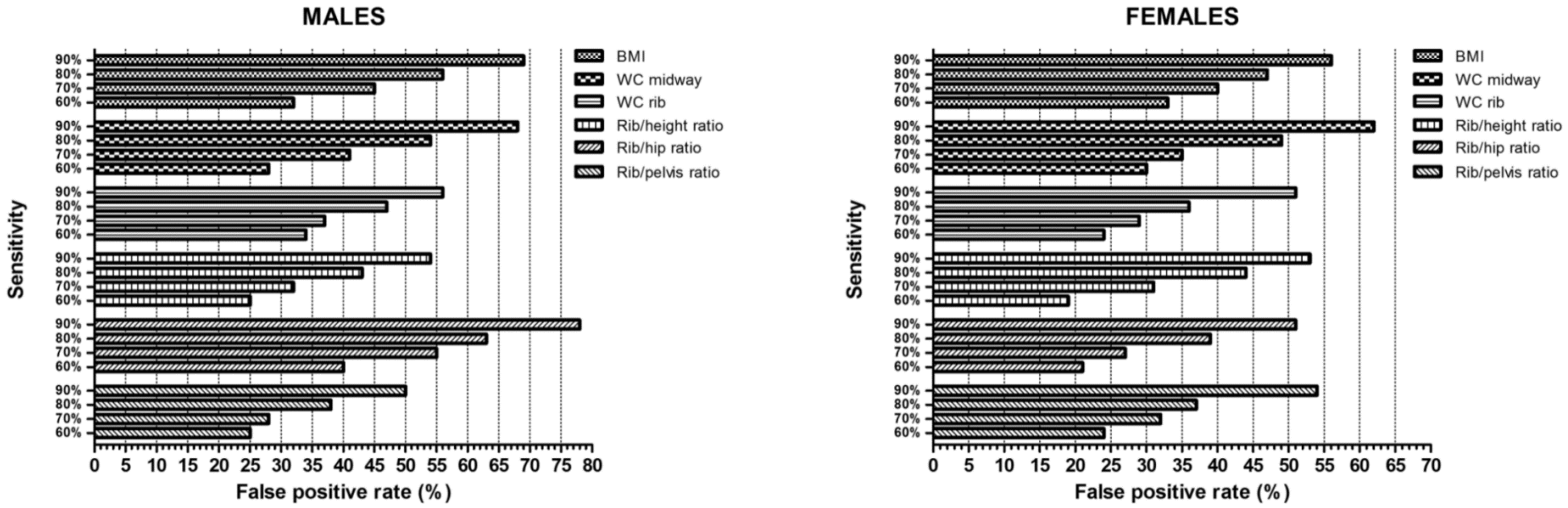

Figure 10—*False positive rates corresponding to 90%, 80%, 70% and 60% sensitivities for selected adiposity measures to classify subjects with type 2 diabetes.* Results are stratified by gender and adjusted for age. Bars represent false positive rates (percentages).

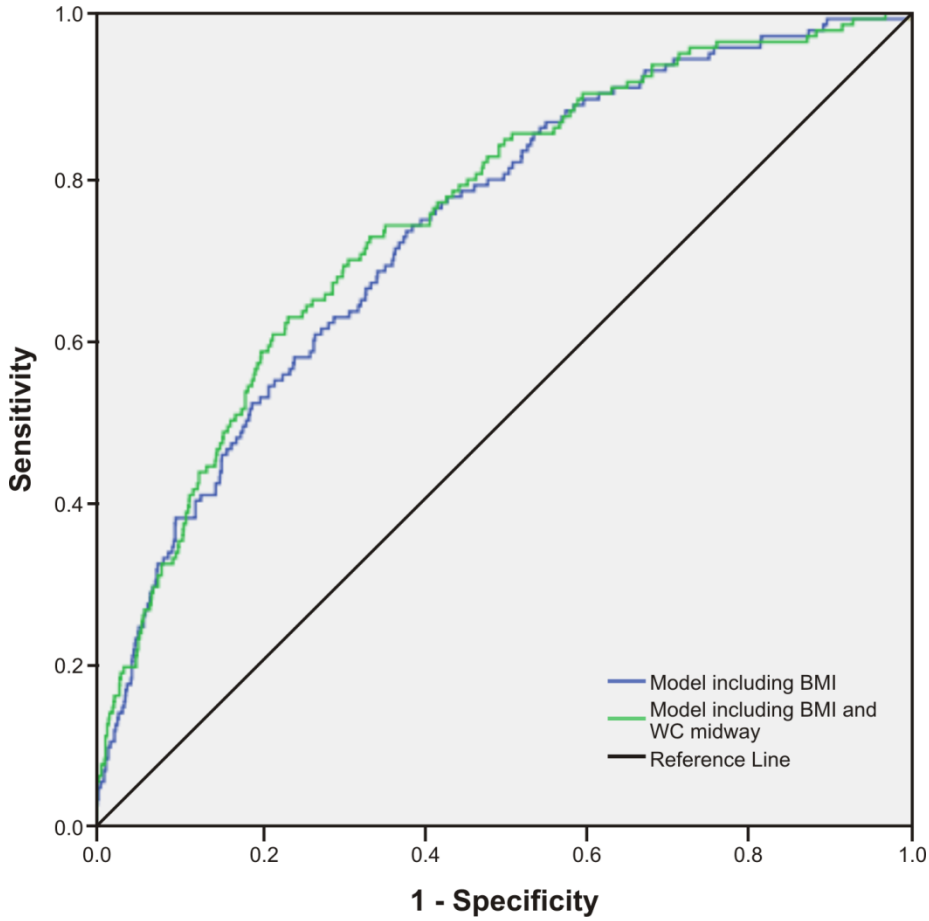

Figure 11—*Receiver operating characteristic curves for index models to discriminate subjects with type 2 diabetes.* The figure shows ROC curves for a model including BMI and a model including BMI and WC midway. All models include age and gender.

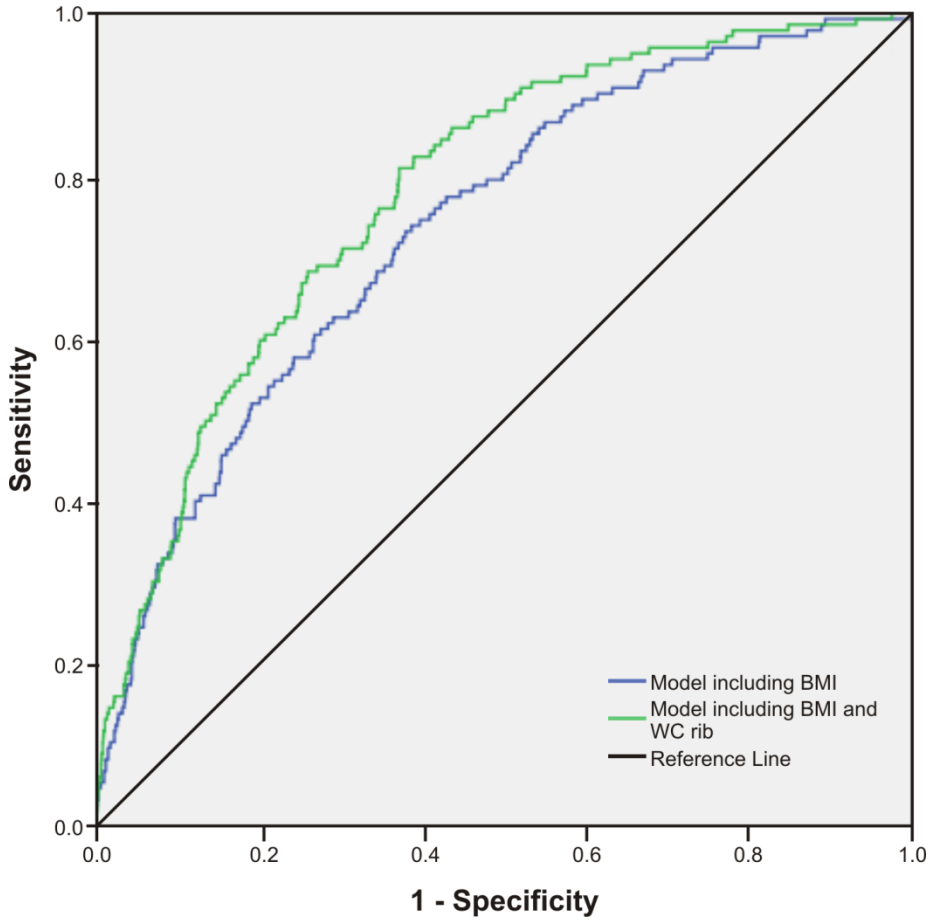

Figure 12—*Receiver operating characteristic curves for index models to discriminate subjects with type 2 diabetes.* The figure shows ROC curves for a model including BMI and a model including BMI and WC rib. All models include age and gender.

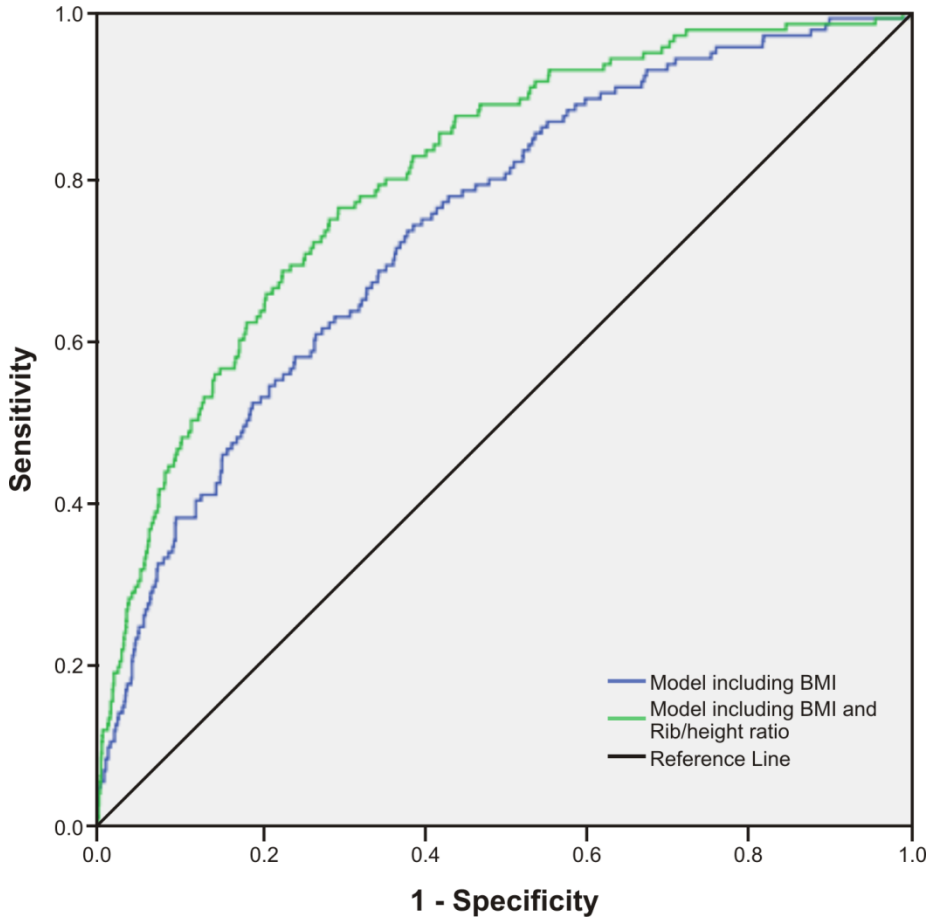

Figure 13—*Receiver operating characteristic curves for index models to discriminate subjects with type 2 diabetes.* The figure shows ROC curves for a model including BMI and a model including BMI and Rib/height ratio. All models include age and gender.

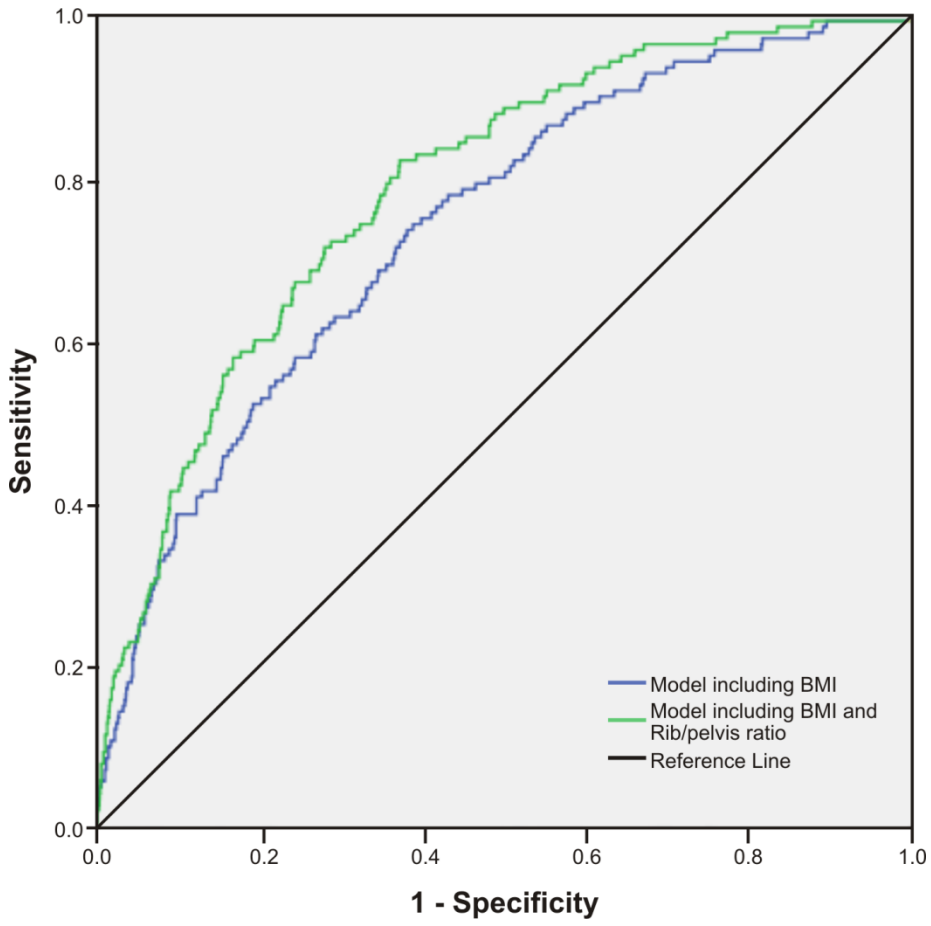

Figure 14—*Receiver operating characteristic curves for index models to discriminate subjects with type 2 diabetes.* The figure shows ROC curves for a model including BMI and a model including BMI and Rib/pelvis ratio. All models include age and gender.

CHAPTER 5

GENERAL AND CENTRAL OBESITY MEASUREMENT ASSOCIATIONS WITH MARKERS OF CHRONIC LOW-GRADE INFLAMMATION AND TYPE 2 DIABETES

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CATHERINE M. PHILLIPS

5.0 Abstract

Background and Objectives

Central obesity defined by waist circumference (WC) measurement is thought to be more strongly related to markers of chronic low-grade inflammation compared to general obesity characterised by body mass index (BMI). However, evidence for this association is still unclear. In this study we compare biomarker relationships with BMI and WC measures, and type 2 diabetes. We examine a range of pro-inflammatory cytokines, acute-phase response proteins, coagulation factors, white blood cell counts and a combination of these markers to determine which anthropometric measure is more strongly associated with diabetes-related chronic inflammation.

Materials and Methods

This was a cross-sectional study involving a random sample of 2,002 men and women aged 46-73 years. Correlation and logistic regression analyses were used to explore general and central adiposity relationships with non-optimal biomarker levels, biomarker combinations and type 2 diabetes.

Results

Waist circumference was more strongly related to a majority of the examined biomarkers of inflammation, adverse biomarker clustering and type 2 diabetes. Associations between markers of inflammation and diabetes were reduced in analyses which adjusted for adiposity variables, with models

including WC showing the greatest attenuation. In a multivariable analysis which included four or more inflammatory markers and which adjusted for age, gender, use of anti-inflammatory medications, physical activity, smoking, alcohol use and metabolic syndrome features, only WC remained significantly associated with type 2 diabetes (OR: 2.19, 95% CI: 1.34-3.58, P=0.002) compared to BMI (OR: 0.65, 95% CI: 0.40-1.03, P=0.069). The relationship between low-grade inflammation and diabetes also persisted (OR: 3.73, 95% CI: 1.97-7.05, P<0.001).

Conclusions

These data suggest that central obesity defined by WC is more strongly associated with obesity-induced inflammation and type 2 diabetes than BMI, and that central adiposity accounts for a greater variance of diabetes-related systemic inflammation. However, our results also imply that relationships between biomarkers of chronic low-grade inflammation and diabetes cannot be completely explained by surrogate measures of adiposity.

5.1 Introduction

Excess body fat has been shown to be a strong risk factor for type 2 diabetes and related cardiovascular complications, partly due to its influence on the prevalence of diabetes-related features such as hypertension, dyslipidaemia and insulin resistance [255]. Increasing evidence has also identified a low-grade but chronic inflammatory state as a potential mechanism linking adipose tissue expansion with cardiometabolic abnormalities [232].

Body mass index (BMI) has traditionally been the chosen surrogate method used to assess body fat. However, as a measure of general obesity, BMI is unable to distinguish between fat and lean mass and elevated BMI may not always indicate higher levels of adiposity or increased cardiometabolic risk [64,98,243].

Research suggests that central obesity may be a more important metabolic health indicator and waist circumference (WC) measurement has been recommended as a method for central obesity assessment [79,83]. Compared with BMI, central adiposity is thought to be more strongly correlated with visceral adipose tissue (VAT) [83]. Proteins released from VAT may contribute to cardiometabolic disease development [79], and increased VAT levels have been shown to be associated with dyslipidaemia, hypertension and diabetes [75,76]. Though numerous studies have suggested WC to be a greater risk factor than BMI for type 2 diabetes [167,246], the mechanism for this association is still unclear.

The aim of this study was to compare inflammatory biomarker relationships with BMI and WC measures and type 2 diabetes. In particular, we examined a range of pro-inflammatory cytokines, acute-phase response proteins, coagulation factors, white blood cell (WBC) counts, and a combination of these markers, to determine whether general or central adiposity is more strongly associated with diabetes-related chronic inflammation. Specifically, we hypothesised that WC would demonstrate stronger associations with markers of inflammation, and that in statistical models which examined relationships between biomarkers and type 2 diabetes, the inclusion of WC would more substantially attenuate observed effects compared to BMI.

5.2 Materials and Methods

5.2.1 Study design

The study design is described in detail in Chapter 1. In summary, the Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000, with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending patients in the 46-73 year age group. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2%

male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). Details regarding the study design, sampling procedures and methods of data collection have been reported previously [183].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

5.2.2 Clinical and laboratory procedures

Study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, use of medications with anti-inflammatory properties (aspirin/statins), physician-diagnosed diabetes, diabetes and blood pressure (BP) medication use and smoking/alcohol behaviours were gathered through a self-completed General Health Questionnaire (GHQ) [170]. Physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ) [171]. Three independent measurements of systolic and diastolic BP were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP.

Glycaemic status and biomarker, lipid and lipoprotein levels were measured by Cork University Hospital Biochemistry Laboratory using standardised procedures. Fasting plasma glucose (FPG) concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium]. Tumour necrosis factor alpha (TNF-α), c-reactive protein (CRP), interleukin 6 (IL-6), adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1) and serum insulin were assessed using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Complement component 3 (C3) was measured by immunoturbidimetric assay (RX Daytona; Randox Laboratories). White blood cell counts were determined by flow cytometry technology as part of a full blood count. Triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were measured on Olympus 5400 biochemistry analysers using Olympus reagents (Olympus Diagnostica GmbH, Hamburg, Germany).

5.2.3 Anthropometric variables

The weight and height of each subject were measured to the nearest 0.1 kg and 0.1 cm respectively by trained researchers. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was assessed using a portable Seca Leicester height/length stadiometer (Seca,

Birmingham, UK) and BMI was calculated as weight divided by the square of height. Waist circumference was measured immediately below the lowest rib at the mid-axillary line. Participants were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. Two independent WC readings were taken and the mean of the two was used in analysis.

5.2.4 Classification of biochemical and blood pressure measurements

According to American Diabetes Association guidelines, type 2 diabetes was defined as HbA_{1c} $\geq$6.5% ($\geq$48 mmol/mol) or FPG $\geq$7.0 mmol/l [23]. Individuals on insulin therapy and subjects indicating a diagnosis of diabetes (either self-reported physician diagnosis or prescription diabetes medication use), but who did not have positive HbA_{1c} or FPG test results, were excluded from analysis (N=45).

As internationally agreed risk cut-points for the examined biomarkers have not been established, low-grade inflammation was determined as a level equal to or above the 75th percentile in the study population for each biomarker (C3, CRP, IL-6, TNF-α, resistin, PAI-1 and WBC) with the exception of adiponectin (equal to or below the 25th percentile). Stepwise forwards and backwards entry elimination logistic regressions were performed to examine non-optimal inflammatory marker associations with type 2 diabetes. Model goodness-of-fit was assessed using the likelihood ratio. Biomarkers selected employing these procedures were used to

construct two variables: (1) *three or more markers* and (2) *four or more markers*.

Lipid, lipoprotein and BP measurements were classified according to National Cholesterol Education Program: Adult Treatment Panel III guidelines [184]. Metabolic syndrome (MetS) features were defined as high triglycerides $\geq$1.7 mmol/l and low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females). High BP was classified as systolic BP $\geq$130 mmHg and/or diastolic BP $\geq$85 mmHg or anti-hypertensive medication use. Insulin resistance was defined as a serum insulin level equal to or above the 75th percentile in the study sample.

5.2.5 Lifestyle data

Lifestyle variables utilised from the IPAQ and GHQ included physical activity levels, smoking status and alcohol use. Self-reported physical activity within the previous six months was collapsed into two categories: *high or moderate* and *no physical exercise*. Subjects were considered to have ever smoked if they smoked cigarettes during the recruitment phase, had smoked within the last 10 years or had smoked more than 100 cigarettes in their lifetime. Alcohol use was assessed by asking study participants how often they consumed alcohol on a monthly or weekly basis, and was dichotomised as follows: “never or less than once a month” and “2-4 times monthly” = *occasional drinker* and “twice or more weekly” = *regular drinker*.

5.2.6 Statistical analysis

Data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) for Windows. Dichotomous features are presented as percentages and continuous data are shown as a mean, plus or minus one standard deviation. Baseline characteristics according to type 2 diabetes status were assessed using linear or logistic models, adjusting for gender. Age is shown as a median and interquartile range with a P value according to a Mann-Whitney U test. The relationships between log transformed biomarkers and anthropometric variables were investigated using partial correlations.

All obesity measures were gender standardised. Logistic regression was employed to examine general and central adiposity associations with non-optimal biomarker levels, biomarker combinations and type 2 diabetes. A final multivariable model explored adverse inflammatory marker clustering relationships with type 2 diabetes adjusting for age, gender, BMI, WC, use of anti-inflammatory medications, physical activity, smoking, alcohol use and MetS features. Seven subjects were excluded from analysis due to missing anthropometric data. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance.

5.3 Results

5.3.1 Descriptive characteristics

Characteristics of the study population according to type 2 diabetes status are shown in Table 12. Subjects with diabetes were significantly more likely to be male, were older, had higher BMI and WC levels and were less likely to be physically active. Prevalence differences were noted for MetS features and higher concentrations of C3, CRP, IL-6, TNF-α, resistin, PAI-1, WBC and for lower levels of adiponectin. A significantly higher percentage of diabetic subjects also displayed non-optimal biomarker clustering, with 53.5% and 22.5% having three or more and four or more adverse inflammatory markers respectively.

5.3.2 Partial correlations between anthropometric variables and log transformed biomarkers

After adjustment for age and gender (Table 13), both BMI and WC were significantly and positively correlated with CRP, IL-6, TNF-α, resistin and PAI-1 concentrations. Significant inverse relationships were noted with adiponectin. Correlations were stronger between WC and a majority of biomarkers with the exception of CRP and resistin.

5.3.3 Associations between adiposity measures and biomarkers and type 2 diabetes

The results from logistic regression analyses examining general and central obesity measurement relationships with individual inflammatory markers are presented in Table 14. After adjustment for age and gender, both BMI and WC displayed positive associations with unfavourable biomarker levels. In models which adjusted for BMI and other features, WC remained significantly associated with high C3, TNF-α, WBC and with low levels of adiponectin, whereas significant relationships with CRP and IL-6 were observed for BMI.

Odds ratios of having adverse biomarker clustering and type 2 diabetes are shown in Figure 15. Both adiposity measures displayed similar attenuation after adjustment. Central adiposity defined by WC was more strongly related to each outcome. Although confidence intervals were wide as a result of the small number of cases within each category, in fully adjusted models which examined three or more markers, four or more markers and type 2 diabetes, odds ratios for a one standard deviation increase of WC were 1.96 (95% CI: 1.69-2.26), 2.53 (95% CI: 2.02-3.18) and 2.17 (95% CI: 1.78-2.64) respectively. The corresponding odds ratios for BMI were 1.84 (95% CI: 1.61-2.12), 2.08 (95% CI: 1.70-2.55) and 1.77 (95% CI: 1.48-2.12).

5.3.4 Relationships between adverse biomarkers and type 2 diabetes

In logistic regression models which examined biomarker clustering relationships with type 2 diabetes (Table 15), having three or more and four

or more adverse inflammatory markers were strongly associated with diabetes; OR: 7.63 (95% CI: 5.31-10.95) and OR: 8.12 (95% CI: 5.02-13.15) respectively. However, relationships between markers of inflammation and type 2 diabetes were reduced in analyses which adjusted for BMI or WC, with models including WC showing the greatest attenuation. Models which adjusted for both adiposity variables together did not display any further attenuation. In a multivariable analysis which included four or more inflammatory markers and which adjusted for age, gender, use of anti-inflammatory medications, physical activity, smoking, alcohol use and MetS features, only WC remained significantly associated with type 2 diabetes (OR: 2.19, 95% CI: 1.34-3.58, P=0.002) compared to BMI (OR: 0.65, 95% CI: 0.40-1.03, P=0.069). The relationship between low-grade inflammation and diabetes also persisted (OR: 3.73, 95% CI: 1.97-7.05, P<0.001).

5.4 Discussion

The aim of this study was to determine whether general or central obesity is more strongly associated with diabetes-related systemic low-grade inflammation. Our results demonstrate that WC was more strongly related to a majority of the examined biomarkers of inflammation, adverse biomarker clustering and type 2 diabetes. These findings also suggest that when compared to general obesity characterised by BMI, a surrogate measure of central adiposity such as WC may provide additional information to help identify individuals at risk of obesity-related chronic disorders.

At a population level, it has been consistently shown that risk of type 2 diabetes development is strongly correlated with an increase in BMI [6,233]. However, though straightforward to measure and easy to calculate, limitations regarding the use of BMI as a method for adiposity appraisal have been widely acknowledged [99,243]. Within a narrow range of BMI levels, individuals may vary considerably with respect to insulin resistance and other MetS features [64,233]. These inter-individual differences have been attributed to variations in body fat distribution and research has indicated that general obesity categorisation based on BMI might be inadequate [61,99].

Compared with BMI, central obesity is thought to be more strongly associated with cardiometabolic disease [19,79,83]. Waist circumference measurement has been adopted by the International Diabetes Federation as a mandatory component for diagnosing the MetS [19] and is the only adiposity variable included in four alternative MetS definitions [21]. Nevertheless, although numerous research has suggested central obesity to be a greater risk factor for type 2 diabetes [246], several studies have also shown that BMI and WC demonstrate similar relationships with cardiometabolic disease markers such as hypertension and dyslipidaemia [139,157,246]. This implies that the association between central adiposity and diabetes may also be explained by other metabolic processes [73].

Over the past three decades, cardiometabolic disease, type 2 diabetes and cardiovascular disorders have been increasingly recognised as inflammatory diseases [7,231,233]. It is currently well accepted that excess adiposity

promotes a state of chronic low-grade inflammation which may be reflected not only in an increased production of pro-inflammatory cytokines, but also in higher levels of acute-phase response proteins, coagulation factors, macrophages and other immune cells [232,233]. Although the exact mechanism between obesity-induced inflammation and type 2 diabetes is still poorly understood, it is hypothesised that cytokines and select proteins secreted by adipose cells may promote a low-grade inflammatory response in adipose and vascular tissue [79,83], thus leading to insulin resistance and β-cell and microvascular dysfunction [233].

Importantly, it has been acknowledged that not all body fat may be harmful and that expansion of fat depots will not necessarily lead to an inflammatory response, insulin resistance or type 2 diabetes [233]. It has also been shown that approximately 10%-30% of obese individuals do not develop insulin resistance, a phenomenon described as “metabolically healthy obese” [256,257]. Though only comprising 10%-15% of total body fat [233], VAT is commonly believed to be a greater risk factor for vascular dysfunction compared to subcutaneous adipose tissue (SAT) [79,83]. Visceral fat demonstrates substantially higher fatty acid fluxes compared to SAT, which may contribute to insulin resistance and β-cell failure [78,258]. It is also characterised by higher secretion of pro-inflammatory cytokines, and lower secretion of adiponectin, the anti-inflammatory adipokine [231,232,259,260]. Although a number of imaging techniques exist which may allow direct quantification of VAT levels, these procedures require expensive apparatus

and specialised personnel, and surrogate measures of body composition are more frequently utilised in research and clinical settings [246].

Consistent with other research, our study also found central obesity to be more strongly related to a majority of the examined markers of inflammation [255] and type 2 diabetes [127,167,246] than BMI, perhaps reflecting a higher correlation between WC measurement and VAT. In particular, WC was more strongly associated with adverse biomarker combinations of C3, IL-6, adiponectin, resistin and WBC concentrations which were found to be strongly related to diabetes. Although individual analysis demonstrated IL-6 and resistin to have comparable associations with both adiposity measures, WC was more strongly related to C3, adiponectin and WBC levels.

Elevated concentrations of C3 have been shown to be correlated with insulin, glucose, insulin resistance and associated with an increased risk of type 2 diabetes [261,262]. It has been suggested that C3 may be a stronger inflammatory marker of insulin resistance than CRP, the acute-phase response protein that is more commonly assessed in epidemiological research [263]. Low levels of adiponectin have also been demonstrated to be related to diabetes development [264]. A recent meta-analysis involving 41,841 subjects showed that adiponectin levels in pre-diabetes patients were lower than that of healthy controls, indicating that the level of circulating adiponectin decreases before the onset of diabetes [265]. In addition, data from mouse models suggest that increased adiponectin levels promote metabolic flexibility of adipose tissue [266]. White blood cell counts, a non-

specific marker of inflammation, have been shown to be an independent risk factor for type 2 diabetes in subjects with increased adiposity. It has also been observed that overweight and obese individuals with relatively low WBC counts have a significantly lower risk of developing diabetes than those with higher levels of leukocytes [267].

In our study it was noted that measured effects in models examining biomarker clustering relationships with type 2 diabetes demonstrated greater attenuation when WC was included compared to BMI. In addition, models which included both adiposity variables together did not display any further attenuation. This suggests that central adiposity may account for a greater variance of diabetes-related systemic inflammation. Although adjusting for BMI and WC together causes problematic issues relating to co-linearity, we found WC to remain a significant risk factor for diabetes even after adjustment for BMI, inflammatory markers and other features.

Nevertheless, both BMI and WC were significantly associated with each individual biomarker, and type 2 diabetes, when examined separately. Moreover, these findings also imply that the relationship between chronic low-grade inflammation and type 2 diabetes cannot be completely explained by surrogate measures of adiposity. Though our results indicate that WC measurement may be a more accurate marker of VAT, previous research by Bosy-Westphal et al. [102] showed that WC was more strongly correlated with SAT than visceral fat. In addition to our results, this finding suggests that alternative adiposity measurement procedures may be needed to more

exactly detect the presence and intensity of the micro-inflammatory process. Equally, it must be allowed that other features apart from adiposity, such as diet [268] or other life-style factors, may also contribute to pathogenesis of the obesity-related chronic inflammatory response.

5.4.1 Limitations of the research

Although our findings are of potential public health and clinical significance in terms of adiposity measurement as a method for assessing cardiometabolic health, several limitations should be considered when examining results from this study. Though we adjusted for age, use of anti-inflammatory medications, physical activity, smoking, alcohol use and MetS features, the possibility that the relationships we observed may be influenced by other factors cannot be discounted. Additionally, given the modest number of outcomes within our sample, we did not stratify models by sex, although the gender variable was accounted for in analysis. Also, cross-sectional data precludes examination of the temporal relationship between variables. Therefore, although our results may suggest associations between adiposity measures, inflammatory markers and type 2 diabetes, they do not indicate the direction of these relationships. Importantly, it has been suggested that in addition to being a mediator, chronic low-grade inflammation is a predictor of weight gain [269,270], and that inflammation may be a common cause of both obesity, and through a separate mechanism, type 2 diabetes [271]. Equally important to consider is that our data were derived from a single primary care based sample. However, random sampling of subjects and the

use of validated methods for data collection ensured internal sample validity and the relationships described may be generalisable to a similar middle-aged population.

Also of concern is that we defined chronic low-grade inflammation according to a range of metabolic markers using arbitrary cut-points. The inflammatory response is complex and involves numerous cytokines, acute-phase reactants and other circulating factors. Consequently it is not clear if one particular inflammatory marker, or specific combination of markers, best reflects the underlying inflammatory state [272]. We classified chronic low-grade inflammation according to a clustering of biomarkers that were selected using forwards and backwards entry elimination regression analyses. It should be noted that the variables selected were the same biomarkers that displayed the strongest associations with both pre-diabetes and type 2 diabetes in individual analyses employing a lower threshold value (Chapter 3). In addition, we conducted a sensitivity analysis which only included the pro-inflammatory cytokines IL-6 and TNF-α. After adjustment for adiposity measures and other variables, similar relationships were observed (Appendix 3, Supporting Table 2). Nevertheless, further longitudinal research will be needed to determine which biomarkers and thresholds most accurately describe the pro-inflammatory condition associated with type 2 diabetes development and obesity-related chronic disorders.

5.5 Conclusions

In summary, our results suggest that central obesity characterised by WC measurement is more strongly associated with diabetes-related chronic low-grade inflammation and type 2 diabetes compared to general obesity defined by BMI. These findings add to the increasing evidence indicating that central adiposity assessment may provide a useful method for evaluating cardiometabolic health. Earlier identification of patients at increased risk could enable earlier targeted interventions or therapies, thus attenuating development of type 2 diabetes and related conditions.

Table 12—*Characteristics of the study population according to type 2 diabetes status.*

Feature	Diabetes (N=142)	No diabetes (N=1860)	P value
Male	92 (64.8)	874 (47.8)	<0.001
Age	61 (57-65)	59 (54-64)	0.001
BMI (kg/m^2)[1]	32.1 ± 5.6	28.3 ± 4.5	<0.001
WC (cm)[1]	104.5 ± 13.1	91.3 ± 12.9	<0.001
On aspirin[1]	62 (43.7)	248 (13.6)	<0.001
On statin medications[1]	83 (58.5)	595 (32.6)	<0.001
No physical exercise[1]	45 (38.5)	309 (19.2)	<0.001
Smoker[1]	78 (54.9)	865 (47.3)	0.246
Regular drinker[1]	38 (27.7)	604 (34.6)	0.013
High C3[1]	71 (51.1)	413 (23.1)	<0.001
High CRP[1]	47 (33.6)	437 (24.3)	0.007
High IL-6[1]	71 (50.7)	412 (22.9)	<0.001
High TNF-α[1]	58 (41.4)	425 (23.6)	<0.001
Low adiponectin[1]	70 (50.0)	418 (23.2)	<0.001
High resistin[1]	54 (38.6)	428 (23.8)	<0.001
High PAI-1[1]	48 (34.3)	435 (24.3)	0.029
High WBC[1]	74 (52.5)	401 (22.2)	<0.001
Three or more markers[1]	76 (53.5)	223 (12.2)	<0.001
Four or more markers[1]	32 (22.5)	59 (3.2)	<0.001
High triglycerides[1]	66 (47.5)	409 (22.8)	<0.001
Low HDL-C[1]	61 (43.0)	271 (15.0)	<0.001
High BP[1]	118 (83.7)	1084 (59.5)	<0.001
Insulin resistance[1]	89 (63.6)	397 (22.0)	<0.001

Mean and ± standard deviation are shown for BMI and WC. Age is shown as a median (interquartile range). % are shown for categorical values. Numbers and (%) may vary as some variables have missing values.

Biomarker combinations: C3, IL-6, adiponectin, resistin and WBC.

[1]P value adjusted for gender.

Table 13—*Partial correlations between anthropometric variables and log transformed biomarkers, adjusted for age and gender.*

Marker	BMI	WC
C3	0.261	**0.275**
CRP	**0.327**	0.325
IL-6	0.252	**0.264**
TNF-α	0.126	**0.144**
Adiponectin	-0.277	**-0.323**
Resistin	**0.117**	0.112
PAI-1	0.133	**0.141**
WBC	0.183	**0.225**

All correlation coefficients are significant ($P<0.05$). The index associated with the highest correlative strength to the variable in the same row is highlighted.

Table 14—*Odds ratios (95% CI) of having non-optimal levels in each biomarker for a one standard deviation increase of BMI and WC.*

Marker	Index	Either BMI or WC[1]	Both BMI and WC[1]	Fully Adjusted Model[2]
		Odds ratios (95% CI)		
High C3				
	BMI	2.21 (1.96-2.48)[3]	1.11 (0.87-1.41)	1.16 (0.89-1.52)
	WC	2.44 (2.15-2.76)[3]	2.23 (1.73-2.87)[3]	2.16 (1.63-2.85)[3]
High CRP				
	BMI	1.90 (1.70-2.12)[3]	1.61 (1.27-2.04)[3]	1.74 (1.34-2.27)[3]
	WC	1.84 (1.65-2.06)[3]	1.20 (0.95-1.53)	1.15 (0.89-1.50)
High IL-6				
	BMI	1.59 (1.43-1.77)[3]	1.27 (1.01-1.61)[3]	1.33 (1.03-1.72)[3]
	WC	1.61 (1.44-1.79)[3]	1.29 (1.02-1.64)[3]	1.16 (0.89-1.51)
High TNF-α				
	BMI	1.29 (1.16-1.43)[3]	0.92 (0.73-1.17)	0.93 (0.72-1.22)
	WC	1.36 (1.22-1.51)[3]	1.46 (1.15-1.85)[3]	1.40 (1.07-1.82)[3]
Low adiponectin				
	BMI	1.59 (1.42-1.78)[3]	0.93 (0.71-1.21)	0.92 (0.69-1.23)
	WC	1.71 (1.52-1.93)[3]	1.83 (1.40-2.40)[3]	1.79 (1.34-2.41)[3]
High resistin				
	BMI	1.23 (1.11-1.36)[3]	1.18 (0.94-1.48)	1.10 (0.85-1.41)
	WC	1.21 (1.09-1.35)[3]	1.05 (0.83-1.32)	1.11 (0.86-1.43)
High PAI-1				
	BMI	1.30 (1.17-1.44)[3]	1.07 (0.85-1.35)	1.17 (0.90-1.52)
	WC	1.32 (1.19-1.47)[3]	1.24 (0.98-1.57)	1.17 (0.90-1.52)
High WBC				
	BMI	1.29 (1.16-1.43)[3]	0.81 (0.64-1.02)	0.96 (0.74-1.24)
	WC	1.39 (1.25-1.55)[3]	1.69 (1.34-2.15)[3]	1.38 (1.06-1.79)[3]

[1]Adjusted for age and gender.

[2]Adjusted for age, gender, use of anti-inflammatory medications, physical activity, smoking and alcohol use.

[3]P<0.05.

Table 15—*Relationships between adverse biomarkers and type 2 diabetes adjusting for either BMI, WC, or both.*

Feature	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
	Odds ratios (95% CI)					
Three or more markers	7.63 (5.31-10.95)[2]	5.58 (3.82-8.15)[2]	4.92 (3.35-7.23)[2]	5.00 (3.40-7.36)[2]	4.39 (2.81-6.88)[2]	3.49 (2.16-5.64)[2]
BMI[1]		1.64 (1.39-1.93)[2]		0.67 (0.45-0.98)[2]	0.67 (0.44-1.04)	0.62 (0.38-1.00)
WC[1]			2.01 (1.67-2.40)[2]	2.94 (1.96-4.41)[2]	2.73 (1.73-4.31)[2]	2.27 (1.37-3.76)[2]
	Odds ratios (95% CI)					
Four or more markers	8.12 (5.02-13.15)[2]	5.18 (3.12-8.59)[2]	4.31 (2.57-7.24)[2]	4.32 (2.57-7.27)[2]	4.59 (2.50-8.43)[2]	3.73 (1.97-7.05)[2]
BMI[1]		1.78 (1.52-2.09)[2]		0.71 (0.49-1.02)	0.72 (0.48-1.07)	0.65 (0.40-1.03)
WC[1]			2.19 (1.83-2.61)[2]	3.05 (2.06-4.51)[2]	2.70 (1.74-4.17)[2]	2.19 (1.34-3.58)[2]

Biomarker combinations: C3, IL-6, adiponectin, resistin and WBC.
Model 1 adjusted for age and gender.
Model 2 adjusted for age, gender and BMI.
Model 3 adjusted for age, gender and WC.
Model 4 adjusted for age, gender, BMI and WC.
Model 5 adjusted for age, gender, BMI, WC, use of anti-inflammatory medications, physical activity, smoking and alcohol use.
Model 6 adjusted for age, gender, BMI, WC, use of anti-inflammatory medications, physical activity, smoking, alcohol use, high triglycerides, low HDL-C, high BP and insulin resistance.
[1]1 SD increase.
[2]P<0.05.

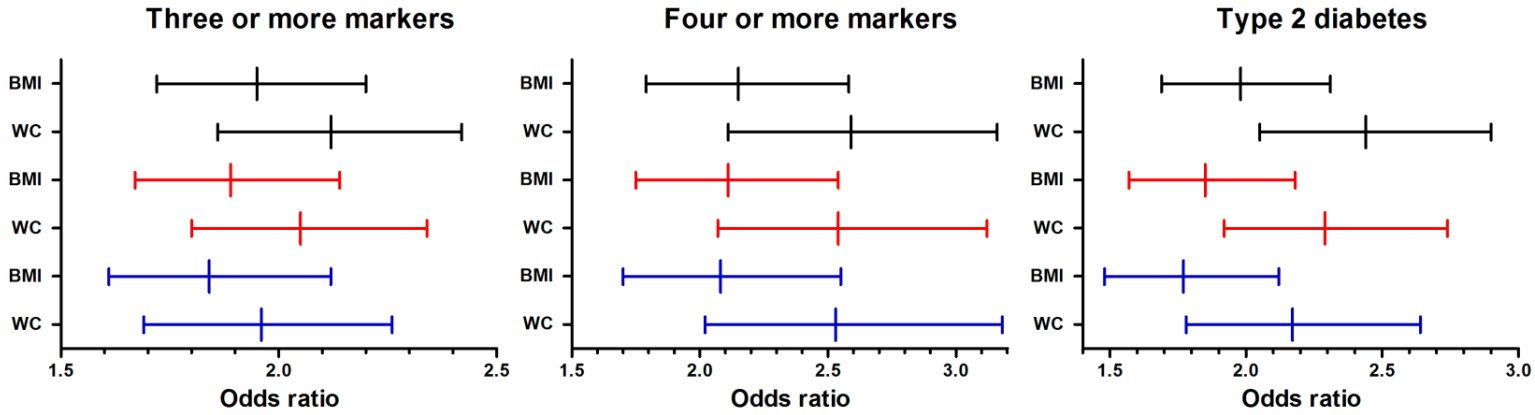

Figure 15—*Odds ratios (95% CI) of having three or more and four or more adverse biomarkers, and type 2 diabetes, for a one standard deviation increase of BMI and WC.*

CHAPTER 6

ASSESSING CARDIOMETABOLIC RISK IN MIDDLE-AGED ADULTS USING BODY MASS INDEX AND WAIST-HEIGHT RATIO – ARE CONCORDANT RESULTS BY TWO INDICES BETTER THAN DISCORDANT RESULTS? A CROSS-SECTIONAL STUDY

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6.0 Abstract

Background and Objectives

A novel obesity classification method has been proposed using body mass index (BMI) and waist-height ratio (WHtR) together. However, the utility of this approach is unclear. In this study we compare the metabolic profiles in subjects defined as overweight or obese by both measures. We examine a range of metabolic syndrome features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell counts to determine whether a combination of both indices more accurately identifies individuals at increased obesity-related cardiometabolic risk.

Materials and Methods

This was a cross-sectional study involving a random sample of 1,856 men and women aged 46-73 years. Metabolic and anthropometric profiles were assessed. Linear and logistic regression analyses were used to compare lipid, lipoprotein, blood pressure, glycaemic and inflammatory biomarker levels between BMI and WHtR tertiles. Multinomial logistic regression was performed to determine cardiometabolic risk feature associations with BMI and WHtR groupings. Receiver operating characteristic curve analysis was used to evaluate index discriminatory ability.

Results

The combination of BMI and WHtR tertiles identified consistent and significant metabolic variable differences relative to those characterised as overweight or obese discordantly by BMI and WHtR. Similarly, odds ratios of having cardiometabolic risk features were noticeably increased in subjects classified as overweight or obese by both measures. Significant discriminatory improvement, using joint measurement, was also observed for detecting individual cardiometabolic disease features and adverse biomarker levels. In a fully adjusted model, only individuals within the highest tertile for both indices displayed a significant and positive association with pre-diabetes (OR: 3.4, 95% CI: 1.9,6.0, P<0.001).

Conclusions

These data provide evidence that the use of BMI and WHtR together may improve body fat classification. Risk stratification using a composite index may provide a more accurate method for identifying high and low-risk subjects.

6.1 Introduction

Excess body fat has been shown to be associated with dyslipidaemia, hypertension, insulin resistance, chronic low-grade inflammation and the development of metabolic syndrome (MetS), type 2 diabetes and cardiovascular complications [6,165,215,232]. Numerous studies have also demonstrated a high mortality rate in subjects with a body mass index (BMI) $\geq$30 kg/m^2 [93]. But because it is a weight-for-height measure, BMI is unable to distinguish between fat and lean mass and elevated BMI may not always indicate higher levels of adiposity or increased cardiometabolic risk [64,98,243].

Evidence suggests that central obesity is a more important metabolic risk factor and waist circumference (WC) measurement has been recommended as a method for central obesity assessment [79,83]. However, as WC diagnostic thresholds are different for men and women, and may vary between ethnic groups [12], the practical utility of WC measurement has been questioned [246].

The waist-height ratio (WHtR) (WC divided by height) has been advocated as an alternative surrogate measure of central adiposity [126]. As a ratio, this index may circumvent problematic issues relating to gender or population-specific risk cut-points [125,273]. But results from studies which have compared BMI and WHtR have been inconclusive, with some showing WHtR to be only marginally superior to BMI as an indicator of obesity-related risk [127,148,156].

The prevalence of obesity has escalated in many world populations [2,5]. Thus, there is an increasing need to identify overweight and obese individuals at highest odds of developing chronic disorders. Recently, a new obesity classification method was proposed, utilising BMI in conjunction with WHtR [273]. Risk stratification using a composite index may provide a more effective method for identifying high and low-risk patients. This could allow earlier diagnosis, thus attenuating metabolic complications and chronic morbidity development.

The aim of this study was to compare the metabolic profiles in subjects defined as overweight or obese, using BMI and WHtR, in a random sample of 1,856 middle-aged men and women. In particular, we examined a range of MetS features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell (WBC) counts to determine whether a combination of both indices more accurately identifies individuals at increased obesity-related cardiometabolic risk.

6.2 Materials and Methods

6.2.1 Study design

The study design is described in detail in Chapter 1. In summary, the Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000,

with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending patients in the 46-73 year age group. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). Details regarding the study design, sampling procedures and methods of data collection have been reported previously [183].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

6.2.2 Clinical and laboratory procedures

Study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, physician-diagnosed diabetes, prescription (Rx) medication use and smoking/alcohol behaviours were gathered through a self-completed General Health Questionnaire (GHQ) [170]. Physical activity levels were assessed using the

International Physical Activity Questionnaire (IPAQ) [171]. Three independent measurements of systolic and diastolic blood pressure (BP) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP.

Triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica GmbH, Hamburg, Germany). Fasting plasma glucose (FPG) concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium]. Serum insulin, c-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), adiponectin, leptin, resistin and plasminogen activator inhibitor-1 (PAI-1) were assessed using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Complement component 3 (C3) was measured by immunoturbidimetric assay (RX Daytona; Randox Laboratories). White blood cell counts were determined by flow cytometry technology as part of a full blood count.

6.2.3 Classification of biochemical and blood pressure measurements

Patients with type 2 diabetes indicated by either HbA_{1c} levels $\geq$6.5% ($\geq$48 mmol/mol) or FPG levels $\geq$7.0 mmol/l [23], a self-reported physician diagnosis, Rx diabetes medication use, or those who were on insulin therapy, were excluded (N=184).

Lipid, lipoprotein and BP measurements were classified according to National Cholesterol Education Program: Adult Treatment Panel III guidelines [184]. Abnormal metabolic risks were defined as high triglycerides $\geq$1.7 mmol/l and low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females). Dyslipidaemia was determined according to both high triglyceride and low HDL-C levels. High BP was classified as systolic BP $\geq$130 mmHg and/or diastolic BP $\geq$85 mmHg or Rx anti-hypertensive medication use.

The Homeostasis Model Assessment Index of Insulin Resistance (HOMA-IR) [247] was derived from FPG and insulin concentrations as [(FPG x fasting serum insulin)/22.5] and insulin resistance was defined as a level equal to or above the 75^{th} percentile in the study sample. Having three or more MetS risk features ($\geq$3 metabolic features) was characterised as any combination of the following: high triglycerides, low HDL-C levels, high BP and insulin resistance. Subjects were classified as having pre-diabetes if they had both elevated HbA_{1c} levels $\geq$5.7% ($\geq$39 mmol/mol) and impaired FPG levels $\geq$5.6 mmol/l [23]. As internationally agreed risk cut-points for the examined biomarkers have not been established, low-grade inflammation was determined as a level equal to or above the 75^{th} percentile for each

biomarker (C3, CRP, IL-6, TNF-α, leptin, resistin, PAI-1 and WBC) with the exception of adiponectin (equal to or below the 25th percentile).

6.2.4 Anthropometric variables

The weight and height of each participant were measured to the nearest 0.1 kg and 0.1 cm respectively. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was measured using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and BMI was calculated as weight divided by the square of height. Waist circumference was measured immediately below the lowest rib at the mid-axillary line on bare skin. Subjects were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. Two independent readings were taken for WC and the mean of the two was used in analysis. The WHtR was calculated as WC divided by height.

Both BMI and WHtR were divided into equal tertiles. Subjects were categorised on the basis of their BMI or WHtR percentiles as *normal weight* (<33%), *overweight* (33-66%) and *obese* (>66%). In our sample these cut-points corresponded to <26.2, 26.2-29.7, >29.7 for BMI and <0.52, 0.52-0.58, >0.58 for WHtR. The BMI and WHtR groups were combined to form a 5-category variable: (1) *normal weight by both*, (2) *overweight by either*, (3) *overweight by both*, (4) *obese by either* and (5) *obese by both*. Overweight

subjects classified as obese by either index were assigned to the higher category. Seven subjects had missing anthropometric values and were excluded from statistical analysis.

6.2.5 Lifestyle data

Lifestyle variables utilised from the IPAQ and GHQ included physical activity levels, smoking status and alcohol use. Self-reported physical activity within the previous six months was collapsed into two categories: *high or moderate (N=1324)* and *no physical exercise (N=312)*. Subjects were considered to be current smokers if they smoked cigarettes during the recruitment phase of the study *(N=257).* Alcohol use was assessed by asking study participants how often they consumed alcohol on a monthly or weekly basis, and was dichotomised as follows: “never or less than once a month” and “2-4 times monthly” = *occasional drinker (N=1165)* and “twice or more weekly” = *regular drinker (N=614).*

6.2.6 Statistical analysis

Data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) and Stata SE Version 13 (Stata Corporation, College Station, TX, USA) for Windows. Descriptive characteristics were examined according to normal weight, overweight and obese defined by BMI and WHtR tertiles. Dichotomous features are presented as percentages and continuous variables are shown as a mean (plus or minus one standard

deviation) or a median and interquartile range for skewed data. Linear and logistic regression (adjusting for gender) were used to examine continuous and dichotomous metabolic variable differences between overweight and obese categories. Skewed continuous data were log transformed. Multinomial logistic regression was performed to determine cardiometabolic risk feature associations with each BMI and WHtR tertile combination. Subjects classified as normal weight by both indices were used as the reference category. All multinomial regression models were adjusted using age, gender, physical activity, smoking status and alcohol use as independent covariates.

The discriminatory ability of BMI, WHtR, and both BMI and WHtR used together, was assessed using receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) provides a scale from 0.5 to 1.0 (with 0.5 representing random chance and 1.0 indicating perfect discrimination) by which to appraise the capacity of an obesity index to detect a positive result [150]. Three separate analyses were performed. The first analysis assessed each anthropometric measure as a continuous variable. The second analysis explored cardiometabolic risk feature discrimination using index tertiles. A final analysis examined the 5-category BMI/WHtR combination variable used in previous regression models. Significant differences between AUC values were determined. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance.

6.3 Results

6.3.1 Descriptive characteristics

The characteristics of the study population were summarised according to BMI and WHtR tertiles (Table 16). A higher tertile level was related to an increased cardiometabolic risk profile as defined by lipid/lipoprotein, BP, glycaemic indicator and biomarker levels, with obese groups showing the highest proportion of cardiometabolic risk factors. In general, cardiometabolic profiles were broadly similar across BMI and WHtR overweight and obese categories, with the percentage of subjects with dyslipidaemia, high BP, insulin resistance, $\geq$3 metabolic features and pre-diabetes showing little variation according to classification by either index.

6.3.2 Cardiometabolic profiles according to classification of normal weight, overweight and obese

The levels of agreement between normal weight, overweight and obese tertiles are shown in Figure 16. Kappa statistics were similar for normal and obese classifications (Kappa: 0.66 for normal weight versus Kappa: 0.68 for obese) with marginal overlap between subjects defined as overweight (Kappa: 0.38). In both overweight and obese groups (Table 17), the combination of BMI and WHtR tertiles identified consistent and significant metabolic variable differences relative to those characterised discordantly. Subjects that were classified as overweight or obese by both indices displayed higher mean BMI, WC and median triglyceride levels, reduced HDL-C and adiponectin concentrations, and a higher percentage had

adverse biomarker levels, insulin resistance, metabolic feature clustering and pre-diabetes.

6.3.3 Associations between cardiometabolic risk features and BMI/WHtR combinations

Table 18 presents results from multinomial logistic regression models examining each BMI and WHtR tertile combination. A clear dose-response relationship was noted, with odds ratios of having cardiometabolic risk features being noticeably increased in subjects classified concordantly by both indices. In univariate analysis (not shown), odds ratios of having pre-diabetes were 0.6 (95% CI: 0.3,1.5), 1.9 (95% CI: 1.1,3.4), 1.8 (95% CI: 1.0,3.3) and 4.1 (95% CI: 2.5,6.7) for subjects categorised as *overweight by either*, *overweight by both*, *obese by either* and *obese by both* measures respectively. In a fully adjusted model, only patients within the highest BMI and WHtR tertile displayed a significant and positive association with pre-diabetes defined by both elevated HbA_{1c} and impaired FPG levels (OR: 3.4, 95% CI: 1.9,6.0, P<0.001).

6.3.4 ROC analysis

In ROC analysis (Table 19), when used as a continuous variable, significantly higher AUC values for WHtR were found to discriminate high triglycerides, $\geq$3 metabolic features, elevated C3 and WBC levels when compared to BMI. BMI displayed a significantly higher AUC for detecting

increased leptin levels compared to WHtR. A combination of both measures displayed significantly greater discriminatory accuracy for high triglycerides, metabolic feature clustering, C3 and CRP compared to BMI, and for leptin compared to WHtR. Significant improvement for detecting insulin resistance and high WBC levels were noted compared to when either BMI or WHtR were used independently.

When indices were examined as tertiles, significant differences between BMI and WHtR remained for discriminating high triglyceride, leptin and WBC concentrations. The BMI/WHtR 5-category variable was a significantly better discriminator of high triglycerides, low HDL-C, pre-diabetes, high C3, CRP, IL-6, TNF-α and WBC levels compared to BMI, and of leptin compared to WHtR. Significantly higher AUC values for detecting insulin resistance and $\geq$3 metabolic features were also found compared to when either measure were used alone.

6.4 Discussion

The aim of this study was to determine whether risk stratification using BMI and WHtR together more accurately identifies individuals at increased obesity-related cardiometabolic risk. Our findings indicate that both measures classify different subjects, particularly within the overweight range. These results also demonstrate that individuals defined as overweight or obese, by both BMI and WHtR, may exhibit different cardiometabolic profiles compared to subjects categorised discordantly. Participants classified

concordantly by both measures demonstrated stronger associations with individual cardiometabolic risk factors, metabolic feature clustering and displayed a profile that was more pro-inflammatory, pro-atherogenic and insulin resistant. Use of both indices also significantly improved discrimination of individual cardiometabolic disease features. These results suggest that joint use of BMI and WHtR may be clinically useful as a method to detect individuals at risk of cardiometabolic abnormalities.

Although it is straightforward to assess, and easy to calculate, limitations regarding the use of BMI as a sole method for adiposity appraisal have been widely acknowledged [98,99,243,246]. Though frequently employed within epidemiological research and healthcare practice, BMI does not discriminate between fat and lean body mass, therefore persons of short stature or muscular build may be misidentified [256]. Research has indicated that general obesity classification based on BMI might be inadequate [61]. A recent large study (N=40,420) which stratified participants by BMI categories demonstrated that nearly half of overweight, 29% of obese and even 16% of obese class II and III subjects were metabolically healthy [64]. Importantly, the finding that 30% of normal weight individuals were cardiometabolically unhealthy, signals that caution should be exercised with regard to how adiposity is defined [256].

Compared with BMI, WC is thought to be more strongly correlated with visceral adipose tissue (VAT) which has been shown to be associated with increased risk of dyslipidaemia, hypertension and type 2 diabetes [75,76].

Though the exact mechanism of association between VAT and metabolic risk is still poorly understood, research has implied that fatty acids released from VAT drain into the liver and skeletal muscle, causing metabolic dysfunction within these organs [78]. Proteins secreted from VAT may also contribute to cardiometabolic disease through inflammation of adipose and vascular tissue [79,83]. Although WC measurement has been recommended as a method for VAT and cardiometabolic risk assessment, controversy exists regarding its clinical efficacy. In particular, the need for gender and ethnic-specific risk cut-points, and the fact that WC does not take whole body fat distribution into account, indicate constraints regarding its practical application and usefulness within a clinical setting [246].

The WHtR is potentially advantageous as it may not require conversion to gender or population-specific cut-offs [125]. It has been previously suggested that a WHtR $\geq$0.5 may serve as a useful boundary for increased cardiometabolic risk, with a WHtR $\geq$0.6 threshold indicating substantially increased risk [273]. Additionally, it has been shown that height has an inverse association with cardiovascular disease mortality and total mortality [274,275], indicating that its use within an adiposity variable may be clinically important. In a recent meta-analysis of 31 prospective or cross-sectional studies, Ashwell et al. demonstrated WHtR to be a better discriminator of hypertension, MetS, type 2 diabetes and cardiovascular disease when compared to BMI [143]. Pooled results showed that WHtR improved discrimination of all outcomes by 4%-5%. However, other studies have

suggested that differences in predictive abilities are minimal, and have questioned the measurement of height in addition to WC [127].

The results from our research suggest that both BMI and WHtR provide important and independent information, and that joint measurement may help refine body fat classification. Within our sample it was noted that participants who were categorised as overweight discordantly also displayed an increased cardiometabolic risk profile. As a proportion of these individuals might be considered normal weight if either measure were used alone, these results indicate that use of both indices may provide a more sensitive method for detecting patients at increased cardiometabolic risk. We also observed noticeably strong associations with cardiometabolic features in subjects who were classified as overweight or obese by both BMI and WHtR together. This suggests that joint measurement may equally provide a more specific procedure for identifying high-risk subjects within overweight and obese categories. In particular, patients within the highest tertile for both indices were at a significantly higher risk compared subjects classified as obese discordantly. In addition, a significant association with pre-diabetes was only observed within this tertile after adjustment for other risk factors. This might imply that the relationship between obesity and diabetes is better indicated at this mode and level of adiposity.

Cardiometabolic disease is thought to be multifactorial, and it has been suggested that subjects with a combination of adverse features are at highest risk of developing type 2 diabetes and obesity-related chronic

disorders [19,230]. Although discriminatory improvements for detecting individual risk features were modest when using joint measurement, overall discrimination of cardiometabolic risk was significantly greater than when either index were used separately. As a degree of measurement error is to be expected during any anthropometric assessment, and as measurement error may limit the minimal detectable difference in a cardiometabolic risk parameter [102], it could be that these findings are due to the greater measurement accuracy that joint BMI and WHtR assessment may provide.

While it is hoped that public health programmes may eventually reduce the prevalence of obesity-related metabolic disorders, current strategies to combat obesity are failing as overweight and obesity rates continue to increase worldwide [2,5]. As a percentage of subjects with increased adiposity are considered to be metabolically healthy [256], there is a need for cheap and non-invasive methods to detect overweight and obese individuals at highest odds of developing type 2 diabetes and atherosclerotic vascular disease.

In previous research we have shown that assessing both bioelectrical impedance-derived body fat percentage and BMI may help to discriminate individuals at greater cardiometabolic risk than BMI alone [245]. Those identified using both tools had a more metabolically unhealthy profile and were non-responsive to dietary changes. These findings suggest that stratification of obese individuals, based on their metabolic health phenotype, could be important in the early identification of those who should be

prioritised for pharmacological and lifestyle interventions [256]. Joint use of BMI and WHtR may provide a convenient and inexpensive means for risk stratification. Such a method might be useful in resource-poor settings where blood sampling is cost-prohibitive, or in populations without regular access to primary health services.

6.4.1 Strengths and limitations of the research

As far as we are aware, our study is the first to comprehensively examine the joint use of BMI and WHtR in a middle-aged European population. Strengths include a high participation rate, the use of questionnaires to assess lifestyle behaviours and inclusion of a wide range of metabolic variables to define cardiometabolic health. Our findings are of potential public health and clinical significance in terms of screening and the use of stratification based on obesity assessment as a method for determining cardiometabolic risk.

Notwithstanding these strengths, methodological limitations should be considered when examining results from this study. Given the modest number of outcomes within our sample, we did not stratify by gender in analysis. Although some studies have implied heterogeneous relationships between measures of adiposity and cardiometabolic disease relating to gender [246], previous work by our group has suggested that these may be partly explained by sex differences in obesity prevalence [167]. In addition, recommended risk cut-points for BMI and WHtR are the same for men and women, and the gender variable was accounted for in analysis.

Also, given the size of our sample, the majority of analyses in this study use an approach comparing concordant results for both BMI and WHtR with subjects classified as overweight or obese discordantly. When combining the results of patients who were discordant by either measure, it is impossible to distinguish whether the poorer performance results exclusively from one or the other index, or from both indices equally. We acknowledge this as a limitation of our approach. Nevertheless, the ROC analysis did compare BMI and WHtR individually, and the results would support our finding that joint measurement may improve cardiometabolic risk classification.

Equally of concern is that we did not use established adiposity index cut-offs and that our data were cross-sectional, as this precludes examination of temporal relationships. Although World Health Organisation cut-points for BMI are commonly used [93], and thresholds for WHtR have been recommended [123,273], for the purposes of this research it was necessary to place both variables on the same scale. Future studies, utilising longitudinal data, will be needed to evaluate the applicability, validity and reliability of joint measurement [273] using established and recommended diagnostic cut-points. In particular, it will be necessary to determine whether employing both BMI and WHtR for risk stratification is clinically useful and superior to currently recommended BMI classification [94].

Finally, our data were derived from a single primary care based sample which may not be representative of the source population. However, Ireland presents a generally ethnically homogeneous population [242]. Thus, the

relationships we observed are likely to be similar in other middle-aged Irish adults. In addition, random sampling of subjects and the use of validated methods for data collection ensured internal sample validity and the results from this research may be generalisable to a similar middle-aged Caucasian-European population.

6.5 Conclusions

In summary, our findings reveal that individuals defined as overweight or obese, by both BMI and WHtR, are at increased cardiometabolic risk when compared to subjects categorised discordantly by BMI and WHtR. Use of both measures also improved discrimination of individual risk features and identified a subset of at-risk patients who might otherwise be missed. Although assessment of WC, in addition to BMI, competes for the limited time available during patient appraisal within clinical practice [83], obtaining two measurements (one for general adiposity, and one for central adiposity) does not entail any extra cost [273]. In light of the increasing prevalence of cardiometabolic disease worldwide [175], effective methods that help to identify subjects at greatest risk are needed. Risk stratification utilising a composite index may provide a simple, cost-effective and more accurate technique for predicting obesity-related chronic disorders. Earlier identification of individuals at risk could enable earlier targeted interventions or therapies, thus attenuating development of cardiovascular complications.

Table 16—*Characteristics of the study population.*

Feature	Normal weight		Overweight		Obese	
	BMI (N=619)	WHtR (N=619)	BMI (N=618)	WHtR (N=618)	BMI (N=619)	WHtR (N=619)
Male	212 (34.2)	145 (23.4)	346 (56.0)	349 (56.5)	327 (52.8)	391 (63.2)
Age	58 (54,63)	57 (54,62)	59 (54,63)	59 (54,64)	60 (55,64)	60 (55,64)
Weight (kg)	64.6 ± 8.5	65.6 ± 9.4	78.3 ± 9.1	78.6 ± 10.2	92.5 ± 12.6	91.2 ± 13.5
BMI (kg/m^2)	23.7 ± 1.8	24.3 ± 2.5	27.9 ± 1.0	27.9 ± 2.2	33.3 ± 3.5	32.7 ± 4.0
WC (cm)	79.7 ± 8.9	77.7 ± 7.2	91.8 ± 8.0	92.2 ± 5.8	102.7 ± 10.0	104.3 ± 8.4
WHtR	0.48 ± 0.04	0.47 ± 0.03	0.55 ± 0.04	0.55 ± 0.02	0.62 ± 0.05	0.62 ± 0.04
Triglycerides (mmol/l)	1.0 (0.8,1.3)	1.0 (0.8,1.3)	1.2 (0.9,1.7)	1.2 (0.9,1.7)	1.4 (1.0,2.0)	1.5 (1.1,2.0)
High triglycerides[1]	67 (11.0)	49 (8.0)	147 (24.5)	152 (25.5)	195 (33.3)	208 (35.1)
HDL-C (mmol/l)	1.7 ± 0.4	1.7 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
Low HDL-C[2]	46 (7.6)	39 (6.4)	74 (12.2)	80 (13.2)	142 (23.8)	143 (24.0)
Dyslipidaemia	12 (2.0)	10 (1.6)	37 (6.1)	37 (6.1)	69 (11.5)	71 (11.9)
Systolic BP (mmHg)	124.9 ± 17.4	124.8 ± 17.2	129.5 ± 15.5	129.2 ± 15.5	133.0 ± 15.9	133.4 ± 16.0
Diastolic BP (mmHg)	77.2 ± 9.6	77.8 ± 9.6	80.7 ± 8.8	80.3 ± 9.4	82.5 ± 9.9	82.3 ± 9.5
High BP[3]	271 (43.8)	263 (42.5)	359 (58.2)	366 (59.3)	471 (76.3)	472 (76.5)
Insulin (μU/ml)	5.3 (3.8,7.9)	5.3 (3.8,7.9)	8.4 (5.7,12.0)	8.3 (5.6,12.2)	12.9 (8.2,18.4)	12.9 (8.4,18.4)
HOMA-IR	1.1 (0.8,1.7)	1.1 (0.8,1.7)	1.8 (1.2,2.7)	1.8 (1.2,2.7)	2.9 (1.8,4.3)	2.9 (1.9,4.3)
Insulin resistance[4]	31 (5.2)	29 (4.9)	121 (20.2)	122 (20.4)	293 (49.4)	294 (49.6)
$\geq$3 metabolic features	16 (2.6)	13 (2.1)	63 (10.2)	59 (9.5)	163 (26.3)	170 (27.5)
HbA_{1c} (%)	5.6 (5.4,4.8)	5.6 (5.4,5.8)	5.7 (5.5,5.8)	5.7 (5.5,5.9)	5.7 (5.5,6.0)	5.7 (5.5,6.0)
FPG (mmol/l)	4.8 (4.5,5.0)	4.7 (4.5,5.0)	4.9 (4.6,5.2)	4.9 (4.7,5.2)	5.1 (4.7,5.4)	5.1 (4.8,5.5)
Pre-diabetes[5]	27 (4.4)	26 (4.3)	49 (8.1)	41 (6.7)	86 (14.2)	95 (15.7)
C3 (mg/dl)	125.7 ± 22.9	125.2 ± 22.4	134.2 ± 22.7	135.2 ± 22.8	144.5 ± 22.9	144.0 ± 22.8
High C3[6]	79 (13.2)	77 (12.8)	133 (22.0)	137 (22.6)	239 (39.8)	237 (39.6)
CRP (ng/ml)	1.1 (0.8,1.6)	1.1 (0.8,1.6)	1.3 (1.0,1.9)	1.3 (1.0,2.0)	1.7 (1.2,3.1)	1.8 (1.2,3.0)
High CRP[6]	91 (15.1)	85 (14.1)	124 (20.5)	136 (22.4)	236 (39.4)	230 (38.5)
IL-6 (pg/ml)	1.4 (1.0,2.3)	1.4 (1.0,2.1)	1.6 (1.2,2.5)	1.7 (1.2,2.5)	2.1 (1.5,3.3)	2.2 (1.5,3.4)
High IL-6[6]	118 (19.5)	102 (16.9)	126 (20.9)	129 (21.3)	207 (34.5)	220 (36.8)
TNF-α (pg/ml)	5.6 (4.6,6.9)	5.5 (4.5,6.6)	5.9 (4.9,7.2)	5.8 (4.9,7.1)	6.3 (5.2,7.5)	6.4 (5.3,7.7)
High TNF-α[6]	117 (19.4)	110 (18.2)	153 (25.4)	140 (23.1)	181 (30.2)	201 (33.6)

Table 16 continued

Feature	Normal weight		Overweight		Obese	
	BMI (N=619)	WHtR (N=619)	BMI (N=618)	WHtR (N=618)	BMI (N=619)	WHtR (N=619)
Adiponectin (ng/ml)	6.6 (4.2,9.8)	6.9 (4.7,10.2)	4.6 (2.9,6.9)	4.6 (2.9,6.9)	4.1 (2.6,6.3)	3.8 (2.5,5.5)
Low adiponectin[6]	78 (12.9)	65 (10.8)	176 (29.1)	167 (27.6)	199 (33.2)	221 (36.9)
Leptin (ng/ml)	1.3 (0.6,2.0)	1.4 (0.8,2.1)	1.8 (1.0,2.7)	1.8 (1.0,2.8)	3.1 (1.9,5.1)	2.7 (1.6,4.7)
High leptin[6]	39 (6.5)	67 (11.1)	109 (18.0)	122 (20.1)	303 (50.5)	262 (43.7)
Resistin (ng/ml)	4.8 (3.9,6.4)	4.9 (3.8,6.4)	4.9 (3.7,6.5)	4.9 (3.8,6.6)	5.3 (4.0,7.0)	5.2 (4.0,6.7)
High resistin[6]	133 (22.0)	136 (22.5)	141 (23.3)	152 (25.1)	178 (29.7)	164 (27.4)
PAI-1 (ng/ml)	24.3 ± 10.3	24.0 ± 10.4	28.1 ± 13.6	27.5 ± 11.7	28.7 ± 12.0	29.7 ± 13.7
High PAI-1[6]	100 (16.6)	94 (15.6)	164 (27.2)	161 (26.6)	187 (31.2)	196 (32.8)
WBC (10^9/l)	5.7 ± 2.4	5.5 ± 1.6	5.8 ± 1.6	5.9 ± 2.3	6.1 ± 1.5	6.3 ± 1.5
High WBC[6]	125 (20.7)	103 (17.0)	149 (24.5)	153 (25.3)	177 (29.7)	195 (32.7)

Mean and ± standard deviation are shown for continuous variables. Age, triglycerides, insulin, HOMA-IR, HbA_{1c}, FPG, CRP, IL-6, TNF-α, adiponectin, leptin and resistin are shown as a median (interquartile range). % (in brackets) for dichotomous variables will vary as some variables have missing values.

[1]Triglycerides $\geq$1.7.

[2]HDL-C <1.03 (males) or HDL-C <1.29 (females).

[3]Systolic BP $\geq$130 and/or diastolic BP $\geq$85 or use of Rx anti-hypertensives.

[4]HOMA-IR $\geq$2.96.

[5]Both HbA_{1c} levels $\geq$5.7% and FPG levels $\geq$5.6.

[6]Threshold: C3 $\geq$148; CRP $\geq$2.25; IL-6 $\geq$2.72; TNF-α $\geq$7.2; adiponectin $\leq$3.1; leptin $\geq$3.07; resistin $\geq$6.6; PAI-1 $\geq$33.66; WBC $\geq$6.6.

Table 17—*Cardiometabolic profiles according to classification of normal weight, overweight and obese defined by BMI, WHtR, or both.*

Feature	Normal weight by both (N=479)	Overweight by either (N=263)	Overweight by both (N=363)	P value[1]	Obese by either (N=264)	Obese by both (N=487)	P value[2]
Male[3]	122 (25.5)	105 (39.9)	231 (63.6)	<0.001	136 (51.5)	291 (59.8)	0.03
Age[4]	58 (54,62)	58 (54,65)	59 (54,63)	0.885	59 (54,64)	60 (55,64)	0.15
Weight (kg)[5]	63.1 ± 8.1	71.9 ± 8.0	79.6 ± 9.2	<0.001	81.8 ± 9.5	94.6 ± 12.6	<0.001
BMI (kg/m^2)[5]	23.3 ± 1.8	26.2 ± 1.5	27.9 ± 1.0	<0.001	29.7 ± 1.9	33.8 ± 3.6	<0.001
WC (cm)[5]	76.6 ± 7.2	85.7 ± 6.7	93.0 ± 5.7	<0.001	95.1 ± 7.2	105.7 ± 8.5	<0.001
WHtR[5]	0.46 ± 0.03	0.52 ± 0.03	0.55 ± 0.02	<0.001	0.57 ± 0.03	0.63 ± 0.05	<0.001
Triglycerides (mmol/l)[5]	0.9 (0.7,1.2)	1.1 (0.8,1.5)	1.3 (0.9,1.7)	0.005	1.3 (1.0,1.8)	1.5 (1.1,2.0)	0.034
High triglycerides[5]	36 (7.6)	43 (16.7)	93 (26.6)	0.031	71 (27.7)	166 (35.8)	0.06
HDL-C (mmol/l)[5]	1.7 ± 0.4	1.6 ± 0.4	1.4 ± 0.3	<0.001	1.4 ± 0.3	1.3 ± 0.3	<0.001
Low HDL-C[5]	31 (6.7)	23 (8.8)	46 (12.9)	0.038	39 (15.1)	123 (26.3)	0.001
Dyslipidaemia[5]	7 (1.5)	8 (3.1)	21 (5.9)	0.059	24 (9.3)	58 (12.3)	0.253
Systolic BP (mmHg)[5]	124.2 ± 17.5	126.4 ± 16.2	129.4 ± 14.7	0.027	132.5 ± 16.4	133.4 ± 15.8	0.52
Diastolic BP (mmHg)[5]	77.1 ± 9.6	78.8 ± 9.4	80.5 ± 8.8	0.003	82.2 ± 9.3	82.5 ± 9.8	0.61
High BP[5]	195 (40.7)	134 (51.0)	213 (58.8)	0.069	175 (66.3)	384 (79.2)	<0.001
Insulin (µU/ml)[5]	5.1 (3.7,7.5)	6.2 (4.3,9.2)	8.8 (6.0,12.1)	<0.001	10.2 (6.8,14.3)	14.0 (9.0,20.2)	<0.001
HOMA-IR[5]	1.1 (0.8,1.6)	1.4 (0.9,2.0)	2.0 (1.3,2.7)	<0.001	2.2 (1.5,3.2)	3.2 (2.0,4.6)	<0.001
Insulin resistance[5]	20 (4.4)	20 (7.8)	72 (20.5)	<0.001	79 (31.1)	254 (54.5)	<0.001
≥3 metabolic features[5]	8 (1.7)	13 (4.9)	35 (9.6)	0.024	39 (14.8)	147 (30.2)	<0.001
HbA_{1c} (%)[5]	5.6 (5.4,5.8)	5.7 (5.5,5.9)	5.7 (5.4,5.8)	0.54	5.7 (5.5,5.8)	5.8 (5.6,6.0)	0.002
FPG (mmol/l)[5]	4.7 (4.5,5.0)	4.8 (4.6,5.1)	4.9 (4.7,5.3)	0.038	4.9 (4.7,5.2)	5.1 (4.8,5.5)	<0.001
Pre-diabetes[5]	22 (4.7)	8 (3.1)	31 (8.7)	0.018	21 (8.1)	80 (16.8)	0.002
C3 (mg/dl)[5]	124.0 ± 23.1	130.2 ± 20.4	134.7 ± 23.0	<0.001	139.2 ± 23.2	145.7 ± 22.9	<0.001
High C3[5]	56 (12.1)	42 (16.2)	77 (21.8)	0.013	76 (29.1)	200 (42.6)	<0.001
CRP (ng/ml)[5]	1.0 (0.8,1.5)	1.3 (1.0,2.0)	1.3 (1.0,1.8)	0.976	1.5 (1.1,2.5)	1.8 (1.2,3.2)	<0.001
High CRP[5]	60 (12.9)	52 (20.1)	67 (18.9)	0.891	78 (30.1)	194 (41.4)	<0.001
IL-6 (pg/ml)[5]	1.3 (1.0,2.1)	1.4 (1.1,2.4)	1.7 (1.2,2.4)	0.317	1.9 (1.3,2.9)	2.3 (1.6,3.5)	0.001
High IL-6[5]	82 (17.6)	49 (18.9)	70 (19.7)	0.99	73 (28.3)	177 (37.7)	0.01
TNF-α (pg/ml)[5]	5.5 (4.6,6.7)	5.8 (4.6,6.9)	5.9 (4.9,7.2)	0.521	5.8 (5.0,7.3)	6.4 (5.3,7.0)	0.067
High TNF-α[5]	87 (18.7)	50 (19.4)	89 (25.1)	0.276	68 (26.4)	157 (33.4)	0.047

Table 17 continued

Feature	Normal weight by both (N=479)	Overweight by either (N=263)	Overweight by both (N=363)	P value[1]	Obese by either (N=264)	Obese by both (N=487)	P value[2]
Adiponectin (ng/ml)[5]	7.0 (4.7,10.3)	5.9 (3.8,9.0)	4.2 (2.7,6.3)	<0.001	4.8 (2.9,6.7)	3.8 (2.5,5.5)	0.023
Low adiponectin[5]	48 (10.3)	43 (16.6)	117 (33.0)	0.007	70 (27.0)	175 (37.2)	0.032
Leptin (ng/ml)[5]	1.3 (0.7,2.0)	1.6 (1.0,2.4)	1.7 (0.9,2.7)	0.001	2.3 (1.3,4.0)	3.2 (1.9,5.2)	<0.001
High leptin[5]	30 (6.5)	39 (15.1)	56 (15.8)	0.009	87 (33.6)	239 (50.9)	<0.001
Resistin (ng/ml)[5]	4.9 (3.8,6.4)	4.8 (3.8,6.7)	4.9 (3.7,6.4)	0.946	4.9 (4.0,6.8)	5.3 (4.0,7.0)	0.274
High resistin[5]	100 (21.5)	66 (25.5)	79 (22.3)	0.452	72 (27.9)	135 (28.7)	0.611
PAI-1 (ng/ml)[5]	23.8 ± 10.2	25.5 ± 10.9	28.1 ± 12.0	0.033	29.3 ± 15.6	29.2 ± 12.1	0.761
High PA1-1[5]	69 (14.8)	52 (20.2)	103 (29.0)	0.046	71 (27.5)	156 (33.2)	0.161
WBC (10^9/l)[5]	5.5 ± 1.7	5.8 ± 3.0	5.9 ± 1.6	0.92	6.1 ± 1.6	6.2 ± 1.4	0.345
High WBC[5]	84 (17.9)	56 (22.0)	88 (24.6)	0.832	74 (28.7)	149 (31.9)	0.418

Mean and ± standard deviation are shown for continuous variables. Age, triglycerides, insulin, HOMA-IR, HbA_{1c}, FPG, CRP, IL-6, TNF-α, adiponectin, leptin and resistin are shown as a median (interquartile range). % (in brackets) for dichotomous variables will vary as some variables have missing values.

[1]P value for difference: overweight by either compared to overweight by both.

[2]P value for difference: obese by either compared to obese by both.

[3]x^2 for difference.

[4]Mann Whitney U for difference.

[5]P value for difference adjusted for gender.

Overweight subjects classified as obese by either index were assigned to the higher category.

Table 18—*Odds ratios (95% CI) of having cardiometabolic risk features according to classification of overweight and obese.*

Feature	Odds ratios (95% CI)[1]							
	Overweight compared to normal weight				Obese compared to normal weight			
	Either BMI or WHtR	P value	Both BMI and WHtR	P value	Either BMI or WHtR	P value	Both BMI and WHtR	P value
High triglycerides	2.1 (1.3,3.5)	0.003	3.5 (2.3,5.4)	<0.001	3.4 (2.1,5.5)	<0.001	5.6 (3.7,8.6)	<0.001
Low HDL-C	1.4 (0.8,2.5)	0.3	2.1 (1.3,3.7)	0.005	2.2 (1.2,3.8)	0.008	5.8 (3.6,9.2)	<0.001
Dyslipidaemia	1.8 (0.6,5.3)	0.263	3.8 (1.5,9.3)	0.004	4.6 (1.9,11.6)	0.001	8.6 (3.7,19.6)	<0.001
High BP	1.5 (1.1,2.1)	0.02	2.1 (1.5,2.8)	<0.001	3.0 (2.1,4.2)	<0.001	5.7 (4.1,7.9)	<0.001
Insulin resistance	1.8 (0.9,3.7)	0.083	5.4 (3.1,9.6)	<0.001	9.5 (5.3,16.8)	<0.001	26.6 (15.5,45.7)	<0.001
≥3 metabolic features	2.6 (1.0,6.6)	0.043	5.4 (2.4,12.0)	<0.001	7.8 (3.5,17.5)	<0.001	22.2 (10.5,47.0)	<0.001
Pre-diabetes	0.6 (0.2,1.4)	0.227	1.6 (0.9,3.1)	0.142	1.6 (0.8,3.2)	0.218	3.4 (1.9,6.0)	<0.001
High C3	1.3 (0.8,2.1)	0.260	2.8 (1.9,4.3)	<0.001	3.3 (2.1,5.0)	<0.001	7.9 (5.4,11.6)	<0.001
High CRP	1.6 (1.0,2.6)	0.032	1.8 (1.2,2.7)	0.007	3.6 (2.4,5.5)	<0.001	6.1 (4.2,8.9)	<0.001
High IL-6	1.0 (0.7,1.6)	0.897	1.1 (0.8,1.7)	0.541	1.7 (1.2,2.6)	0.008	2.7 (1.9,3.8)	<0.001
High TNF-α	1.1 (0.7,1.6)	0.738	1.4 (1.0,2.0)	0.089)	1.2 (0.8,1.9)	0.323	2.2 (1.5,3.1)	<0.001
Low adiponectin	1.4 (0.8,2.3)	0.204	2.7 (1.8,4.2)	<0.001	2.6 (1.6,4.2)	<0.001	3.9 (2.6,6.0)	<0.001
High leptin	3.6 (2.1,6.3)	<0.001	5.9 (3.5,9.9)	<0.001	15.7 (9.3,26.6)	<0.001	46.6 (27.9,77.6)	<0.001
High resistin	1.3 (0.9,1.9)	0.205	1.3 (0.9,1.9)	0.194	1.5 (1.0,2.2)	0.046	1.8 (1.2,2.5)	0.001
High PAI-1	1.2 (0.8,1.8)	0.460	2.0 (1.3,2.9)	<0.001	1.9 (1.3,2.9)	0.002	2.7 (1.9,3.9)	<0.001
High WBC	1.5 (1.0,2.4)	0.073	1.9 (1.2,2.9)	0.003	2.7 (1.7,4.2)	<0.001	3.2 (2.2,4.8)	<0.001

[1]Multinomial logistic regression, reference category: normal weight by both BMI and WHtR.
Overweight subjects classified as obese by either index were assigned to the higher category.
All models adjusted for age, gender, physical activity, smoking and alcohol use.

Table 19—*Area under the receiver operating characteristic curve values (95% CI) for index models to discriminate cardiometabolic risk features.*

Feature	As a continuous variable						As a categorical variable (tertiles)						Overweight and obese by either or both[1]	
	BMI alone		WHtR alone		Both BMI and WHtR		BMI alone		WHtR alone		Both BMI and WHtR			
	AUC	95% CI	AUC	95% CI	AUC	95% CI	AUC	95% CI	AUC	95% CI	AUC	95% CI	AUC	95% CI
High triglycerides	0.68	0.65,0.71	0.71[2]	0.68,0.73	0.71[2]	0.68,0.74	0.68	0.65,0.71	0.70[4]	0.67,0.73	0.70[4]	0.68,0.73	0.70[4]	0.67,0.73
Low HDL-C	0.67	0.63,0.70	0.68	0.65,0.72	0.68	0.65,0.71	0.66	0.62,0.69	0.67	0.64,0.71	0.68[4]	0.65,0.71	0.68[4]	0.64,0.71
Dyslipidaemia	0.68	0.64,0.73	0.70	0.65,0.74	0.70	0.65,0.74	0.69	0.64,0.73	0.69	0.65,0.73	0.70	0.66,0.74	0.70	0.66,0.74
High BP	0.70	0.67,0.72	0.70	0.67,0.72	0.70	0.68,0.72	0.69	0.67,0.72	0.69	0.67,0.71	0.70	0.67,0.72	0.70	0.67,0.72
Insulin resistance	0.80	0.78,0.82	0.80	0.78,0.83	0.81[2,3]	0.79,0.83	0.78	0.76,0.80	0.77	0.75,0.80	0.80[4,5]	0.77,0.82	0.79[4,5]	0.77,0.82
≥3 metabolic features	0.76	0.73,0.79	0.78[2]	0.75,0.81	0.78[2]	0.75,0.81	0.75	0.72,0.78	0.75	0.72,0.78	0.77[4,5]	0.74,0.80	0.77[4,5]	0.74,0.80
Pre-diabetes	0.70	0.66,0.74	0.70	0.66,0.74	0.70	0.66,0.74	0.67	0.63,0.71	0.68	0.64,0.72	0.69	0.65,0.73	0.69[4]	0.65,0.73
High C3	0.70	0.67,0.73	0.72[2]	0.69,0.75	0.72[2]	0.69,0.75	0.69	0.66,0.71	0.70	0.67,0.73	0.71[4]	0.68,0.74	0.71[4]	0.68,0.73
High CRP	0.69	0.66,0.72	0.69	0.67,0.72	0.70[2]	0.67,0.72	0.67	0.64,0.70	0.68	0.65,0.71	0.69[4]	0.66,0.72	0.69[4]	0.66,0.72
High IL-6	0.66	0.63,0.69	0.67	0.64,0.69	0.67	0.64,0.69	0.65	0.62,0.68	0.66	0.63,0.69	0.66	0.63,0.69	0.66[4]	0.63,0.69
High TNF-α	0.63	0.60,0.66	0.63	0.60,0.66	0.63	0.60,0.66	0.62	0.59,0.65	0.63	0.61,0.66	0.63	0.61,0.66	0.63[4]	0.60,0.66
Low adiponectin	0.79	0.77,0.81	0.79	0.77,0.81	0.79	0.77,0.81	0.79	0.77,0.81	0.79	0.76,0.81	0.79	0.77,0.81	0.79	0.77,0.81
High leptin	0.86[3]	0.84,0.87	0.84	0.82,0.86	0.86[3]	0.84,0.88	0.84[5]	0.82,0.86	0.81	0.79,0.83	0.85[5]	0.83,0.87	0.84[5]	0.82,0.86
High resistin	0.57	0.54,0.60	0.56	0.53,0.59	0.57	0.54,0.60	0.57	0.54,0.60	0.56	0.53,0.59	0.57	0.54,0.60	0.56	0.53,0.60
High PAI-1	0.61	0.58,0.64	0.62	0.59,0.65	0.62	0.59,0.65	0.62	0.59,0.64	0.62	0.59,0.65	0.62	0.59,0.65	0.62	0.59,0.65
High WBC	0.59	0.56,0.62	0.61[2]	0.58,0.64	0.63[2,3]	0.60,0.66	0.58	0.55,0.61	0.60[4]	0.57,0.63	0.61[4]	0.58,0.64	0.59[4]	0.56,0.62

All models include age and gender.
[1]5-category variable: (1) *normal weight by both*, (2) *overweight by either*, (3) *overweight by both*, (4) *obese by either* and (5) *obese by both*.
Overweight subjects classified as obese by either index were assigned to the higher category.
[2]P<0.05 compared to BMI (continuous).
[3]P<0.05 compared to WHtR (continuous).
[4]P<0.05 compared to BMI (categorical).
[5]P<0.05 compared to WHtR (categorical).

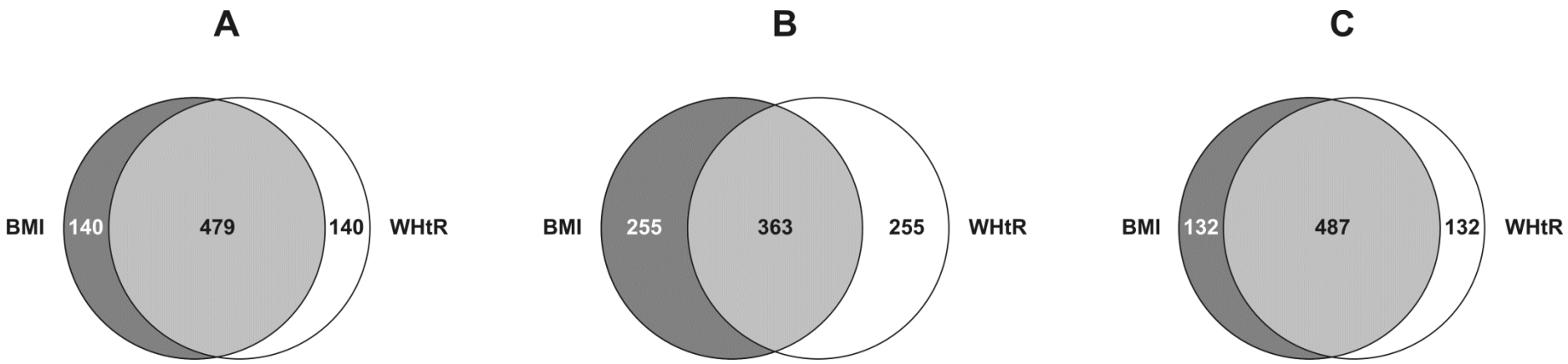

Figure 16—*Overlap of normal weight, overweight and obese defined by BMI and WHtR.* The figure shows Venn diagrams illustrating overlap of BMI and WHtR tertiles for (A) normal weight, (B) overweight and (C) obese.

CHAPTER 7

DISCUSSION AND RECOMMENDATIONS

7.0 Introduction

This thesis aimed to contribute to the current evidence base regarding the use of anthropometric measures of adiposity to detect patients with type 2 diabetes, and those at increased obesity-related cardiometabolic risk. This chapter outlines the major findings from this thesis and discusses them in the context of the current literature. The clinical implications of the research are considered, and the main strengths and limitations of the work are high-lighted. Areas for future study are also proposed.

7.1 Main Findings

7.1.1 Literature review

As discussed in Chapter 1, despite a large number of studies which have compared adiposity indices with features of cardiometabolic disease, type 2 diabetes and cardiovascular and mortality outcomes [127,138-149], controversy still exists as to which surrogate measure of adiposity better indicates obesity-related risk. In particular, uncertainty exists as to whether central adiposity indices provide additional information, beyond that which is assessed by BMI, and whether more accurate adiposity measurement within healthcare practice might be clinically useful.

Limitations regarding previous research were identified. These included statistical procedures used to evaluate adiposity measures, methods used for disease classification and the lack of a universal protocol for WC measurement. These limitations were investigated in the thesis chapters.

7.1.2 Diabetes prevalence and rationale for adiposity measurement

Chapter 2 examined the prevalence of undiagnosed and diagnosed type 2 diabetes within our sample. The total prevalence of diabetes was found to be 8.5%, which is comparable to the estimate determined from previous nationally representative research using data from the SLÁN 2007: Survey of Lifestyle, Attitudes and Nutrition (8.9%) [35,178]. It is also similar to the 9.5% prevalence rate reported from a recent study utilising data from The Irish Longitudinal Study on Ageing (TILDA) [276]. Notably, within our sample, a high percentage of diabetes cases were undiagnosed (41%). This is greater than the 30% indicated by the SLÁN survey, and considerably higher than the prevalence of undiagnosed diabetes suggested by the TILDA data (0.9%). As geographic location was significantly associated with having undiagnosed diabetes in the TILDA study, these results suggest that in certain areas within Ireland, between one-third and almost 50% of middle-aged adult diabetes cases are undiagnosed.

It was observed that undiagnosed subjects displayed a less-optimal cardiometabolic profile compared to diagnosed patients. Access to primary healthcare may partly account for these differences, as undiagnosed study participants were less likely to have private medical insurance, a finding which has since been replicated using the TILDA data [276]. However, within our sample, a majority of undiagnosed patients did have health insurance (either private or state-assisted), indicating that other factors, such as better methods to detect high-risk subjects, are needed.

We evaluated clinically relevant variables to identify undiagnosed cases. Models which included health insurance status, physical activity levels and BMI displayed a higher discriminatory capacity to detect subjects with undiagnosed diabetes compared to diagnosed individuals. Subsequently, these findings suggest a rationale for accurate adiposity measurement; as a procedure to help identify undiagnosed, high-risk patients within clinical practice.

7.1.3 Defining cardiometabolic risk

Chapter 3 explored metabolic feature relationships with type 2 diabetes and pre-diabetes in order to determine how diabetes and cardiometabolic risk should be diagnosed within clinical practice and epidemiological research. Specifically, it compared diabetes and pre-diabetes classifications using HbA_{1c} and FPG. In keeping with previous findings, the results of this research suggest discordance between these two methods for diagnosing type 2 diabetes and assessing cardiometabolic risk [24,192,196,220]. Markedly, the prevalence of both diabetes and pre-diabetes was higher using HbA_{1c}. The prevalence of type 2 diabetes diagnosed by HbA_{1c} was almost double that compared to the estimate determined using FPG. This finding is important regarding implied increases in diabetes prevalence within Ireland. Studies which compare current prevalence estimates with those obtained before 2010 [169] need to acknowledge that part of the reason for observed higher prevalence rates may be due to the use of a different diagnostic test [34,35,276].

Nevertheless, we found that the cardiometabolic profiles in subjects diagnosed with type 2 diabetes, by either assay, were broadly similar. This suggests that both methods are valid for diagnosing diabetes within clinical practice. These data also indicated that either test was acceptable for classifying diabetes within our sample. Both methods were used to define this outcome in subsequent chapters.

However, when pre-diabetes cases were examined, it was noted that subjects diagnosed by HbA_{1c} alone did not display a moderate or strong association with any of the examined cardiometabolic features. These individuals were also clearly less obese compared to those diagnosed by either FPG, or both HbA_{1c} and FPG together. These results were surprising given that numerous studies have shown HbA_{1c} to strongly predict incident diabetes [24,226,227,277]. Nevertheless, these findings have implications regarding how type 2 diabetes and cardiometabolic risk should be defined within epidemiology, in particular within our study, as correct classification of high-risk subjects is needed in order to properly assess relationships between exposure variables and outcomes.

These results are also important regarding future diabetes estimates within Ireland, as accurate assessment of progression rates to type 2 diabetes is needed for efficient allocation of resources in order to optimise public health prevention strategies. Notably, although prevalence rates for type 2 diabetes in middle-aged Irish adults are comparable between our sample, the SLÁN survey [35] and the TILDA study [276], prevalence estimates for pre-diabetes

using HbA_{1c} vary widely (47.9% for our study, versus 19.8% for SLÁN [36], versus 5.5% for TILDA [276]). These findings suggest both validity and reliability concerns with HbA_{1c} measurement, and that standardisation issues with regard to different procedures used for assessing HbA_{1c} levels need to be addressed.

Consistent with previous research, we found high BP, atherogenic dyslipidaemia, insulin resistance and adverse cardiometabolic feature clustering to be significantly associated with both type 2 diabetes and pre-diabetes [19]. Subjects with these outcomes also displayed a chronic low-grade pro-inflammatory profile as indicated by the examined biomarkers. Relationships between adiposity measures and these metabolic markers were explored in following chapters.

7.1.4 General adiposity compared to central adiposity

In Chapter 4, adiposity variable relationships with features of cardiometabolic disease and type 2 diabetes were examined. In particular, we compared BMI with central adiposity indices. In logistic regression models it was observed that central adiposity measures displayed stronger associations with adverse cardiometabolic features, metabolic feature clustering and type 2 diabetes. Central adiposity indices were also noticeably better discriminators of patients with type 2 diabetes. These findings concur with previous research which suggest that central adiposity, as defined by WC, WHR or WHtR, is a

better indicator of cardiometabolic disease and type 2 diabetes than BMI [58,115,143].

However, it was also noted that the utility of these additional measures was significantly influenced by measurement procedure. When compared to BMI, our results imply that, within our middle-aged population, the WHO and IDF-recommended midway WC measurement protocol provides little additional information. As this is the most commonly employed method used for assessing WC within clinical practice and epidemiological research [107], these results have implications for future study and how previous research should be assessed. Importantly, the results from studies [13] which report obesity prevalence rates using midway WC thresholds, and compare them to BMI overweight and obese classifications, must be treated with caution, as this is not necessarily demonstrating a higher level of risk, but rather a different way of dividing the risk continuum.

In this chapter we have provided evidence which suggest that rib-level WC measurement is superior to midway WC measurement in terms of identifying subjects with type 2 diabetes. However, whether rib-level measurement is the optimal procedure for assessing WC could not be determined in our sample as we did not have other WC measurement sites to contrast. Nonetheless, our findings do suggest an urgent need for a universal WC measurement protocol. This would make comparisons with other epidemiological studies, and across multiple populations, more intuitive and facilitate interpretation of the clinical utility of central adiposity variables for

obesity-related risk stratification [106]. Also, as observed in our sample and previous research [278], as the magnitude of WC is also influenced by measurement procedure, small differences may be amplified using dichotomous cut-points, such as used in MetS definitions and diabetes risk scores, thus diagnosing different patients as being at risk and leading to misclassification.

7.1.5 Adiposity and chronic low-grade inflammation

Although it was noted in Chapter 4 that central adiposity measures displayed stronger associations with commonly assessed cardiometabolic disease features, we also observed that relationships between central adiposity indices and type 2 diabetes were particularly strong when compared to BMI. This suggested that the relationship between central adiposity and diabetes may also be mediated by other metabolic processes beyond those captured through commonly assessed cardiometabolic disease markers.

Chapter 5 compared BMI and WC relationships with biomarkers of inflammation. We found that WC measurement was more strongly related to a majority of the examined biomarkers and adverse biomarker clustering. The association between low-grade inflammation and diabetes was also reduced in analyses including either BMI or WC, with models incorporating WC showing the greatest attenuation. It was also noted that models which included both adiposity variables together did not display any further attenuation. Collectively, these findings suggest that central adiposity is more

strongly associated with obesity-induced inflammation than BMI, and that central adiposity may also account for a greater variance of diabetes-related systemic low-grade inflammation.

Previous research by our group which examined metabolically healthy and unhealthy obese and non-obese phenotypes indicated that favourable inflammatory status may be positively associated with metabolic health. Metabolically healthy subjects presented lower concentrations of inflammatory biomarkers compared to their metabolically unhealthy counterparts [232]. A recent Korean study also demonstrated that chronic low-grade inflammation may be an important early marker of risk of developing type 2 diabetes, as metabolically healthy obese subjects with systemic inflammation were at increased risk [279]. Although the added value of novel biomarkers in type 2 diabetes risk prediction algorithms has yet to be established [280-283], the results from our study support the theory that central adiposity measures may be better markers of visceral fat. However, as the association between chronic low-grade inflammation and diabetes was not completely attenuated in models which included adiposity variables, these results also indicated that alternative adiposity measurement procedures may be needed to better assess the pro-inflammatory state associated with type 2 diabetes and obesity-related disorders.

7.1.6 Assessing the utility of a composite index

In Chapter 6, we investigated the utility of composite index for assessing cardiometabolic risk. This research addressed the hypothesis that the use of two indices to define overweight and obese classifications might provide additional and independent information. Body mass index is the most commonly used method for assessing general adiposity, and our previous findings suggested WHtR to be the best overall method for determining central obesity. Therefore, we examined a range of cardiometabolic disease features and inflammatory markers to explore whether joint measurement might improve cardiometabolic risk classification.

It was found that a combination of BMI and WHtR tertiles identified consistent and significant metabolic variable differences relative to those characterised as overweight or obese discordantly by BMI and WHtR. Similarly, associations with cardiometabolic features and inflammatory biomarkers were noticeably increased in subjects classified as overweight or obese by both measures. This indicated that joint measurement may provide a more specific procedure for identifying high-risk patients within overweight and obese BMI/WHtR categories. It was also noted that in a logistic regression model which adjusted for age, gender, physical activity, smoking and alcohol use, only individuals who were classified as obese by both indices displayed a significant and positive association with pre-diabetes. This inferred that the relationship between obesity and diabetes is better indicated at this mode and level of adiposity. In addition, it was observed that patients classified as overweight discordantly also displayed an increased

cardiometabolic risk profile. As a proportion of these individuals might be considered normal weight if either index were used alone, this suggested that joint measurement could also provide an effective procedure for identifying normal weight subjects at increased risk who would not otherwise be detected.

Although our study is the first to explore the utility of a composite index using BMI and WHtR together in a middle-aged Irish population, a number of studies have evaluated joint use of general and central adiposity measures to assess cardiometabolic risk. In a cross-sectional analysis of 2,924 elderly men, Wannamethee et al. [284] found BMI and WC to be of similar value for identifying adverse cardiometabolic feature clustering in ROC analysis. However, it was noted that within normal weight and overweight BMI categories, elevated WC was associated with increased odds of having MetS, leading the authors to recommend using BMI cut-offs for initial assessment, and WC as a complimentary indicator of health risk. In contrast, Ardern et al. [285] found the odds associated with having MetS to be increased in both overweight and obese women with a high WC, but not men, in a study of 7,981 subjects aged 20-74 years. Park et al. [120] showed that the prevalence of metabolic risk factors and MetS were significantly increased across quartiles of WHtR in normal weight men and women aged 20-79 years (N=2,952). Similarly, using a WHtR ≥0.5 threshold value, Ashwell et al. [286] observed that male and female subjects classified as “healthy weight” using BMI had significantly higher cardiometabolic risk factors compared to a group with a healthy BMI and WHtR below 0.5, in a

recent analysis using data from the United Kingdom National Diet and Nutrition Survey (N=1,453).

Contrary to other studies [138], we found that joint measurement significantly improved discrimination of individual cardiometabolic disease features and adverse inflammatory biomarker levels when indices were examined as both continuous and categorical variables, though discriminatory differences were modest. It was also noted that although WHtR was a better overall discriminator of cardiometabolic risk than BMI when compared as a linear variable, significant discriminatory differences between WHtR and BMI were reduced when examined as tertiles. This indicates that both adiposity variables are more likely to have independent effects on cardiometabolic risk when a threshold approach is used, such as employed in a clinical setting. These results suggest that use of BMI and WHtR together may help refine body fat classification. However, as diagnostic risk cut-offs for both indices are defined arbitrarily, optimal categorical thresholds for joint measurement should be examined. The utility of combined univariable cut-points based on linear combinations of both measures should also be explored.

Although the focus of Chapter 6 was on assessing cardiometabolic risk, it was also noted in Chapter 4 that a discrimination model for type 2 diabetes, using age, gender, BMI and WHtR displayed an AUC value of 0.80 (95% CI: 0.76-0.84). However, further analysis (not shown) indicated that this was only marginally higher than the AUC for a model including age, gender and WHtR alone (AUC=0.79, 95% CI: 0.75-0.83), a difference which was not statistically

significant (P=0.08). Nevertheless, both AUCs were significantly higher than models which only included age and gender (AUC=0.63, 95% CI: 0.58-0.67, P<0.001 for both) and are comparable to AUC values obtained in studies which have evaluated non-invasive diabetes risk scores to screen for undiagnosed type 2 diabetes [287-292]. As diabetes risk scores typically include a number of variables, some of which must be subjectively assessed or may be unknown, the findings from Chapters 4 and 6 suggest that accurate adiposity measurement within a clinical setting might provide useful non-subjective, diagnostic or prognostic information a clinician could obtain by performing three, relatively simple, non-invasive procedures.

7.2 Strengths and Limitations of the Research

This section provides a summary of the overall strengths and limitations of this research. The individual strengths and limitations of the five papers included in this thesis have also been acknowledged and discussed in the previous chapters.

As one of the largest cross-sectional studies performed to date within the Republic of Ireland, the Cork and Kerry Diabetes and Heart Disease Study sample size is comparable to other related Irish studies. Strengths include a high participation rate, the use of a range questionnaires to assess lifestyle behaviours, the use of two procedures to classify glycaemic status and the inclusion of a wide range of metabolic markers and anthropometric measurements to define cardiometabolic health.

This thesis addressed a timely and relevant research area within Ireland with regard to adiposity measurement, cardiometabolic disease, type 2 diabetes and the need for effective tools to help identify high-risk patients within clinical practice. The findings from this work are of potential public health and clinical significance in terms of screening, and in particular, the use of stratification based on accurate adiposity measurement as a method for identifying patients with type 2 diabetes and for assessing cardiometabolic risk. The relevance of this work is perceptible in that our results have been presented at a number of scientific conferences, both nationally and internationally (Appendix 4), and have also attracted attention from online media [293-297]. To date, four of the included papers have been published in peer-reviewed scientific journals (Appendix 6) [165-168].

Notwithstanding these strengths, methodological limitations must be considered when assessing findings from this work. The data used in this thesis were derived from a single primary care based sample. In addition, a potential limitation of any cohort study is potential bias because of initial non-response. The current study was based in a single primary care centre which serves a well defined catchment area. The response rate of 67% is typical of response rates currently achieved in developed country settings. Additionally the response rate was similar in men and women and likewise for age groups. Nevertheless, although prevalence rates for overweight/obesity and type 2 diabetes found within our sample are comparable to those observed in other recent nationally representative Irish

studies [13,35,276], the possibility that these data are not representative of the source population must be acknowledged.

However, as mentioned in preceding chapters, it is estimated that 98% of Irish adults are registered with a GP, and that, even in the absence of universal patient registration system, it is possible to perform population-based epidemiological studies that are representative using our methods [210]. Also, as previously discussed, Ireland is an ethnically homogeneous population [242]. Hence, there is little reason to believe that the metabolic relationships we observed would be any different in other middle-aged Irish adults. As random sampling of subjects and the use of validated methods for data collection ensured internal sample validity, it is equally possible that our findings may be generalisable to a similar middle-aged Caucasian-European population.

Of greater concern is that our data were cross-sectional, as this precludes examination of temporal relationships. Thus, although this research suggests a potential rationale for accurate adiposity measurement as a procedure for identifying patients with type 2 diabetes, and also for assessing cardiometabolic disease risk, it does not demonstrate that surrogate measures of adiposity would be clinically useful to predict future diabetes cases. Equally, it does not show that they would be useful for predicting future CVD events or mortality outcomes. These limitations, in particular, will need to be addressed in future work utilising longitudinal data.

7.3 Recommendations

Although central adiposity measurement has been recommended as a method for assessing cardiometabolic health, a recent straw poll of clinicians in the United Kingdom showed that only 10% regularly measured WC [298]. In addition, the need for any type of adiposity measurement within healthcare practice has been questioned, as it has been suggested that a simple "eyeball test" is sufficient in most instances [299]. The findings from this thesis suggest that non-subjective assessment of adiposity provides important information regarding disease and risk status. However, our results also demonstrate that a reluctance to measure WC in clinical practice is not misplaced, as such an assessment may provide additional information compared to BMI, or almost none at all, according to the procedure used for estimating WC. For this reason, we cannot recommend regular WC measurement within healthcare practice in Ireland at the present time.

We have just begun a re-screen of the Mitchelstown Cohort (5 year follow-up) which includes two further WC measurements (iliac crest and umbilical level), in addition to the two previously examined. This will allow us to determine whether rib-level measurement is the optimal procedure for assessing WC in our population, and also to further examine adiposity variable relationships with features of cardiometabolic disease. Importantly, it will also allow us to ascertain whether a composite index, using measures of general and central adiposity, might be clinically useful as a method for predicting type 2 diabetes.

This work will need to be duplicated in other nationally representative Irish studies. The need for a universal WC measurement protocol will also have to be explored in research utilising data from other ethnic populations. In addition, although we suggest that WC measurement would not entail any extra cost when compared to invasive blood testing, it would compete for the limited time a GP or clinician has available during patient appraisal. Therefore, examination of feasibility and cost-implications regarding central adiposity measurement is warranted and should be a focus of future health services research.

Consistent with other research [121,128,143,156], our findings suggest that of the currently used surrogate measures of central adiposity, the WHtR may be the most accurate method for assessing obesity-related risk. Future epidemiological studies which examine adiposity relationships with cardiometabolic features, type 2 diabetes, obesity-related diseases and mortality outcomes should concentrate on this index and BMI. The WHtR is also probably the most practical method for determining central adiposity as it may allow the use of universal risk thresholds [125,300]. Notably, we did not determine cut-points for use within our population as we felt that, in the absence of an agreed WC measurement protocol, this would be premature. Optimal cut-offs using WHtR may differ according to the disease to be predicted. It is also possible that WHtR measurement may only be useful as a pre-screening diagnostic tool to help identify current type 2 diabetes cases within clinical practice.

Nevertheless, results from this thesis suggest that accurate adiposity measurement (through anthropometry or other means) might provide a greater amount of predictive information regarding a patient's future health status than that provided by any other single cardiometabolic disease feature (with the exception of glycaemic status) or many non-invasive variables typically included in diabetes risk scores. However, this is speculative and remains to be determined. Although anthropometry continues to be the preferred method for assessing adiposity within healthcare practice, future studies should also investigate the use of other more sophisticated procedures for measuring body composition, such as bioelectrical impedance or photonic scanning [156]. The development of other low-cost, clinically practical methods for assessing adiposity should also be explored.

Finally, our results may have implications regarding how excess adiposity should be defined within epidemiological research. We noted that a percentage of individuals who might be classified as normal weight be either BMI or WHtR also displayed an increased cardiometabolic risk profile. In addition, cardiometabolic profiles for subjects within each overweight and obese category varied considerably. This suggests a possible explanation for the "obesity paradox", as such individuals might be misclassified and included in a reference category, or a low or high-risk group, in studies which investigate adiposity relationships with morbidity and mortality outcomes [61,99,149]. Therefore, research examining overweight and obese associations with these outcomes should consider defining adiposity using joint measurement. Studies which adjust for adiposity should also

contemplate using both general and central obesity variables in analysis to prevent residual confounding.

7.4 Conclusions

Public health prevention strategies should be prioritised as a method for reducing the prevalence of obesity and obesity-related diseases within Ireland. However, clinicians should be aware that a high percentage of overweight and obese patients will not have type 2 diabetes [67-70] or ever develop the condition and related cardiovascular complications [71,72]. As the prevalence of obesity continues to rise within Ireland [13,14] and other world populations [5], classification of excess adiposity based on BMI measurement alone will increasingly identify a majority of the population as being at risk. In addition, a percentage of subjects who are defined as normal weight by BMI may be at high-risk of obesity-induced chronic disorders [64,164,243,301].

Though no level of increased adiposity should necessarily be considered to be “healthy” at a population level, accurate classification of high-risk subjects is desirable. This is particularly pertinent within healthcare practice, as losing weight is difficult and clinical interventions should be targeted to those most at risk and who would benefit most from earlier identification. Accurate adiposity assessment, using surrogate measures of both general and central adiposity, may be a useful tool for stratifying high and low-risk patients. Other novel adiposity measurement procedures might also prove beneficial. Earlier

identification of subjects at increased risk, and those with type 2 diabetes, could allow earlier targeted interventions to be implemented, thus improving the quality of life of patients affected. Collectively, the results from this thesis may help in improving the characterisation of obesity-related health risk in order to reduce premature mortality and financial costs associated with the obesity epidemic within Ireland.

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APPENDICES

Appendix 1: Supporting Table 1

Univariate odds ratios (95% CI) of having undiagnosed or diagnosed type 2 diabetes compared to no diabetes.

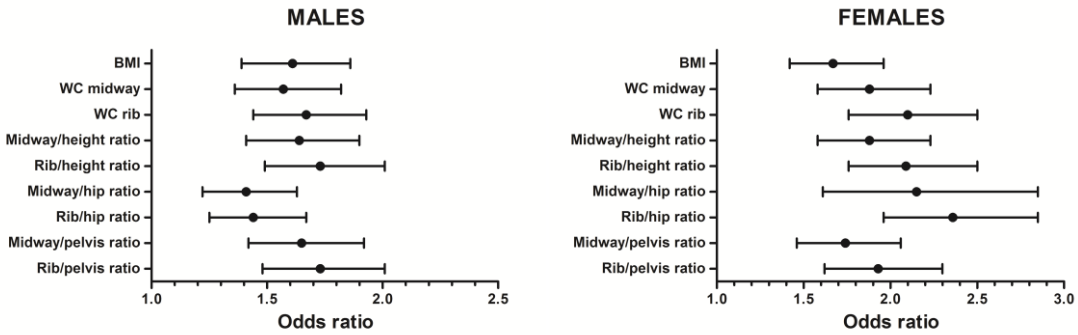
Feature	Odds ratio (95% CI) of having undiagnosed diabetes compared to no diabetes[1]		Odds ratio (95% CI) of having diagnosed diabetes compared to no diabetes[2]	
	Odds ratio	95% CI	Odds ratio	95% CI
Health conditions				
Male	1.6	(1.0-2.6)	2.3	(1.5-3.6)
Age ≥60 years	1.3	(0.8-2.0)	2.0	1.3-3.1)
On Rx for hypertension	2.3	(1.4-3.6)	5.1	(3.4-7.8)
On Rx for cholesterol	1.9	(1.2-3.1)	3.9	(2.6-5.9)
BMI category:				
<25	1		1	
25-29.9	3.1	(1.1-8.9)	11.0	(2.7-45.6)
≥30	8.7	(3.1-24.3)	22.5	(5.5-92.8)
Family diabetes history	2.0	(1.2-3.4)	5.4	(3.6-8.2)
CVD	2.9	(1.6-5.2)	4.0	(2.5-6.3)
Socio-economic				
Education:				
Bachelor or higher	1		1	
Diploma	1.1	(0.3-4.0)	0.9	(0.3-2.9)
Secondary	1.6	(0.6-4.5)	1.6	(0.6-4.2)
Primary only	2.6	(0.9-7.5)	3.3	(1.3-8.5)
Social class:				
High income	1		1	
Middle income	1.6	(0.7-4.0)	1.4	(0.7-2.9)
Low income	1.9	(0.7-4.8)	1.5	(0.8-2.9)
Medical cover				
Health insurance:				
Private insurance	1		1	
State insurance	3.0	(1.8-5.1)	2.4	(1.6-3.6)
No insurance	3.0	(1.6-5.6)	0.8	(0.3-1.7)
Health behaviours				
Physical activity:				
High	1		1	
Moderate	2.8	(1.3-6.1)	1.7	(1.0-2.7)
No physical exercise	7.0	(3.4-14.7)	1.9	(1.1-3.3)
Smoker	1.2	(0.8-2.0)	1.5	(1.0-2.3)
Alcohol use:				
Non-drinker	1		1	
Occasional drinker	0.7	(0.4-1.3)	1.	(0.7-1.8)
Regular drinker	0.6	(0.4-1.1)	0.5	(0.3-0.8)
Metabolic				
Triglycerides ≥1.7	3.7	(2.3-6.0)	2.0	(1.3-3.1)
Non-optimal HDL-C[3]	4.9	(3.0-7.9)	4.8	(3.2-7.3)
Dyslipidaemia[4]	7.2	(4.3-12.2)	3.9	(2.3-6.5)
Hypertension[5]	1.6	(1.0-2.5)	0.9	(0.6-1.4)

[1]Models excluding subjects with diagnosed diabetes.
[2]Models excluding subjects with undiagnosed diabetes.
[3]HDL-C <1.03 (males) or <1.29 (females).
[4]Dyslipidaemia: triglycerides ≥1.7 and HDL-C <1.03 (males) or <1.29 (females).
[5]Hypertension: SBP ≥140 and/or DBP ≥90.

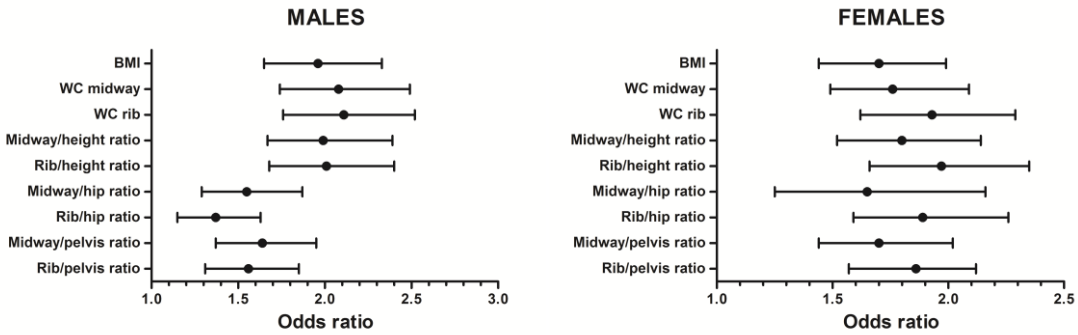
Appendix 2: Supporting Figure 1

Odds ratios (95% CI) of having cardiometabolic risk features for a one standard deviation increase in each adiposity measure.

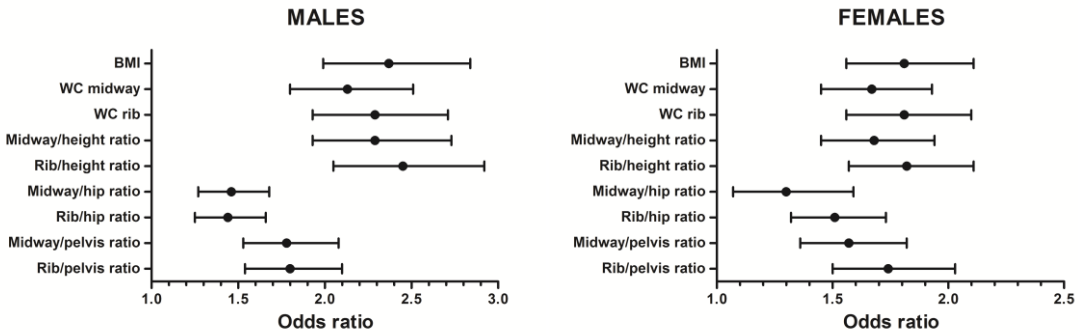
HIGH TRIGLYCERIDES



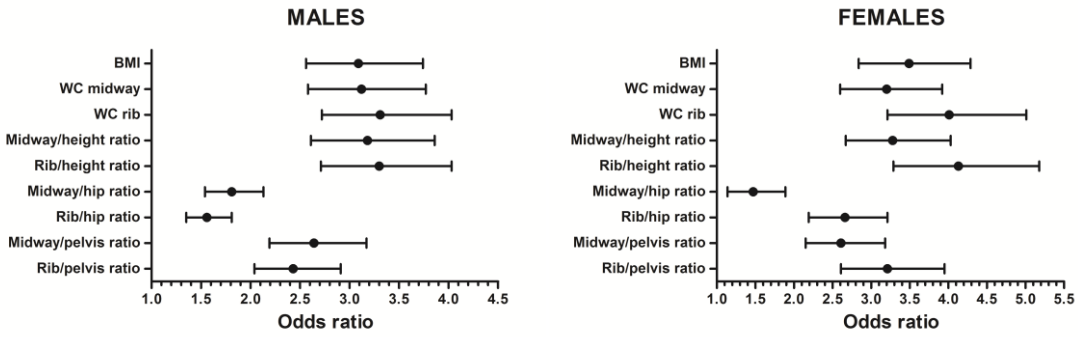
LOW HDL-C



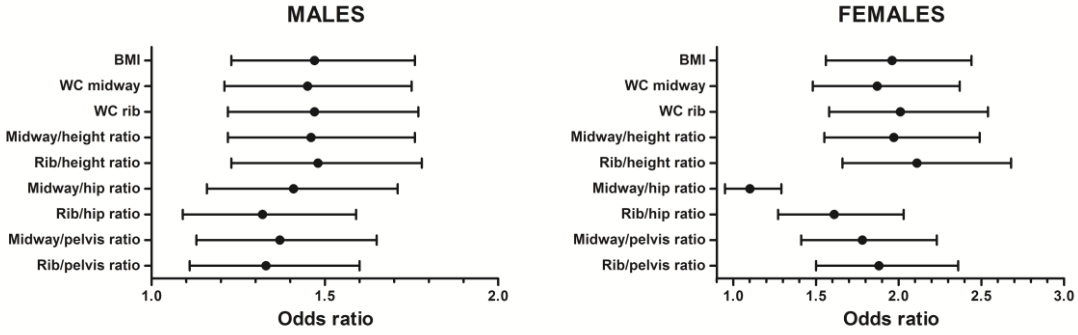
HIGH BP



INSULIN RESISTANCE



IMPAIRED FPG[1]



[1]FPG ≥5.6 mmol/l. Models exclude subjects with type 2 diabetes.

Appendix 3: Supporting Table 2

Relationships between adverse cytokine levels and type 2 diabetes adjusting for either BMI, WC, or both.

Feature	Model 1	Model 2	Model 3	Model 4	Model 5
	Odds ratios (95% CI)				
Either high IL-6 or high TNF-α	2.80 (1.94-4.04)[2]	2.17 (1.48-3.18)[2]	2.02 (1.38-2.97)[2]	2.07 (1.41-3.05)[2]	1.95 (1.26-3.02)[2]
BMI[1]		1.88 (1.60-2.21)[2]		0.70 (0.48-1.03)	0.74 (0.48-1.14)
WC[1]			2.26 (1.89-2.69)[2]	3.13 (2.11-4.64)[2]	2.68 (1.71-4.20)[2]
	Odds ratios (95% CI)				
Both high IL-6 and high TNF-α	3.16 (2.09-4.78)[2]	2.33 (1.50-3.61)[2]	2.03 (1.30-3.18)[2]	2.05 (1.31-3.21)[2]	2.27 (1.36-3.78)[2]
BMI[1]		1.92 (1.63-2.26)[2]		0.73 (0.50-1.07)	0.76 (0.50-1.17)
WC[1]			2.30 (1.93-2.75)[2]	3.07 (2.06-4.57)[2]	2.66 (1.70-4.17)[2]

Model 1 adjusted for age and gender.
Model 2 adjusted for age, gender and BMI.
Model 3 adjusted for age, gender and WC.
Model 4 adjusted for age, gender, BMI and WC.
Model 5 adjusted for age, gender, BMI, WC, use of anti-inflammatory medications, physical activity, smoking and alcohol use.
[1]1 SD increase.
[2]P<0.05.

Appendix 4: Research Outputs and Dissemination

Thesis-related journal articles

Connor JM, Millar SR, Buckley CM, Kearney PM, Perry IJ. (2013) *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* PLOS ONE.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. (2015) *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* PLOS ONE.

Millar SR, Perry IJ, Phillips CM. (2015) *HbA_{1c} Alone Is a Poor Indicator of Cardiometabolic Risk in Middle-Aged Subjects with Pre-Diabetes but is Suitable for Type 2 Diabetes Diagnosis: A Cross-Sectional Study.* PLOS ONE.

Millar SR, Perry IJ, Phillips CM. (2015) *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist–Height Ratio: Are Two Indices Better Than One? A Cross-Sectional Study.* Diabetology & Metabolic Syndrome.

Other journal articles

Gaye A, Marcon Y, Isaeva J, LaFlamme P, Turner A, Jones EM, Minion J, Boyd AW, Newby CJ, Nuotio ML, Wilson R, Butters O, Murtagh B, Demir I, Doiron D, Giepmans L, Wallace SE, Budin-Ljøsne I, Schmidt CO, Boffetta P, Boniol M, Bota M, Carter KW, deKlerk N, Dibben C, Francis RW, Hiekkalinna T, Hveem K, Kvaløy K, Millar SR, Perry IJ, Peters A, Phillips CM, Popham F, Raab G, Reischl E, Sheehan N, Waldenberger M, Perola M, Heuvel EVD, Macleod J, Knoppers BM, Stolk RP, Fortier I, Harris JR, Woffenbuttel BHR, Murtagh MJ, Ferretti V and Burton PR. (2014) *DataSHIELD: Taking the Analysis to the Data, not the Data to the Analysis.* International Journal of Epidemiology.

O'Reilly MA, Millar SR, Buckley, CM, Harrington JM, Perry IJ, Cahill MR. (2015) *Smoking as an Independent Risk Factor for Macrocytosis in Middle-Aged Adults. A Population-Based Observational Study.* American Journal of Hematology.

Published abstracts

Millar SR, Perry IJ, Broeck JVD, Phillips CM. (2014) *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* Journal of Epidemiology and Community Health.

Millar SR, Perry IJ, Phillips CM. (2014) *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* Journal of Epidemiology and Community Health.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. (2014) *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* Diabetes and Primary Care.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. (2014) *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* Diabetes and Primary Care.

Millar SR, Perry IJ, Phillips CM. (2014) *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist–Height Ratio: Are Two Indices Better Than One?* Diabetes and Primary Care.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. (2015) *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* Appetite.

Millar SR, Perry IJ, Phillips CM. (2015) *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist–Height Ratio: Are Two Indices Better Than One?* Appetite.

O Connor D, Griffin DL, O'Sullivan JS, Millar SR, O'Keefe J, Bird BR, Deady S, Murphy CG (2015) *Pre-Surgical Neutrophil-to-Lymphocyte Ratio (NLR) is a Prognostic Indicator of Recurrence Free and Overall Survival in Breast Cancer Patients Undergoing Primary Surgery.* Cancer Research.

Millar SR, Perry IJ, Phillips CM. (2015) *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* European Journal of Epidemiology.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. (2015) *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* European Journal of Epidemiology.

Millar SR, Perry IJ, Phillips CM. (2015) *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist–Height Ratio: Are Two Indices Better Than One?* European Journal of Epidemiology.

Millar SR, Perry IJ, Phillips CM. (2015) *General and Central Obesity Measurement Associations with Markers of Chronic Low-Grade Inflammation and Type 2 Diabetes.* European Journal of Epidemiology.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. (2015) *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* Journal of Epidemiology and Community Health.

Millar SR, Perry IJ, Phillips CM. (2015) *General and Central Obesity Measurement Associations with Markers of Chronic Low-Grade Inflammation and Type 2 Diabetes.* Journal of Epidemiology and Community Health.

Millar SR, Perry IJ, Phillips CM. (2015) *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist–Height Ratio: Are Two Indices Better Than One?* Journal of Epidemiology and Community Health.

Oral presentations

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* 21st European Congress on Obesity, Sofia, Bulgaria; 05/2014.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* ASO UK Congress on Obesity, Birmingham, UK; 09/2014.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* New Horizons in Medical Research Conference, Cork, Ireland; 12/2014.

Oral presentations continued

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* Society for Social Medicine 59th Annual Scientific Meeting, Dublin, Ireland; 09/2015.

Poster presentations

Millar SR, Broeck JVD, Perry IJ, Phillips CM. *Optimal Waist Circumference Measurement Site for Assessing Metabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* 20th European Congress on Obesity, Liverpool, UK; 05/2013.

Millar SR, Broeck JVD, Perry IJ, Phillips CM. *Optimal Waist Circumference Measurement Site for Assessing Metabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* CoMH Research Day, Cork, Ireland; 06/2013.

Millar SR, Broeck JVD, Perry IJ, Phillips CM. *Optimal Waist Circumference Measurement Site for Assessing Metabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* HRB Centre for Health and Diet Research Conference, Cork, Ireland; 10/2013.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* CoMH Research Day, Cork, Ireland; 06/2014.

Millar SR, Perry IJ, Phillips CM. *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* CoMH Research Day, Cork, Ireland; 06/2014.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* CoMH Research Day, Cork, Ireland; 06/2014.

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* CoMH Research Day, Cork, Ireland; 06/2014.

Poster presentations continued

Millar SR, Perry IJ, Phillips CM. *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* 3rd International Congress on Personalized Medicine, Prague, Czech Republic; 06/2014.

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* 3rd International Congress on Personalized Medicine, Prague, Czech Republic; 06/2014.

Millar SR, Broeck JVD, Perry IJ, Phillips CM. *Optimal Waist Circumference Measurement Site for Assessing Metabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* 20th IEA World Congress of Epidemiology, Anchorage, Alaska, USA; 08/2014. 2nd Place Student Award.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* 20th IEA World Congress of Epidemiology, Anchorage, Alaska, USA; 08/2014.

Millar SR, Perry IJ, Phillips CM. *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* Society for Social Medicine 58th Annual Scientific Meeting, Oxford, UK; 09/2014.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* Society for Social Medicine 58th Annual Scientific Meeting, Oxford, UK; 09/2014.

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* ASO UK Congress on Obesity, Birmingham, UK; 09/2014.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* 10th National Conference of the Primary Care Diabetes Society, Birmingham, UK; 11/2014.

Poster presentations continued

Millar SR, Perry IJ, Broeck JVD, Phillips CM. *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* 10th National Conference of the Primary Care Diabetes Society, Birmingham, UK; 11/2014.

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* 10th National Conference of the Primary Care Diabetes Society, Birmingham, UK; 11/2014.

Millar SR, Perry IJ, Phillips CM. *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* New Horizons in Medical Research Conference, Cork, Ireland; 12/2014.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* New Horizons in Medical Research Conference, Cork, Ireland; 12/2014.

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* New Horizons in Medical Research Conference, Cork, Ireland; 12/2014.

Millar SR, Perry IJ, Phillips CM. *General and Central Obesity Measurement Associations with Markers of Chronic Low-Grade Inflammation and Type 2 Diabetes.* 22nd European Congress on Obesity, Prague, Czech Republic; 05/2015.

Millar SR, Perry IJ, Phillips CM. *Cardiometabolic Risk Profiles in Pre-Diabetes and Diabetes Defined by Fasting Plasma Glucose and HbA_{1c} Levels in Middle-Aged Adults.* European Congress of Epidemiology, Maastricht, Netherlands; 06/2015.

Millar SR, Perry IJ, Broeck JVD, Phillips CM. *Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults.* European Congress of Epidemiology, Maastricht, Netherlands; 06/2015.

Poster presentations continued

Millar SR, Perry IJ, Phillips CM. *Assessing Cardiometabolic Risk in Middle-Aged Adults Using Body Mass Index and Waist-Height Ratio – Are Two Indices Better Than One?* European Congress of Epidemiology, Maastricht, Netherlands; 06/2015.

Millar SR, Perry IJ, Phillips CM. *General and Central Obesity Measurement Associations with Markers of Chronic Low-Grade Inflammation and Type 2 Diabetes.* European Congress of Epidemiology, Maastricht, Netherlands; 06/2015.

Millar SR, Connor JM, Buckley CM, Kearney PM, Perry IJ. *The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults.* Society for Social Medicine 59th Annual Scientific Meeting, Dublin, Ireland; 09/2015.

Millar SR, Perry IJ, Phillips CM. *General and Central Obesity Measurement Associations with Markers of Chronic Low-Grade Inflammation and Type 2 Diabetes.* Society for Social Medicine 59th Annual Scientific Meeting, Dublin, Ireland; 09/2015.

Appendix 5: Anthropometric Measurement Procedures

1.0 Weight Measurements

1.1 Introduction

Height and weight measurements provide the necessary details to calculate the body mass index of participants.

1.2 Responsibilities

Research nurses trained in the method are responsible for recording weight measurements for all participants.

1.3 Equipment

- Portable electronic TANITA WB-100MA weighing scale
- Calibration weights (75kgs)

1.4 Methods

1.4.1 Set up requirements:

- Make sure the scales are placed on a firm, flat surface. Place the display unit on an even surface nearby. Do not place the scales on carpet, sloping surfaces or rough, uneven surfaces.

1.4.2 Calibration

- It will be necessary to calibrate the scales on a regular basis to ensure it accurately records weight on an ongoing basis.
- The weighing scales will have been calibrated by the suppliers before the study commenced (June 2008).
- Further calibration will be carried out weekly in the department using the 75kg calibration weights. Any calibration drifts should be recorded in the calibration record book and the research nurse co-ordinator informed (Vera Mc Carthy).

1.4.3 Procedure:

- Ask the participant to remove heavy outer garments (jackets, coats etc) and footwear (shoes, slippers, sandals etc) and socks.
- Ask the participant to step onto the centre of the scale with one foot on each side of the scale (not having weight distributed evenly may affect measurement)
- Ask the participant to:
    - stand still
    - face forward
    - place arms by their side and
    - wait until asked to step off
- Record the weight in kilograms on the Clinical Report Form.

Important guidelines regarding the use of the weighing scales:

- Use only the adapter provided with the scale. This adapter should have TANITA written on it.
- Do not wrap cables around the screen part of the scale.
- Place the scales in the bag with the screen facing down.

2.0 Height Measurements

2.1 Introduction

Height should be measured in all selected respondents, except wheelchair bound individuals, persons who have difficulty standing steady or straight, and participants with a hairstyle or headdress (e.g. turban) that prevent proper use of the height measuring equipment.

2.2 Responsibilities

Research nurses trained in the method are responsible for recording weight measurements for all participants.

2.3 Equipment

- Portable Seca Leicester height/length measuring rule

2.4 Methods

2.4.1 Requirements for examination

- Ask the participant to remove their:
  - footwear (shoes, slippers, sandals etc)
  - heavy outer clothes (coat, jackets etc)
  - head gear (hat, cap, hair bows, comb, ribbons, etc). Note: If it would be insensitive to seek removal of a scarf or veil, the measurement may be taken over light fabric.
- Ask the participant to stand on the Standiometer Platform facing you with:
  - feet together
  - knees straight
- Ask the participant to look straight ahead and not look up (make sure eyes are the same level as the ears).
- Move the measure arm gently down onto the head of the participant and ask the participant to breathe in and stand tall.
- Record the height measurement in centimetres on the Clinical Report Form.

3.0 Central Adiposity Measurements: Waist Circumference, Hip Circumference and Pelvic Width

3.1 Introduction

A variety of different measurements can be used to predict body density or fat content. For this study we will assess waist circumference, hip circumference and pelvic width.

3.2 Responsibilities

Research nurses trained in the method are responsible for recording waist circumference for all participants.

3.3 Equipment

- Seca 200 measuring tape
- Marker pen
- Plastic 15cm ruler

3.4 Methods

3.4.1 Calibration

- Calibration should be conducted with a steel tape measure on the last day of each month.
- Set the tape measure to obtain a reading of 50.0cm and then measure the actual distance with the steel tape measure. Repeat this procedure for 100.0cm, 150.0, and 200.0 cm.
- All readings should be recorded in the Tape Measure Calibration Log (Appendix 4).
- If a discrepancy is identified contact the research nurse co-ordinator (Ms Vera Mc Carthy) who will organise a replacement tape.

3.4.2 Set up requirements

Ideally, this measurement should be taken against the skin, but if participant prefers, it may be taken over thin layer of clothing e.g. a vest or t-shirt. It must not be taken over thick or bulky clothing.

How to take the measurement:

This measurement should be taken:

- At the end of a normal expiration [breath out]
- With the arms relaxed at the sides
- Under the midline of the participant's armpit, at the midpoint between the lower part of the last rib and the top of the hip.

3.5 Procedure for (Midway) Waist Circumference

- Ask the participant to:
  - stand with their feet together and pointing forward,
  - place their arms at their side with the palms of their hands facing inwards, and
  - breathe gently and relax the abdomen [If you feel the respondent is trying to 'hold in' their abdomen, engage them in conversation so that they relax].
  - Feel for the subject's lower rib margin in the mid-axillary line and make a mark (with the marker pen) on the skin at this point.
  - Palpate the iliac crest in the mid-axillary line and make a mark on the skin surface.
  - Using the plastic ruler, measure the distance between these two points. Now make a distinct mark half-way between these two points on the skin surface.
  - Ensure that you make the marks on both sides of the body as this improves the reproducibility of the measurement.
  - Apply the metric tape horizontally around the subject's body, line the tape over the two middle marks and ensure that it is sitting evenly, not tilted up on one side. Tighten/loosen the tape so that it sits comfortably around the subject's body. The tape should be tight enough so that you can just put two fingers underneath it.
  - Instruct the subject to breathe in and then breathe out and hold, at the time the measurement is taken.
  - Record the measurement in centimetres in the clinical report form. Take two measurements on each participant to test for reproducibility.

NOTE:	**Use the side of the tape measure which begins at 5cm-this side takes into account the 5cm for the box**

3.6 Procedure for Waist Circumference - Lowest Rib

- Ask the participant to:
  - stand with their feet together and pointing forward,
  - place their arms at their side with the palms of their hands facing inwards, and
  - breathe gently and relax the abdomen [If you feel the respondent is trying to 'hold in' their abdomen, engage them in conversation so that they relax].
- Feel for the subject's lower rib margin in the mid-axillary line and make a mark (with the marker pen) on the skin at this point.
- Apply the metric tape horizontally around the subject's body, line the tape over mark at the lower rib margin. Tighten/loosen the tape so that it sits comfortably around the subject's body. The tape should be tight enough so that you can just put two fingers underneath it.
- Instruct the subject to breathe in and then breathe out and hold, at the time the measurement is taken.

- Record the measurement in centimetres in the clinical report form. Take two measurements on each participant to test for reproducibility.

NOTE:	**Use the side of the tape measure which begins at 5cm-this side takes into account the 5cm for the box**

3.7 Procedure for Hip circumference

- Following on from the waist circumference, ensure the subject remains in the same position and breathing normally.
- With the metric tape, measure the point yielding the maximum circumference around the hips. As before the tape should sit horizontally around the body, without a tilt and should allow two fingers to slide under it.
- Record the measurement in centimetres in the clinical report form. Take two measurements on each subject to test for reproducibility.
- Participation in the procedure is now complete.

3.8 Procedure for Pelvic Width Measurement

- Bi-iliac breadth, is the maximum diameter between right and left iliac crests measured from the rear of the participant. Ask the participant to:
  - stand with their feet approximately 5cm apart to prevent them from swaying.
  - fold their arms across their chest,
  - measurement, using the calipers, is done from behind the participant.
  - palpate the landmarks, the outer edges of the upper iliac bones,
  - apply the caliper to these landmarks at a 45^{o} angle ensuring that the maximum breadth is recorded and applying gentle pressure
  - take two separate measurements on each participant for reproducibility and record on the clinical report form.

3.9 Additional information

To ensure the reproducibility of anthropometric readings it is crucial to maintain the standards laid down in the anthropometric standard operating procedures document.

Appendix 6: Published Papers

OPEN ACCESS Freely available online

PLOS ONE

The Prevalence and Determinants of Undiagnosed and Diagnosed Type 2 Diabetes in Middle-Aged Irish Adults

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Abstract

Background: The prevalence of type 2 diabetes within the Republic of Ireland is poorly defined, although a recent report suggested 135,000 cases in adults aged 45+, with approximately one-third of these undiagnosed. This study aims to assess the prevalence of undiagnosed and diagnosed diabetes in middle-aged adults, and compare features related to either condition, in order to investigate why certain individuals remain undetected.

Methods: This was a cross-sectional study involving a sample of 2,047 men and women, aged between 50–69 years, randomly selected from a large primary care centre. Univariate logistic regression was used to explore socio-economic, metabolic and other health related variable associations with undiagnosed or diagnosed diabetes. A final multivariate analysis was used to determine odds ratios and 95% confidence intervals for having undiagnosed compared to diagnosed diabetes, adjusted for gender, age and significant covariates determined from univariate models.

Principle Findings: The total prevalence of diabetes was 8.5% (95% CI: 7.4%–8.8%); 72 subjects (3.5%) had undiagnosed diabetes (95% CI: 2.8%–4.4%) and 102 subjects (5.0%) had diagnosed diabetes (95% CI: 4.1%–6.0%). Obesity, dyslipidaemia, and family history of diabetes were positively associated with both undiagnosed and diagnosed type 2 diabetes. Compared with diagnosed subjects, study participants with undiagnosed diabetes were significantly more likely to have low levels of physical activity and were less likely to be on treatment for diabetes-related conditions or to have private medical insurance.

Conclusions: The prevalence of diabetes within the Cork and Kerry Diabetes and Heart Disease Study is comparable to recent estimates from the Slán National Health and Lifestyle Survey, a study which was nationally representative of the general population. A considerable proportion of diabetes cases were undiagnosed (41%), emphasising the need for more effective detection strategies and equitable access to primary healthcare.

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Introduction

Type 2 Diabetes mellitus (T2DM), a chronic disease which causes significant morbidity and mortality, was the ninth leading cause of death worldwide in 2008 [1]. Diabetes is associated with obesity, dyslipidaemia and hypertension, and is characterised by chronic hyperglycaemia due to insufficient insulin release, impaired insulin action, or a combination of both [2]. Importantly, the persistent hyperglycaemia that is associated with diabetes may cause serious health complications such as cardiovascular disease (CVD) and impairment and malfunction of the renal, ophthalmic, vascular and nervous systems. These complications pose significant financial burdens on healthcare services; research conducted in 2006, which examined economic consequences related to T2DM, estimated that almost 10% of total health expenditure was spent on diabetes care in the Republic of Ireland (ROI) alone [3].

The prevalence of T2DM is increasing globally, representing a key public health issue [4]. There is a lack of research relating to diabetes in Ireland, although recent studies have indicated that the condition may be reaching epidemic proportions [5,6]. In 1998, the prevalence of T2DM amongst subjects in a primary care based sample was estimated to be 3.9% [7]. A recent report from the Irish Institute of Public Health (IPH) [8] based on findings from the 2007 Slán National Health and Lifestyle Survey [9], suggested a prevalence of 8.9% in adults aged 45+. This estimate consisted of 94,000 subjects who had clinically diagnosed T2DM and 41,000 with undiagnosed diabetes. While the efficacy and cost-effectiveness of routine screening for diabetes in primary care has not been established [10–12], there is an ongoing need for contemporary data on the prevalence of T2DM, in population and primary care settings, in order to guide policy in this area. This could help formulate strategies that further develop effective diabetes prevention, detection and management, as individuals with undiagnosed T2DM are at high risk of diabetic complications [13].

The aim of this study was to estimate the prevalence of both undiagnosed and diagnosed T2DM in a sample of men and women aged 50–69 years, drawn from a primary care setting similar to that studied in 1998 [7], using the same field survey procedures and methods. In particular, we determined the extent

to which the probability of T2DM diagnosis is influenced by access to primary care as defined by health insurance status.

Methods

Study population

This research makes use of data from the Cork and Kerry Diabetes and Heart Disease Study (Phase II), a single centre, cross-sectional study conducted between 2010 and 2011. A population representative random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic includes eight General Practitioners (GP) and serves a population of approximately 20,000, with a mix of urban and rural residents. The name, address, gender and date of birth were provided for all registered attending patients and stratified random sampling by age and sex was employed to recruit equal numbers of men and women in four quartiles between the ages of 50 and 69 years. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). The status of non-responders included individuals refusing to participate (59.4%) and those who did not reply (40.6%). Male subjects accounted for 53.7% of non-responders while 43.5% (vs. 42.8% of responders) were >60 years of age. All non-responders were followed up with a phone call where possible and otherwise with a single postal reminder. Details regarding the study design, sampling procedures and methods of data collection have been reported previously [14].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

Clinical and laboratory measurements

The weight and height of each subject were measured to the nearest 0.1 kg and 0.1 cm respectively by trained researchers. Study participants were asked to remove heavy outer clothing and footwear. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was measured using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK). Two measurements of weight and height were taken for each subject and the mean value of these were used in the analysis. Three measurements of systolic and diastolic blood pressure (SBP and DBP respectively) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered as a subject's blood pressure. After an overnight fast, all participants were invited to attend the clinic for the sampling of blood between 8 and 10 A.M. Triglyceride (TAG), high density lipoprotein cholesterol (HDL-C) and fasting plasma glucose (FPG) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica Gmbh, Hamburg, Germany). Glucose concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland). Glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 (Tosoh HLD-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium). A self-administered General Health Questionnaire (GHQ) was used to collect supplementary information which included medication use, demographic characteristics, medical cover, family T2DM history, past medical history of CVD and smoking and alcohol behaviours. Physical activity levels were assessed using the validated International Physical Activity Questionnaire (IPAQ) [15].

Metabolic and anthropometric classifications

Metabolic features were categorised according to International Diabetes Federation metabolic syndrome (MetS) criteria cut-points [16]. Abnormal metabolic risks were defined as FPG ≥5.6 mmol/L, TAG ≥1.7 mmol/L and HDL-C <1.03 mmol/L in males and HDL-C <1.29 mmol/L in females. Dyslipidaemia was determined according to elevated TAG and low HDL-C levels. Hypertension was defined as SBP ≥140 mmHg) and/or DBP ≥90 mmHg [17]. Body Mass Index (BMI) was calculated by dividing a subject's weight by the square of their height and was categorised as <25= *Normal-weight*, 25–29.9= *Overweight*, and ≥30= *Obese* [18,19].

Morbidity

Type 2 diabetes was defined as HbA_{1c} ≥6.5% (N = 146). Undiagnosed diabetes was determined if subjects had positive HbA_{1c} tests but did not report a medical diagnosis of T2DM or oral medication use for the condition (N = 72). Diagnosed diabetes was classified according to positive test results and self-reported doctor diagnosis or diabetes medication use (N = 74), or by diagnosis or medication use alone (N = 28, total diagnosed = 102). The presence of CVD was obtained from the GHQ by asking study participants if they had been diagnosed with one of the following seven conditions: *Heart Attack* (including coronary thrombosis or myocardial infarction), *Heart Failure*, *Angina*, *Aortic Aneurysm*, *Hardening of the Arteries*, *Stroke* or any other *Heart Trouble*. Subjects indicating a diagnosis of any of these disorders were classified as having CVD.

Covariates

Covariates utilised from the GHQ included gender, age, use of prescription (Rx) anti-hypertensive and cholesterol-lowering medication, family T2DM or CVD history, education, social class, medical cover, physical activity levels, smoking status and alcohol use. Age was included either as a dichotomous (<60/≥60 years of age) or continuous variable in univariate or multivariate regression models. Education was divided into four categories: *Primary*, *Secondary*, *Diploma* and *Bachelor or Higher*. Social class was defined according to the European Socio-economic Classification System (ESeC) [20,21], and collapsed into three groups: *High Income*, *Middle Income and Low Income*. The health service variables – *Private Insurance*, *No Insurance*, and means-tested, state-assisted *General Practice Visit Card* (GPC) and *Full Medical Card* (FMC) – were transformed into a dummy variable: *Private Insurance*, *State Insurance*, *No Insurance*. Subjects reporting more than one insurance type were assigned to the higher insurance category. Self-reported physical activity within the previous six months, measured using the IPAQ questionnaire [15], was divided into three categories: *High*, *Moderate* and *No Physical Exercise*. Alcohol use was assessed by asking study participants how often they consumed alcohol on a monthly or weekly basis, and was classified as follows: 'never or less than once a month' - *Non-drinker*, '2–4 times monthly' - *Occasional Drinker*, and 'twice or more weekly' - *Regular Drinker*.

Subjects were considered current smokers if they smoked cigarettes during the recruitment phase, had smoked within the last 10 years or had smoked more than 100 cigarettes in their lifetime, and non-smokers if they had smoked less than this or had never smoked.

Statistical analysis

The descriptive statistics (mean, median, standard deviation, inter-quartiles and percentage distribution) of the study population were examined by diabetes status. Gender differences in T2DM prevalence were compared using chi-square tests. The relationships between health conditions, health behaviours, health insurance status and metabolic/socio-economic factors for individuals with undiagnosed or diagnosed T2DM were explored through multiple univariate binary logistic regressions. Diagnosed subjects were excluded from models examining undiagnosed T2DM, while models investigating associations between features and diagnosed diabetes excluded undiagnosed cases. Distinctions between undiagnosed and diagnosed T2DM were explored in univariate analyses excluding non-diabetic participants.

To further compare feature/morbidity relationships and strengths of association to either undiagnosed or diagnosed T2DM, multivariate logistic regressions were performed. To select independent predictor variables (IPV) to be included in analysis, IPVs that had a P-value of less than 0.2 in univariate models were included in stepwise forward and backwards entry elimination multivariate analysis, with model stability assessed using the likelihood ratio (LR). Variables indicating a significant relationship ($P<0.05$) with either condition were then entered sequentially, by order of magnitude of the chi-square association, into two independent logistic regressions, adjusted for gender and age as a dichotomous ($<60/\geq 60$) variable. Using the same procedures, a final multivariate model comparing undiagnosed to diagnosed T2DM was determined, adjusted for gender and age as a continuous measure.

The discriminatory properties of specific IPVs indentified in multivariate analysis were evaluated. Models including these variables were assessed for their ability to detect undiagnosed or diagnosed T2DM using the *c* statistic. The *c* statistic is identical to the area under the receiver operating characteristic curve (AUC) with values ranging from 0.5 (no better than chance) to 1.0 (indicating perfect discrimination) [22].

Primary data analysis was conducted using PASW Statistics version 18 (SPSS, Chicago, IL, USA) for Windows. Confidence intervals for prevalence proportions were calculated using the VasserStats statistical website [23]. For all analyses, a P-value (two-tailed) of less than 0.05 was considered to indicate statistical significance. Glycated haemoglobin A_{1c} test results and diagnostic status information were available for 1,995 (97.5%) and 1,999 (97.7%) subjects respectively. Missing dichotomous predictor data values were assumed to be negative, while missing continuous data were assumed to be ignorable and missing at random.

Results

The baseline characteristics of the study population for participants with undiagnosed, diagnosed and no diabetes are shown in table 1. The total prevalence of T2DM was 8.5% (95% CI: 7.4%–8.8%); 102 (5.0%) subjects had diagnosed diabetes (95% CI: 4.1%–6.0%) and 72 (3.5%) had undiagnosed diabetes (95% CI: 2.8%–4.4%), representing 41.4% of all diabetes cases. A significantly greater proportion of male subjects 11.1% ($N=112$) had T2DM compared to females 6.0% ($N=62$, $P<0.001$), and a greater proportion of males had both undiagnosed and diagnosed T2DM. A high proportion of diabetes cases were overweight or obese, used Rx anti-hypertensive and cholesterol-lowering medications, had a family history of diabetes or previous history of CVD, finished education at primary level and reported having low levels of physical activity within the previous six months. Variations in health insurance were also noted, with a greater proportion of T2DM subjects having state-assisted healthcare.

In univariate analysis (table S1), overweight and obesity, family diabetes and CVD history, elevated TAG, low HDL-C and dyslipidaemia were significantly associated with both undiagnosed and diagnosed T2DM. Associations between reduced physical activity levels and T2DM were noticeably strong, with seven-fold and approximate two-fold increased odds for undiagnosed or diagnosed T2DM respectively. With regard to health services related factors, there was a two-fold increased likelihood of undiagnosed T2DM in patients on treatment for hypertension versus a five-fold increased odds for diagnosed diabetes. Similarly, the odds of having undiagnosed T2DM were approximately two-fold higher in patients on treatment with cholesterol-lowering therapy versus an approximate four-fold increase for diagnosed diabetes. The probability of both undiagnosed and diagnosed T2DM was significantly reduced in patients with private medical insurance, whilst the odds of having undiagnosed diabetes were significantly increased in subjects with no medical insurance (OR: 3.0, 95% CI: 1.6–5.6).

Multivariate analysis (table 2) revealed overweight and obesity, use of cholesterol-lowering medication, family T2DM history and dyslipidaemia to be associated with both undiagnosed and diagnosed T2DM. Low level physical activity (OR: 5.8, 95% CI: 2.7–12.5) and health service variables remained significant determinants of undiagnosed diabetes, with odds that were approximately two-fold higher in subjects with state-assisted healthcare and for participants without medical insurance. Characteristics associated with diagnosed T2DM included CVD history, Rx anti-hypertensive therapy and alcohol use. In addition, male subjects were statistically more likely to have diagnosed diabetes compared to females (OR: 2.5, 95% CI: 1.5–4.1).

Table 3 shows univariate odds ratios for undiagnosed compared to diagnosed T2DM. Within this sub-sample of diabetes cases, significant effects were observed for medication use, family T2DM history, TAG levels and dylipidaemia. Both health insurance and physical activity IPVs demonstrated strong associations for having undiagnosed T2DM, with approximate four-fold increased odds in subjects without healthcare insurance and in those reporting low levels of physical activity. Individuals with undiagnosed T2DM were also more likely to have a higher BMI. Overall, metabolic features were less optimal in undiagnosed cases, and a greater proportion had uncontrolled hypertension.

Results from multivariate analysis comparing undiagnosed to diagnosed T2DM are presented in table 4. Significant associations were noted for BMI (continuous) and physical inactivity. Undiagnosed participants were significantly less likely to be on treatment for hypertension or to have a family history of T2DM relative to subjects with diagnosed diabetes.

Figures S1 and S2 show AUCs for models to discriminate undiagnosed or diagnosed T2DM (compared to no diabetes). Models which included both health insurance and physical activity IPVs showed a higher discriminatory capacity to detect undiagnosed T2DM (*c*: 0.735, 95% CI: 0.668–0.801) compared to diagnosed T2DM (*c*: 0.608, 95% CI: 0.544–0.671). A model including health insurance, physical activity and BMI (continuous) displayed further improved discrimination, (*c*: 0.814, 95% CI: 0.758–0.871) for undiagnosed T2DM vs. (*c*: 0.698, 95% CI: 0.646–0.750) for diagnosed subjects.

Table 1. Characteristics of the study population.[1]

Feature	No diabetes	Undiagnosed diabetes	Diagnosed diabetes
	N = 1873 (91.5%)	N = 72 (3.5%)	N = 102 (5.0%)
Health conditions			
Male	893 (47.8)	43 (59.7)	69 (67.6)
Age	59.0 (54.0–64.0)	60.0 (56.3–65.0)	62.0 (57.0–65.0)
Age ≥60	875 (46.9)	38 (52.8)	65 (63.7)
On Rx for hypertension	486 (26.0)	32 (44.4)	66 (64.7)
On Rx for cholesterol	609 (32.6)	35 (48.6)	67 (65.7)
BMI (kg/m^2)	28.29±4.6	33.06±6.3	31.19±4.4
BMI category:			
<25	439 (23.6)	4 (5.6)	2 (2.0)
25–29.9	857 (46.0)	24 (33.3)	43 (42.2)
≥30	566 (30.4)	44 (61.1)	57 (55.9)
Family history of T2DM	315 (16.9)	21 (29.2)	54 (52.9)
CVD	167 (8.9)	16 (22.2)	29 (28.4)
Socio-economic			
Education:			
Bachelor or higher	175 (10.0)	4 (5.9)	5 (5.3)
Diploma	239 (13.7)	6 (8.8)	6 (6.3)
Secondary	863 (49.5)	31 (45.6)	40 (42.1)
Primary only	466 (26.7)	27 (39.7)	44 (46.3)
Social class:			
High income	244 (18.2)	6 (11.5)	11 (13.3)
Middle income	396 (29.5)	18 (34.6)	25 (30.1)
Low income	704 (52.4)	28 (53.8)	47 (56.6)
Medical cover			
Health insurance:			
Private insurance	1196 (64.0)	27 (37.5)	51 (50.0)
State insurance	437 (23.4)	29 (40.3)	44 (43.1)
No insurance	236 (12.6)	16 (22.2)	7 (6.9)
Health behaviours			
Physical activity:			
High	795 (48.4)	10 (17.5)	31 (34.8)
Moderate	536 (32.6)	19 (33.3)	35 (39.3)
No physical exercise	313 (19.0)	28 (49.1)	23 (25.8)
Smoker	889 (47.6)	38 (52.8)	60 (58.8)
Alcohol use:			
Non-drinker	800 (44.7)	38 (55.1)	54 (53.5)
Occasional drinker	367 (20.5)	12 (17.4)	27 (26.7)
Regular drinker	623 (34.8)	19 (27.5)	20 (19.8)
Metabolic			
FPG (mmol/L)	4.90 (4.6–5.3)	6.60 (5.6–7.5)	7.50 (5.7–9.4)
FPG ≥5.6	238 (13.1)	58 (80.6)	80 (80.8)
TAG (mmol/L)	1.19 (0.9–1.6)	1.80 (1.3–2.4)	1.36 (1.0–2.0)
TAG≥1.7	417 (23.0)	37 (52.9)	36 (37.5)
HDL-C (mmol/L)	1.48±0.4	1.22±0.3	1.18±0.3
HDL-C (non-optimal)[2]	267 (14.7)	32 (45.7)	45 (45.0)
Dyslipidaemia[3]	122 (6.7)	24 (34.3)	21 (21.0)
SBP (mmHg)	129.25±16.7	134.18±19.3	132.94±16.4
DBP (mmHg)	80.20±9.7	80.12±10.9	78.79±9.5

Table 1. Cont.

Feature	No diabetes	Undiagnosed diabetes	Diagnosed diabetes
	N = 1873 (91.5%)	N = 72 (3.5%)	N = 102 (5.0%)
Hypertension[4]	552 (29.7)	28 (39.4)	28 (27.5)

[1]Mean and ± SD are shown for continuous and % are shown for categorical variables. Age, FPG and TAG are shown as a median (interquartile range). Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.
[2]HDL-C: <1.03 (MALES) <1.29 (FEMALES).
[3]Dyslipidaemia: TAG≥1.7 and HDL-C: <1.03 (MALES) <1.29 (FEMALES).
[4]Hypertension: SBP≥140 and/or DBP≥90.
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Discussion

The results from previous research investigating the prevalence of T2DM within the ROI are conflicting. In 1998 a study conducted by Perry et al. [7] suggested an overall prevalence of 3.9%, 30% of whom were undiagnosed, whereas research in 2003, examining T2DM in primary care [24], estimated a population prevalence of 9.2%, with undiagnosed subjects representing 23.5% of all cases. The variance between these studies is possibly explained by the differences in age groups assessed, or by methods used for diabetes detection. The higher prevalence of undiagnosed T2DM identified within the present study population may be due to use of the HbA_{1c} procedure as compared to the FPG test that was more commonly employed in the ROI before 2010. Research conducted in Germany and the United States (US), which compared FPG and Oral Glucose Tolerance Test methods, reported that overall prevalence of T2DM would have been lower had diabetes been classified by FPG [25,26]. The present study also observed that 14 (19%) undiagnosed subjects (who were positively identified according to HbA_{1c} concentrations) had FPG levels that were less than 5.6 mmol/L, and would have been classified as non-diabetic if this method had been used for diagnostic purposes within the Cork and Kerry Study. This finding is consistent with other studies which have reported variations between HbA_{1c} and FPG [27–29]. Although a recent report from the US implied that use of the HbA_{1c} assay would not significantly alter T2DM prevalence and that diabetes categorisation would remain unchanged in 97.7% of subjects [30], evidence is still equivocal [31]. Several studies have shown poor concordance between HbA_{1c} and FPG [27], in particular regarding pre-diabetes classification [32–34]. Additionally, factors such as age or ethnicity are thought to influence results [27,28,35]. Nevertheless, as discussed by Bonora et al., comparisons between diagnostic methods for T2DM detection are ambiguous, as a true gold standard test is unavailable [36].

The recent Irish IPH report [8], based on the nationally representative 2007 Slán National Health and Lifestyle Survey [9] (which also used the HbA_{1c} test), estimated the prevalence of T2DM in adults 45+ to be 8.9%, which is similar to the result suggested by this study. In the IPH report, undiagnosed diabetes prevalence was determined to be 2.7% (30% of all diabetes cases). Of note, however, is that the IPH research estimated the prevalence of both undiagnosed and diagnosed T2DM in adults aged between 55–64 to be 4.6% and 6.3% respectively, which are comparable to outcomes attained from this study population (3.6% and 5.6%), for the same age group. Also of interest, is that results from the Slán data are consistent with the present study's finding that the prevalence of T2DM in middle-aged subjects within the ROI is higher in men. Although this gender disparity may be a consequence of selection bias due to non-response, similarity in outcomes between the 2007 Slán survey and the Cork and Kerry Diabetes and Heart Disease Study imply that observed prevalence estimates are valid. It is possible that the lower prevalence of T2DM in women may be as a result of random opportunistic screening due to higher GP consultation rates observed in females [37]. An alternative explanation may be the higher prevalence of overweight and obesity observed in male subjects within this population (males: 85.8% vs. females: 70.6%, P for difference <0.001), a relationship noted in previous research examining obesity within Ireland [37,38].

As numerous studies have indicated, non-optimal metabolic risk factors such as elevated TAG and low HDL-C were significantly and positively associated with both undiagnosed and diagnosed T2DM [25,26,39]. A noticeable feature of undiagnosed subjects was the higher percentage of cases with uncontrolled hypertension, increased TAG concentrations and dyslipidaemia, perhaps reflecting access to treatment, as a greater proportion of diagnosed subjects used Rx anti-hypertensive and cholesterol-lowering medications. Undiagnosed individuals were also less likely to have a family history of T2DM and CVD, or to engage in regular physical activity compared to diagnosed subjects. Nevertheless, unfavourable lipid profiles, T2DM history, low level physical activity and CVD were all positively associated with both undiagnosed and diagnosed diabetes. The inverse association between diagnosed T2DM and alcohol intake was also of interest as correlations between alcohol use and MetS have been reported previously [40]. Markedly, 96.6% (N = 168) of study participants with both undiagnosed and diagnosed T2DM were either overweight or obese, confirming results from previous research which suggests that obesity is a primary and significant risk factor related to diabetes development [41]. Screening for T2DM may be more efficient within these subgroups, particularly individuals with a combination of these features.

Within the ROI, residents accessing public healthcare are divided into two categories: (1) those who hold a medical card (either a FMC or GPC) and thus qualify for means-tested, state-assisted healthcare insurance. A FMC entitles individuals to free GP services, Rx medications, public hospital services, dental, optical and aural services, community care and personal social services. A GPC entitles individuals to free GP care; (2) non-card holders, who are entitled to free public hospital services but who must pay for GP care and may also have to pay in-patient and out-patient hospital charges. In addition to the public health system there is also a large private healthcare market [42]. Results from the present study suggest that within this population, subjects with private medical insurance are less likely to have undiagnosed or diagnosed diabetes. This may indicate that these individuals have greater financial resources and access to screening and healthcare, or an increased awareness of risk factors related to T2DM. This awareness could be due to higher educational levels, as it was also

Table 2. Odds ratios (95% CI) of having undiagnosed or diagnosed type 2 diabetes compared to no diabetes – multivariate logistic regression adjusted for gender, age and all significant covariates.

Feature	Odds ratio	95% CI
Undiagnosed T2DM compared to no diabetes[1]		
Male	1.4	(0.8–2.5)
Age≥60	1.0	(0.6–1.9)
On Rx for cholesterol	2.2	(1.2–3.9)
BMI category:		
<25	1	
25-29.9	4.5	(1.0–19.5)
≥30	6.8	(1.6–29.4)
Family history of T2DM	1.9	(1.0–3.6)
Health insurance:		
Private insurance	1	
State insurance	2.2	(1.2–4.2)
No insurance	2.3	(1.0–5.2)
Physical activity:		
High	1	
Moderate	1.9	(0.8–4.2)
No physical exercise	5.8	(2.7–12.5)
Dyslipidaemia[3]	4.3	(2.3–8.3)
Diagnosed T2DM compared to no diabetes[2]		
Male	2.5	(1.5–4.1)
Age≥60	1.4	(0.9–2.3)
On Rx for hypertension	2.7	(1.7–4.4)
On Rx for cholesterol	2.0	(1.2–3.3)
BMI category:		
<25	1	
25–29.9	8.2	(1.9–34.6)
≥30	9.4	(2.2–40.3)
Family history of T2DM	5.9	(3.7–9.4)
CVD	2.0	(1.1–3.5)
Alcohol use:		
Non-drinker	1	
Occasional drinker	1.3	(0.7–2.2)
Regular drinker	0.4	(0.2–0.7)
Dyslipidaemia[3]	1.9	(1.0–3.5)

[1]Model excludes subjects with diagnosed T2DM. Final model covariates entered in order: dyslipidaemia, BMI category, physical activity, health insurance, on Rx for cholesterol, family history of T2DM, gender and age.
[2]Model excludes subjects with undiagnosed T2DM. Final model covariates entered in order: family history of T2DM, on Rx for hypertension, BMI category, on Rx for cholesterol, CVD, dyslipidaemia, alcohol use, gender and age.
[3]Dyslipidaemia: TAG≥1.7 and HDL-C: <1.03 (MALES) <1.29 (FEMALES).
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noted that study participants who had only completed education to a primary level were more likely to have diabetes. Although social class (defined by the ESeC) was not an associated risk factor for having either undiagnosed or diagnosed T2DM, it is possible that the lower prevalence of diabetes amongst subjects with private medical insurance was due to socio-economic inequalities, as study participants in receipt of state-assisted medical insurance were notably at a higher risk. These findings suggest that diabetes cases occur disproportionately amongst individuals who are economically deprived and have lower education levels, and this concurs with previous research which found significant correlations between social deprivation and T2DM [43].

Importantly, these results also imply that health service inequalities are significant determinants of diagnostic status, as a greater proportion of undiagnosed cases indicated having no state-assisted or private healthcare insurance. This is consistent with outcomes observed in previous studies which have examined relationships between healthcare inequities and T2DM [13,44]. Univariate analysis suggested three-fold and four-fold increased odds of having undiagnosed T2DM in subjects without medical insurance when compared to individuals with no diabetes (table S1) or diagnosed T2DM (table 3) respectively. This association was also noted in a multivariate logistic regression comparing undiagnosed to non-diabetic individuals (table 2) but was not observed in multivariate analysis restricted to subjects with T2DM (table 4).

To investigate this discrepancy, we forced the health insurance IPV into a model and entered covariates independently to assess confounder-adjusted relationships. In a logistic regression which controlled for family T2DM history, Rx anti-hypertensives, BMI, age and gender, having no healthcare insurance remained strongly associated with undiagnosed T2DM (OR: 3.5, 95% CI: 1.2–10.4, $P = 0.025$) although this was attenuated when the physical activity IPV was included (OR: 2.4, 95% CI: 0.7–8.9, $P = 0.184$). This may indicate a relationship between physical activity and both health insurance and undiagnosed T2DM or that physical activity levels explain most of the variance. Equally possible is that missing data from the IPAQ questionnaire resulted in a loss of statistical power.

We further explored health insurance/physical activity relationships with undiagnosed/diagnosed T2DM using the LR. Tests for model assessment included significant covariates, age, gender and either health insurance or physical activity IPVs. Both models implied similar goodness of fit (LR chi-square: 33.29, $P<0.001$ for a model with health insurance vs. LR chi-square: 32.68, $P<0.001$ for a model with physical activity) in full models against a constant, indicating that both predictors may be clinically relevant. In addition, it was noted that models including health insurance and physical activity IPVs displayed variations in discriminatory ability to detect either undiagnosed or diagnosed T2DM (figures S1 and S2). This suggests that use of these variables in T2DM risk prediction scores may be useful for identifying a subset of diabetes cases.

Strengths and limitations

As one of the largest cross-sectional studies performed to date within the ROI, the Cork and Kerry Diabetes and Heart Disease Study sample size is comparable to other related Irish studies. Selection bias was minimised as a similar number of male and female subjects, aged between 50–69 years of age, were randomly selected from a register of patients within a single primary care based sample. Furthermore, non-responders had similar numbers for both males and females and likewise for age groups. Few studies have assessed the prevalence of undiagnosed or diagnosed T2DM within one broadly representative population sample or compared features between undiagnosed and diagnosed subjects. Finally, use of the HbA_{1c} measurement provided prevalence rates

Table 3. Univariate odds ratios (95% CI) of having undiagnosed compared to diagnosed type 2 diabetes.[1]

Feature	Undiagnosed diabetes	Diagnosed diabetes	Odds ratio	95% CI
	N = 72 (41.4%)	N = 102 (58.6%)		
Health conditions				
Female	29 (40.3)	33 (32.4)	1	
Male	43 (59.7)	69 (67.6)	0.7	(0.4–1.3)
Age <60 years	34 (47.2)	37 (36.3)	1	
Age≥60 years	38 (52.8)	65 (63.7)	0.6	(0.3–1.2)
Not on Rx for hypertension	40 (55.6)	36 (35.3)	1	
On Rx for hypertension	32 (44.4)	66 (64.7)	0.4	(0.2–0.8)
Not on Rx for cholesterol	37 (51.4)	35 (34.3)	1	
On Rx for cholesterol	35 (48.6)	67 (65.7)	0.5	(0.3–0.9)
BMI (kg/m^2)	33.06±6.3	31.19±4.4	1.1	(1.0–1.1)
BMI category:				
<25	4 (5.6)	2 (2.0)	1	
25–29.9	24 (33.3)	43 (42.2)	0.3	(0.1–1.7)
≥30	44 (61.1)	57 (55.9)	0.4	(0.1–2.2)
No family history of T2DM	51 (70.8)	48 (47.1)	1	
Family history of T2DM	21 (29.2)	54 (52.9)	0.4	(0.2–0.7)
No CVD	56 (77.8	73 (71.6)	1	
CVD	16 (22.2)	29 (28.4)	0.7	(0.4–1.5)
Socio-economic				
Education:				
Bachelor or higher	4 (5.9)	5 (5.3)	1	
Diploma	6 (8.8)	6 (6.3)	1.3	(0.2–7.1)
Secondary	31 (45.6)	40 (42.1)	1.0	(0.2–3.9)
Primary only	27 (39.7)	44 (46.3)	0.8	(0.2–3.1)
Social class:				
High income	6 (11.5)	11 (13.3)	1	
Middle income	18 (34.6)	25 (30.1)	1.3	(0.4–4.2)
Low income	28 (53.8)	47 (56.6)	1.1	(0.4–3.3)
Medical cover				
Health insurance:				
Private insurance	27 (37.5)	51 (50.0)	1	
State insurance	29 (40.3)	44 (43.1)	1.2	(0.6–2.4)
No insurance	16 (22.2)	7 (6.9)	4.3	(1.6–11.8)
Health behaviours				
Physical activity:				
High	10 (17.5)	31 (34.8)	1	
Moderate	19 (33.3)	35 (39.3)	1.7	(0.7–4.2)
No physical exercise	28 (49.1)	23 (25.8)	3.8	(1.5–9.3)
Non-smoker	34 (47.2)	42 (41.2)	1	
Smoker	38 (52.8)	60 (58.8)	0.8	(0.4–1.4)
Alcohol use:				
Non-drinker	38 (55.1)	54 (53.5)	1	
Occasional drinker	12 (17.4)	27 (26.7)	0.6	(0.3–1.4)
Regular drinker	19 (27.5)	20 (19.8)	1.4	(0.6–2.9)
Metabolic				
TAG (mmol/L)	1.80 (1.3–2.4)	1.36 (1.0–2.0)	1.5	(1.1–2.0)
TAG <1.7	33 (47.1)	60 (62.5)	1	
TAG≥1.7	37 (52.9)	36 (37.5)	1.9	(1.0–3.5)

Table 3. Cont.

Feature	Undiagnosed diabetes	Diagnosed diabetes	Odds ratio	95% CI
	N = 72 (41.4%)	N = 102 (58.6%)		
HDL-C (mmol/L)	1.22±0.3	1.18±0.3	1.7	(0.6–4.7)
Optimal HDL-C	38 (54.3)	55 (55.0)	1	
Non-optimal HDL-C[2]	32 (45.7)	45 (45.0)	1.0	(0.6–1.9)
No dyslipidaemia	46 (65.7)	79 (79.0)	1	
Dyslipidaemia[3]	24 (34.3)	21 (21.0)	2.0	(1.0–3.9)
SBP (mmHg)	134.18±19.3	132.94±16.4	1.0	(0.99–1.0)
DBP (mmHg)	80.12±10.9	78.79±9.5	1.0	(0.98–1.0)
No hypertension	43 (60.6)	74 (72.5)	1	
Hypertension[4]	28 (39.4)	28 (27.5)	1.7	(0.9–3.3)

[1]Mean and ± SD are shown for continuous variables. TAG is shown as a median (interquartile range). Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.
[2]HDL-C: <1.03 (MALES) <1.29 (FEMALES).
[3]Dyslipidaemia: TAG≥1.7 and HDL-C: <1.03 (MALES) <1.29 (FEMALES).
[4]Hypertension: SBP≥140 and/or DBP≥90.
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comparable to those from a recent nationally representative study: the 2007 Slán National Health and Lifestyle Survey [9].

Notwithstanding these strengths, several limitations can be identified. The use of self-reported questionnaires is subject to potential inaccuracies, recall and reporting bias [45,46]. Misclassification of diabetes from self-reporting is a recognised limitation present in all surveys, and is a particular restraint in the ROI due to the absence of a unique health identifier within the Irish healthcare system [47]. This makes linkage with other records, such as disease registries or death records problematic [14]. Nonetheless, several studies have indicated a reasonable or high degree of concordance between T2DM prevalence and self-reporting [45,48–50] and whenever possible empirical methods were used in analysis. Additionally, within this sample there was a high level of agreement between self-reported doctor diagnosis of T2DM and Rx diabetes medication use (Kappa: 0.854, 95% CI: 0.796–0.912, P<0.001).

Table 4. Odds ratios (95% CI) of having undiagnosed compared to diagnosed type 2 diabetes – multivariate logistic regression adjusted for all significant covariates.[1]

Feature	Model 1		Model 2[2]	
	Odds ratio	95% CI	Odds ratio	95% CI
BMI (kg/m^2)	1.1	(1.0–1.2)	1.1	(1.0–1.2)
On Rx for hypertension	0.3	(0.2–0.7)	0.3	(0.1–0.7)
Family history of T2DM	0.4	(0.2–0.8)	0.4	(0.2–0.8)
Physical activity:				
High	1		1	
Moderate	1.6	(0.6–4.3)	1.6	(0.6–4.3)
No physical exercise	3.5	(1.3–9.3)	3.4	(1.3–9.1)

[1]Final model covariates entered in order: family history of T2DM, physical activity, on Rx for hypertension and BMI.
[2]Adjusted for gender and age.
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Equally of concern is that prevalence estimates were derived from a single primary care based sample which may not be representative of the general Irish population. However, previous research suggests that approximately 98% of Irish adults are registered with a GP and that, even in the absence of a universal patient registration system, it is possible to perform population based epidemiological studies that are representative of the general population using these methods [51]. Further studies are needed to definitively confirm this conclusion. If correct, it may indicate that findings from the Cork and Kerry Diabetes and Heart Disease Study are generalisable to the Irish population aged between 50–69 years. Also, as this research makes use of cross-sectional data, interpretations of these findings are compromised by the inability to infer causal relationships. Nevertheless, the relationships described have been extensively replicated in other prospective cohort studies. Finally, with regard to statistical procedures employed in analysis, the possibility of model over-fitting or type II errors cannot be discounted, and results should be considered preliminary and exploratory, as future studies with larger sample sizes and greater statistical power might find other relationships [52].

Conclusions

The prevalence of T2DM within the ROI is consistent with trends worldwide [53,54], and is primarily driven by the increasing obesity epidemic [4,55]. Despite policies and continued investment in services which promote awareness and knowledge of a disease that is largely preventable, the prevalence of diabetes in Ireland is rising [8].

Socio-economic and health service inequalities are significant risk factors for having undiagnosed T2DM. The results from this study indicate that subjects with state-subsidised healthcare insurance, and those without private or state-assisted medical cover, are more likely to be undetected. These findings suggest that individuals from lower socio-economic backgrounds should be targeted. Observed low levels of physical activity, obesity level assessment and recognition of untreated cardiovascular conditions may also improve identification of T2DM cases within clinical practice. Finally, as a successful programme to detect subjects with T2DM may depend on regular General Practice attendance, a

strategic approach which identifies individuals without access to primary health services and which furthers efforts to promote affordable and equitable healthcare, is needed to prevent predictable sequelae for affected individuals and populations.

Supporting Information

Figure S1 Area under the receiver operating characteristic curves (AUC) for models to discriminate undiagnosed type 2 diabetes compared to no diabetes. The figure shows area under the curves for models to detect undiagnosed type 2 diabetes. The *c* statistics values were: (1) *c*: 0.735, (95% CI: 0.668–0.801) for a model including health insurance and physical activity; (2) *c*: 0.814, (95% CI: 0.758–0.871) for a model including health insurance, physical activity and BMI (continuous).
(DOCX)

Figure S2 Area under the receiver operating characteristic curves (AUC) for models to discriminate diagnosed type 2 diabetes compared to no diabetes. The figure shows area under the curves for models to detect diagnosed type 2 diabetes. The *c* statistics values were: (1) *c*: 0.608, (95% CI: 0.544–0.671) for a model including health insurance and physical activity; (2) *c*: 0.698, (95% CI: 0.646–0.750) for a model including health insurance, physical activity and BMI (continuous).
(DOCX)

Table S1 Univariate odds ratios (95% CI) of having undiagnosed or diagnosed type 2 diabetes compared to no diabetes. The table displays univariate associations for socio-economic, metabolic and other health related variables with either undiagnosed or diagnosed diabetes. Diagnosed subjects were excluded from models examining undiagnosed diabetes. Undiagnosed subjects were excluded from models examining diagnosed diabetes.
(DOCX)

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Author Contributions

Conceived and designed the experiments: SRM IJP. Analyzed the data: SRM. Wrote the paper: JMO SRM CMB PMK IJP.

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RESEARCH ARTICLE

HbA_{1c} Alone Is a Poor Indicator of Cardiometabolic Risk in Middle-Aged Subjects with Pre-Diabetes but Is Suitable for Type 2 Diabetes Diagnosis: A Cross-Sectional Study

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Abstract

Objectives

Glycated haemoglobin A_{1c} (HbA_{1c}) measurement is recommended as an alternative to fasting plasma glucose (FPG) for the diagnosis of pre-diabetes and type 2 diabetes. However, evidence suggests discordance between HbA_{1c} and FPG. In this study we examine a range of metabolic risk features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell counts to determine which assay more accurately identifies individuals at increased cardiometabolic risk.

Materials and Methods

This was a cross-sectional study involving a random sample of 2,047 men and women aged 46-73 years. Binary and multinomial logistic regression were employed to examine risk feature associations with pre-diabetes [either HbA_{1c} levels 5.7-6.4% (39-46 mmol/mol) or impaired FPG levels 5.6-6.9 mmol/l] and type 2 diabetes [either HbA_{1c} levels >6.5% (>48 mmol/mol) or FPG levels >7.0 mmol/l]. Receiver operating characteristic curve analysis was used to evaluate the ability of HbA_{1c} to discriminate pre-diabetes and diabetes defined by FPG.

Results

Stronger associations with diabetes-related phenotypes were observed in pre-diabetic subjects diagnosed by FPG compared to those detected by HbA_{1c}. Individuals with type 2 diabetes exhibited cardiometabolic profiles that were broadly similar according to diagnosis by either assay. Pre-diabetic participants classified by both assays displayed a more pro-inflammatory, pro-atherogenic, hypertensive and insulin resistant profile. Odds ratios of having three or more metabolic syndrome features were also noticeably increased (OR: 4.0, 95% CI: 2.8-5.8) when compared to subjects diagnosed by either HbA_{1c} (OR: 1.4, 95% CI: 1.2-1.8) or FPG (OR: 3.0, 95% CI: 1.7-5.1) separately.

Conclusions

In middle-aged Caucasian-Europeans, HbA_{1c} alone is a poor indicator of cardiometabolic risk but is suitable for diagnosing diabetes. Combined use of HbA_{1c} and FPG may be of additional benefit for detecting individuals at highest odds of type 2 diabetes development.

Introduction

The prevalence of type 2 diabetes, a chronic disease which causes significant mortality, has increased considerably in world populations, representing a major public health issue [1]. Diabetes is associated with a clustering of cardiometabolic features including obesity, dyslipidaemia, hypertension, insulin resistance, chronic low-grade inflammation [2, 3], and may lead to severe cardiovascular complications [4].

Pre-diabetes, a condition defined by glycaemic profiles that are higher than normal but which do not meet thresholds for diabetes, is a strong risk factor for type 2 diabetes and related complications [5]. The American Diabetes Association (ADA) classifies type 2 diabetes as a fasting plasma glucose (FPG) level $\geq$7.0 mmol/l and pre-diabetes as impaired FPG levels between 5.6–6.9 mmol/l [2]. In 2009 the International Expert Committee recommended glycated haemoglobin A_{1c} (HbA_{1c}) as an alternative marker [6], and in 2010 the ADA introduced HbA_{1c} cut-points of $\geq$6.5% ($\geq$48 mmol/mol) for diabetes diagnosis and between 5.7–6.4% (39–46 mmol/mol) as a criterion to identify individuals at a high-risk state of developing diabetes [2]. Perceived benefits of the use of HbA_{1c} measurement, over FPG, include greater pre-analytical stability, lower biological variability and that the assay may be performed in non-fasting blood samples [7, 8]. However, use of HbA_{1c} as a screening tool has been controversial, with research showing discordance between HbA_{1c} and FPG [9–12], and several studies suggesting that factors such as age or ethnicity may influence diagnostic performance [13–15].

The aim of this study was to compare the metabolic profiles in subjects with pre-diabetes and type 2 diabetes, using ADA-recommended HbA_{1c} and FPG diagnostic thresholds, in a random sample of 2,047 middle-aged men and women. In particular, we examined a range of diabetes risk factors, metabolic syndrome (MetS) features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell (WBC) counts to determine which assay more accurately identifies individuals at increased cardiometabolic risk.

Materials and Methods

Study population

The Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000 Caucasian-European subjects, with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending patients in the 46–73 year age group. In total, 3,807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). The status of non-responders included individuals refusing to participate (59.4%) and those who did not reply (40.6%).

Male subjects accounted for 53.7% of non-responders while 43.5% (vs. 42.8% of responders) were >60 years of age. Details regarding the study design, sampling procedures and methods of data collection have been reported previously [16].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

Clinical and laboratory procedures

All study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, family diabetes history, physician-diagnosed type 2 diabetes and prescription (Rx) medication use were gathered through a self-completed General Health Questionnaire. Triglyceride and high density lipoprotein cholesterol (HDL-C) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica GmbH, Hamburg, Germany). Fasting glucose concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and HbA_{1c} levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium]. Serum insulin, c-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), adiponectin, leptin, resistin and plasminogen activator inhibitor-1 (PAI-1) were assessed using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Complement component 3 (C3) was measured by immunoturbidimetric assay (RX Daytona; Randox Laboratories). White blood cell counts were determined by flow cytometry technology as part of a full blood count.

Three independent measurements of systolic and diastolic blood pressure (BP) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP. The weight and height of each participant were measured to the nearest 0.1 kg and 0.1 cm respectively. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was measured using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and body mass index (BMI) was calculated as weight divided by the square of height. A BMI ≥30 kg/m^2 was classified as obese. Waist circumference (WC) was measured between the lowest rib and iliac crest on bare skin. Subjects were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. Two independent measurements of WC were taken and the mean of the two was used in analysis. Central obesity was defined as a WC level ≥102 cm for males and ≥88 cm for females.

Classification of biochemical and blood pressure measurements

Lipid, lipoprotein and BP measurements were categorised according to National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATP III) guidelines [17]. Abnormal metabolic risks were defined as high triglycerides ≥1.7 mmol/l and low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females). Dyslipidaemia was determined according to both high triglyceride and low HDL-C levels. Elevated BP was classified as systolic BP ≥130 mmHg and/or diastolic BP ≥85 mmHg or Rx anti-hypertensive medication use. High serum insulin was

defined as a level equal to or above the 75th percentile in the study sample. Metabolic syndrome was determined according to a modified version of the NCEP: ATP III criterion, substituting serum insulin 75th percentile for impaired FPG. Three or more MetS features (≥3 MetS) was characterised as any combination of the following: obesity defined by WC, high triglyceride levels, low HDL-C, elevated BP and high insulin concentrations. According to ADA guidelines, pre-diabetes was classified as elevated HbA_{1c} levels between 5.7–6.4% (39–46 mmol/mol) or impaired FPG levels between 5.6–6.9 mmol/l. Type 2 diabetes was defined as HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l [2]. As internationally recognised risk cut-points for the examined biomarkers have not been established, we classified inflammation and raised immune activation as a level above the study population median for each biomarker (C3, CRP, IL-6, TNF-α, leptin, resistin, PAI-1 and WBC) with the exception of adiponectin (below median level).

Statistical analysis

Descriptive characteristics were examined according to diagnosis of pre-diabetes and type 2 diabetes. Categorical features are presented as percentages and continuous variables are displayed as a mean (plus or minus one standard deviation) or a median and interquartile range for skewed data. Binary logistic regression was used to explore diabetes-related risk factor and inflammatory biomarker relationships with pre-diabetes (compared to normoglycaemic subjects) and type 2 diabetes (compared to individuals without diabetes) defined using HbA_{1c} and FPG diagnostic cut-points. Models examining metabolic feature associations with pre-diabetes excluded patients with type 2 diabetes indicated by either HbA_{1c} or FPG, a physician diagnosis or Rx diabetes medication use. Risk feature relationships with pre-diabetes (either HbA_{1c} alone, FPG alone or dual categorisation by both HbA_{1c} and FPG) were further evaluated using multinomial logistic regression. Subjects classified as normoglycaemic by both assays were used as the reference category.

The ability of HbA_{1c} to discriminate pre-diabetes (defined by impaired FPG) and type 2 diabetes (defined by FPG levels ≥7.0 mmol/l) was assessed using receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) provides a scale from 0.5 to 1.0 (with 0.5 representing random chance and 1.0 indicating perfect discrimination) by which to compare the ability of a marker to detect a positive result [18]. The diagnostic properties of different HbA_{1c} thresholds were contrasted by determining sensitivity and false positive rates (FPR). Levels of agreement between diagnostic methods were ascertained using Cohen's kappa coefficient (K).

Primary data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) for Windows. Confidence intervals for prevalence proportions were calculated using the VasserStats statistical website [19]. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance. Assay results for HbA_{1c} and FPG were available for 1,995 (97.5%) and 1,994 (97.4%) subjects. Participants missing either HbA_{1c} or FPG data were excluded from multinomial and ROC analyses. Low-level missing values were found within most independent variables. Sensitivity analysis indicated a similar percentage of missing data according to either HbA_{1c} or FPG pre-diabetes and diabetes classifications. Missing independent variable data were thus assumed to be ignorable and missing at random.

Results

Descriptive characteristics

Characteristics of the study population according to pre-diabetes and type 2 diabetes classifications are presented in Table 1. The prevalence of pre-diabetes was 49.1% (95% CI: 46.9%-

Table 1. Characteristics of the study population according to pre-diabetes and type 2 diabetes status.

Feature	Full cohort	Pre-diabetes[1]		Type 2 diabetes[2]	
	(N = 2047)	HbA_{1c} (N = 980)	FPG (N = 230)	HbA_{1c} (N = 146)	FPG (N = 85)
Male	1008 (49.2)	441 (45.0)	150 (65.2)	95 (65.1)	59 (69.4)
Age	59.0 (55.0–64.0)	60.0 (55.0–64.0)	61.0 (56.0–65.0)	60.0 (57.0–65.0)	61.0 (56.5–64.5)
Age ≥60	981 (47.9)	510 (52.0)	125 (54.3)	83 (56.8)	51 (60.0)
Diagnosed diabetes	101 (4.9)	-	-	73 (50.0)	51 (60.0)
On Rx for diabetes	78 (3.8)	-	-	60 (41.1)	41 (48.2)
On Rx for hypertension	584 (28.5)	307 (31.3)	98 (42.6)	81 (55.5)	48 (56.5)
On Rx for cholesterol	711 (34.7)	385 (39.3)	93 (40.4)	88 (60.3)	49 (57.6)
BMI (kg/m2)	28.60 ± 4.7	28.80 ± 4.7	30.45 ± 5.2	32.17 ± 5.5	31.81 ± 5.5
BMI ≥30	668 (32.7)	345 (35.2)	109 (47.4)	85 (58.2)	49 (57.6)
WC (cm)	97.04 ± 13.2	97.08 ± 12.9	102.44 ± 12.8	107.91 ± 13.7	108.52 ± 13.9
WC (HIGH)	1119 (54.8)	562 (57.4)	150 (65.2)	119 (81.5)	66 (77.6)
Family diabetes history	390 (19.1)	176 (18.0)	46 (20.0)	62 (42.5)	41 (48.2)
Triglycerides (mmol/l)	1.22 (0.9–1.7)	1.23 (0.9–1.7)	1.41 (1.0–2.0)	1.58 (1.2–2.3)	1.68 (1.2–2.3)
Triglycerides ≥1.7	490 (24.6)	230 (23.8)	85 (37.9)	65 (45.5)	40 (48.8)
HDL-C (mmol/l)	1.45 ± 0.4	1.45 ± 0.4	1.32 ± 0.3	1.17 ± 0.3	1.17 ± 0.4
HDL-C (LOW)	353 (17.6)	165 (17.0)	59 (26.1)	66 (45.2)	35 (41.2)
Dyslipidaemia	168 (8.4)	78 (8.0)	32 (14.0)	37 (25.3)	22 (25.9)
Systolic BP (mmHg)	129.60 ± 16.8	130.10 ± 16.1	134.78 ± 15.5	134.19 ± 17.3	136.24 ± 17.4
Diastolic BP (mmHg)	80.12 ± 9.7	80.24 ± 9.6	82.25 ± 9.1	79.50 ± 10.3	80.72 ± 10.5
BP ≥130/85	1045 (51.3)	521 (53.4)	155 (67.7)	89 (61.4)	56 (66.7)
HbA1c (%)	5.7 (5.5–6.0)	5.9 (5.7–6.0)	5.8 (5.6–6.1)	7.0 (6.7–8.1)	7.6 (6.8–9.0)
HbA1c (mmol/mol)	39 (37–42)	41 (39–42)	40 (38–43)	53 (50–65)	60 (51–75)
FPG (mmol/l)	4.90 (4.7–5.4)	5.00 (4.7–5.3)	5.80 (5.7–6.1)	6.90 (6.0–9.0)	8.50 (7.6–10.8)
Insulin (μU/ml)	8.65 (5.3–14.1)	8.98 (4.6–11.8)	12.67 (7.4–19.5)	18.27 (10.6–31.9)	19.21 (12.1–30.9)
Insulin 75th percentile	497 (25.0)	238 (24.6)	98 (43.2)	94 (65.7)	59 (70.2)
≥3 MetS features[3]	606 (29.6)	298 (30.4)	112 (48.7)	103 (70.5)	63 (74.1)
C3 (mg/dl)	135.92 ± 24.7	138.85 ± 24.5	141.41 ± 25.8	148.13 ± 28.6	149.20 ± 24.9
CRP (ng/ml)	1.35 (1.0–2.3)	1.43 (1.0–2.4)	1.38 (1.0–2.3)	1.79 (1.1–3.2)	1.91 (1.2–3.0)
IL-6 (pg/ml)	1.81 (1.2–2.9)	1.91 (1.3–3.0)	2.02 (1.5–3.0)	2.92 (1.7–4.8)	2.83 (1.8–4.6)
TNF-α (pg/ml)	5.97 (4.9–7.3)	6.02 (5.0–7.3)	5.94 (4.9–7.5)	6.99 (5.5–8.3)	7.09 (5.6–8.1)
Adiponectin (ng/ml)	4.75 (2.9–7.5)	4.92 (3.1–7.5)	3.63 (2.4–5.6)	2.82 (1.7–4.6)	2.73 (1.9–4.7)
Leptin (ng/ml)	1.95 (1.1–3.1)	2.09 (1.3–3.5)	2.06 (1.3–3.8)	2.28 (1.3–3.9)	2.09 (1.1–3.4)
Resistin (ng/ml)	5.07 (3.9–6.7)	4.93 (3.8–6.6)	4.89 (3.7–6.7)	6.15 (4.6–7.3)	5.53 (4.5–7.3)
PAI-1 (ng/ml)	27.38 ± 12.6	27.87 ± 12.0	29.56 ± 13.2	31.35 ± 15.9	30.03 ± 11.0
WBC (109/l)	6.00 ± 1.9	6.12 ± 2.1	6.33 ± 1.72	7.39 ± 2.4	7.21 ± 1.9

Mean and ± standard deviation are shown for continuous variables. Age, triglycerides, HbA_{1c}, FPG, insulin, CRP, IL-6, TNF-α, adiponectin, leptin and resistin are shown as a median (interquartile range). Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.

[1]Pre-diabetes: HbA_{1c} levels 5.7–6.4% (39–46 mmol/mol) or FPG levels 5.6–6.9 mmol/l.

[2]Type 2 diabetes: HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l.

[3]MetS features: WC (HIGH), triglycerides ≥1.7, HDL-C (LOW), BP ≥130/85 or Rx and insulin 75th percentile.

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51.3%) by elevated HbA_{1c} and 11.5% (95% CI: 10.2%-13.0%) by impaired FPG. Subjects categorised as pre-diabetic using HbA_{1c} had lower BMI and WC levels, lower triglyceride and insulin concentrations, higher HDL-C levels, were less hypertensive, and a greater proportion were female when compared to individuals with pre-diabetes defined by FPG.

Logistic regression

In binary logistic regression analyses (Table 2), associations between commonly assessed diabetes risk factors and pre-diabetes were stronger in subjects diagnosed by FPG. Odds ratios for pre-diabetes indicated by HbA_{1c} were non-significant for having a family diabetes history and elevated triglyceride levels, while there was a three-fold increased likelihood (OR: 3.0, 95% CI: 2.2–3.9) of having ≥3 MetS features in participants identified by FPG compared to an odds ratio of 1.6 (95% CI: 1.3–2.0) in pre-diabetes by HbA_{1c}. In contrast, metabolic risk factor relationships with type 2 diabetes were generally comparable according to diagnosis by either assay, with odds ratios of having ≥3 MetS features being 6.1 (95% CI: 4.2–8.8) and 6.8 (95% CI: 4.1–11.2) for subjects diagnosed by HbA_{1c} and FPG respectively. Regardless of definition,

Table 2. Odds ratios (95% CI) of having risk factors according to diagnosis of pre-diabetes and type 2 diabetes by HbA_{1c} or FPG.

Feature	Odds ratios (95% CI)[1]							
	Pre-diabetes compared to normoglycaemia[2]				Type 2 diabetes compared to no diabetes[3]			
	HbA_{1c}	P value	FPG	P value	HbA_{1c}	P value	FPG	P value
Male	0.8 (0.6–0.9)	<0.001	2.3 (1.7–3.0)	<0.001	2.0 (1.4–2.9)	<0.001	2.5 (1.5–3.9)	<0.001
Age ≥60	1.6 (1.3–1.9)	<0.001	1.4 (1.1–1.9)	0.011	1.5 (1.1–2.2)	0.018	1.7 (1.1–2.7)	0.017
Family diabetes history	1.2 (0.9–1.5)	0.182	1.4 (1.0–2.1)	0.043	4.1 (2.9–5.9)	<0.001	5.2 (3.3–8.1)	<0.001
BMI ≥30	1.8 (1.4–2.2)	<0.001	2.2 (1.7–3.0)	<0.001	3.1 (2.2–4.3)	<0.001	2.8 (1.8–4.4)	<0.001
WC (HIGH)	1.5 (1.2–1.9)	0.001	2.0 (1.4–3.1)	0.001	5.4 (2.5–11.8)	<0.001	7.4 (2.3–23.5)	0.001
Triglycerides ≥1.7	1.2 (0.9–1.5)	0.134	2.1 (1.5–2.8)	<0.001	2.5 (1.8–3.6)	<0.001	2.8 (1.8–4.4)	<0.001
HDL-C (LOW)	1.4 (1.1–1.8)	0.018	2.3 (1.7–3.3)	<0.001	4.6 (3.2–6.6)	<0.001	3.6 (2.3–5.7)	<0.001
Dyslipidaemia	1.6 (1.1–2.4)	0.019	2.6 (1.7–4.1)	<0.001	4.3 (2.8–6.5)	<0.001	4.1 (2.4–6.9)	<0.001
BP ≥130/85 or Rx	1.4 (1.2–1.7)	<0.001	2.5 (1.8–3.5)	<0.001	3.0 (1.9–4.8)	<0.001	4.4 (2.2–8.6)	<0.001
Insulin 75th percentile	1.6 (1.3–2.0)	<0.001	3.1 (2.3–4.2)	<0.001	6.5 (4.5–9.4)	<0.001	7.2 (4.4–11.7)	<0.001
≥3 MetS features[4]	1.6 (1.3–2.0)	<0.001	3.0 (2.2–3.9)	<0.001	6.1 (4.2–8.8)	<0.001	6.8 (4.1–11.2)	<0.001
C3[5]	1.8 (1.5–2.2)	<0.001	1.4 (1.0–1.8)	0.032	3.3 (2.2–4.9)	<0.001	3.1 (1.9–5.0)	<0.001
CRP[5]	1.4 (1.1–1.7)	0.001	1.2 (0.9–1.5)	0.293	1.5 (1.1–2.2)	0.02	1.6 (1.0–2.6)	0.032
IL-6[5]	1.6 (1.3–1.9)	<0.001	1.5 (1.1–2.0)	0.005	2.8 (1.9–4.1)	<0.001	2.8 (1.7–4.6)	<0.001
TNF-α[5]	1.2 (1.0–1.4)	0.078	1.0 (0.7–1.3)	0.738	2.3 (1.6–3.3)	<0.001	2.7 (1.6–4.4)	<0.001
Adiponectin[5]	1.4 (1.1–1.7)	0.004	2.0 (1.4–2.7)	<0.001	4.0 (2.5–6.2)	<0.001	3.2 (1.8–5.6)	<0.001
Leptin[5]	1.5 (1.2–1.8)	<0.001	1.4 (1.1–1.9)	0.014	1.5 (1.0–2.1)	0.026	1.2 (0.8–1.8)	0.48
Resistin[5]	0.9 (0.8–1.1)	0.305	0.9 (0.7–1.2)	0.391	2.4 (1.7–3.5)	<0.001	1.8 (1.1–2.8)	0.012
PAI-1[5]	1.3 (1.1–1.6)	0.005	1.3 (1.0–1.7)	0.108	1.5 (1.0–2.1)	0.028	1.5 (1.0–2.4)	0.078
WBC[5]	1.7 (1.4–2.1)	<0.001	1.6 (1.2–2.1)	0.001	3.4 (2.3–5.0)	<0.001	3.3 (2.0–5.5)	<0.001

[1]Binary logistic regression. Gender adjusted for age (continuous), age ≥60 adjusted for gender, all other variables adjusted for age (continuous) and gender.

[2]Pre-diabetes: HbA_{1c} ≥5.7% (≥39 mmol/mol) or FPG ≥5.6 mmol/l, models exclude subjects with type 2 diabetes: HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l or physician diagnosis or Rx diabetes medication use.

[3]Models exclude 24 subjects that indicated a physician diagnosis or Rx diabetes medication use but who did not have positive HbA_{1c} or FPG test results.

[4]MetS features: WC (HIGH), triglycerides ≥1.7, HDL-C (LOW), BP ≥130/85 or Rx and insulin 75th percentile.

[5]Threshold: above median level in the study population except adiponectin (below median level).

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Table 3. Odds ratios (95% CI) of having risk factors according to diagnosis of pre-diabetes[1] by HbA_{1c} alone, FPG alone, or by both HbA_{1c} and FPG together.

Feature	Odds ratios (95% CI)[2]					
	HbA_{1c} alone (N = 814)	P value	FPG alone (N = 62)	P value	HbA_{1c} & FPG (N = 162)	P value
Male	0.8 (0.6–0.9)	0.006	3.3 (1.8–5.9)	<0.001	1.6 (1.2–2.3)	0.005
Age ≥60	1.6 (1.3–1.9)	<0.001	1.4 (0.8–2.3)	0.251	2.0 (1.4–2.8)	<0.001
Family diabetes history	1.1 (0.8–1.4)	0.474	1.2 (0.6–2.4)	0.651	1.7 (1.1–2.6)	0.013
BMI ≥30	1.6 (1.3–2.0)	<0.001	1.7 (1.0–3.0)	0.051	3.4 (2.4–4.9)	<0.001
WC (HIGH)	1.4 (1.2–1.8)	<0.001	2.0 (1.2–3.4)	0.011	2.6 (1.8–3.7)	<0.001
Triglycerides ≥1.7	1.2 (0.9–1.5)	0.267	2.5 (1.4–4.3)	0.001	2.3 (1.4–4.3)	<0.001
HDL-C (LOW)	1.3 (1.0–1.7)	0.095	2.5 (1.3–4.7)	0.004	2.8 (1.8–4.3)	<0.001
Dyslipidaemia	1.6 (1.0–2.5)	0.041	3.5 (1.6–7.8)	0.002	3.5 (2.0–6.2)	<0.001
BP ≥130/85 or Rx	1.3 (1.0–1.6)	0.012	2.2 (1.2–3.9)	0.009	3.3 (2.2–5.1)	<0.001
Insulin 75th percentile	1.5 (1.2–2.0)	0.002	3.4 (2.0–5.9)	<0.001	4.1 (2.8–5.9)	<0.001
≥3 MetS features[3]	1.4 (1.2–1.8)	0.003	3.0 (1.7–5.1)	<0.001	4.0 (2.8–5.8)	<0.001
C3[4]	1.8 (1.5–2.3)	<0.001	1.4 (0.9–2.4)	0.17	2.2 (1.5–3.1)	<0.001
CRP[4]	1.4 (1.1–1.7)	0.002	1.1 (0.7–2.0)	0.640	1.5 (1.1–2.2)	0.017
IL-6[4]	1.5 (1.2–1.9)	<0.001	1.4 (0.8–2.4)	0.212	2.1 (1.5–3.0)	<0.001
TNF-α[4]	1.2 (1.0–1.5)	0.096	0.8 (0.5–1.4)	0.524	1.1 (0.8–1.6)	0.446
Adiponectin[4]	1.3 (1.0–1.6)	0.043	1.3 (0.7–2.3)	0.373	2.6 (1.8–3.9)	<0.001
Leptin[4]	1.4 (1.2–1.8)	<0.001	1.3 (0.8–2.2)	0.345	2.0 (1.4–2.9)	<0.001
Resistin[4]	1.0 (0.8–1.2)	0.626	1.3 (0.7–2.1)	0.389	0.8 (0.5–1.1)	0.139
PAI-1[4]	1.3 (1.1–1.6)	0.008	1.4 (0.8–2.4)	0.2	1.6 (1.1–2.2)	0.014
WBC[4]	1.6 (1.3–2.0)	<0.001	1.3 (0.7–2.2)	0.371	2.6 (1.8–3.7)	<0.001

[1]Pre-diabetes: HbA_{1c} ≥5.7% (≥39 mmol/mol) or FPG ≥5.6 mmol/l, models exclude subjects with type 2 diabetes: HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l or physician diagnosis or Rx diabetes medication use.

[2]Multinomial logistic regression, reference category: normoglycaemia by both HbA_{1c} and FPG. Gender adjusted for age (continuous), age ≥60 adjusted for gender, all other variables adjusted for age (continuous) and gender.

[3]MetS features: WC (HIGH), triglycerides ≥1.7, HDL-C (LOW), BP ≥130/85 or Rx and insulin 75th percentile.

[4]Threshold: above median level in the study population except adiponectin (below median level).

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patients with pre-diabetes and type 2 diabetes displayed a chronic pro-inflammatory profile as characterised by elevated C3, IL-6, WBC levels and reduced adiponectin concentrations.

The results from multinomial regression models exploring risk factor relationships with pre-diabetes classified by HbA_{1c} alone, FPG alone, or by both HbA_{1c} and FPG together are displayed in Table 3. Odds ratios for obesity, elevated BP, increased insulin concentrations and MetS were higher in participants classified by both assays, with four-fold increased odds (OR: 4.0, 95% CI: 2.8–5.8) of having ≥3 MetS features, compared to either HbA_{1c} (OR: 1.4, 95% CI: 1.2–1.8) or FPG (OR: 3.0, 95% CI: 1.7–5.1) alone. Stronger associations with markers of inflammation were also observed in subjects identified by both criteria.

ROC analysis

Receiver operating characteristic curves for HbA_{1c} to detect pre-diabetes and type 2 diabetes are shown in Figs 1 and 2. The ability of HbA_{1c} to discriminate pre-diabetes characterised by impaired FPG was low (AUC: 0.668, 95% CI: 0.627–0.710). The HbA_{1c} ≥5.7% (≥39 mmol/mol) pre-diabetes threshold demonstrated marginal sensitivity (72%) and a high FPR (52%).

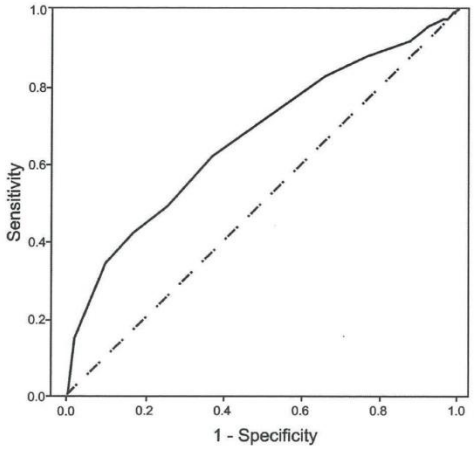

Fig 1. Receiver operating characteristic curve for HbA_{1c} to discriminate subjects with pre-diabetes. The figure shows an ROC curve for HbA_{1c} (continuous) to discriminate subjects with pre-diabetes (impaired FPG ≥5.6 mmol/l). The area under the curve value was AUC: 0.668, (95% CI: 0.627–0.710).

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The level of agreement between both diagnostic methods was also poor (K: 0.084). Discriminatory capacity for type 2 diabetes defined by FPG ≥7.0 mmol/l was high (AUC: 0.941, 95% CI: 0.902–0.980). Sensitivity, FPR and kappa for the ADA-recommended HbA_{1c} ≥6.5% (≥48 mmol/mol) cut-off were 84%, 4% and 0.60 respectively.

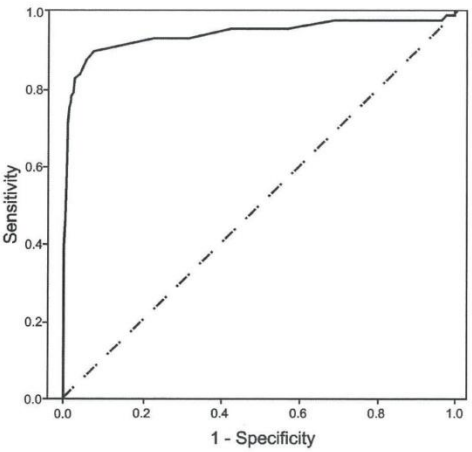

Fig 2. Receiver operating characteristic curve for HbA_{1c} to discriminate subjects with type 2 diabetes. The figure shows an ROC curve for HbA_{1c} (continuous) to discriminate subjects with type 2 diabetes (FPG ≥7.0 mmol/l). The area under the curve value was AUC: 0.941, (95% CI: 0.902–0.980).

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Discussion

In this study of 2,047 middle-aged Caucasian-European men and women we show that subjects with HbA_{1c} levels 5.7–6.4% (39–46 mmol/mol) or FPG levels 5.6–6.9 mmol/l may exhibit different cardiometabolic profiles. Stronger relationships with diabetes-related risk features were found using impaired FPG compared to elevated HbA_{1c} to diagnose pre-diabetes. Conversely, the metabolic profiles of patients with type 2 diabetes, defined by either HbA_{1c} ≥6.5% (≥48 mmol/mol) or FPG ≥7.0 mmol/l concentrations, were broadly similar. In addition, it was noted that associations with risk factors and inflammatory markers were higher in pre-diabetic individuals classified by both assays. These results suggest that a combination of both criteria may be useful for detecting subjects at increased cardiometabolic risk.

Noticeably, within this population, a higher percentage of patients were identified as having pre-diabetes by HbA_{1c} (49.1% vs. 11.5% for FPG). A higher prevalence of pre-diabetes by HbA_{1c} in a United Kingdom cohort (N = 8,696) was also noted by Mostafa et al. [20], who reported a prevalence of 44.9% in participants diagnosed by HbA_{1c} compared to 16.2% in subjects detected by an oral glucose tolerance test (OGTT). Similar findings were determined using FPG as the glucose-based criterion. Our results are also consistent with those reported in a recent Chinese study (N = 2,318) and from research examining a Palestinian Arab population (N = 1,370). Du et al. [21] and Kharroubi et al. [22] found reasonable or moderate concordance between HbA_{1c} and FPG for type 2 diabetes, but a higher prevalence by HbA_{1c} and limited overlap for pre-diabetes using ADA-designated thresholds.

However, our results contrast with findings reported in the United States by the Insulin Resistance Atherosclerosis Study (N = 855), which found a higher prevalence of pre-diabetes by FPG (31.1% vs. 10.6% for HbA_{1c}) [23]. Similarly, research utilising data from the National Health and Nutrition Examination Survey (1999–2006) found the prevalence of pre-diabetes in a sample of 7,029 adults to be 28.2% and 12.6% using FPG and HbA_{1c} respectively [24]. Possible reasons for observed prevalence disparities between HbA_{1c} and FPG may include age, gender or ethnic differences in examined populations [10, 14, 15]. In addition, as glucose continues to be metabolized in blood cells even after sampling, discrepancies may be due to biochemical analysis intervals within different studies [7, 22].

Longitudinal research has suggested that combined use of HbA_{1c} and FPG may be beneficial for identifying high-risk subjects. In two Asian studies, Inoue et al. [25] and Heianza et al. [26] demonstrated hazard ratios for type 2 diabetes to be greater for subjects classified by both assays when compared to those diagnosed by either HbA_{1c} or FPG separately. Findings from the Kansai Healthcare Study showed that joint use of both methods improved predictive ability [27]. In ROC analysis, AUCs for models including both HbA_{1c} and FPG were larger than those for HbA_{1c} (0.853 vs. 0.771; $P<0.001$) or FPG (0.853 vs. 0.818; $P<0.001$) alone. Recent research by Lipska et al. also revealed that addition of HbA_{1c} to a model with impaired FPG improved discrimination and calibration [28]. The results from the present study imply that the mechanism for this association is that individuals with diabetes-related phenotypes are more accurately identified using combined criteria.

Established risk factors for type 2 diabetes include obesity, raised triglyceride and low HDL-C levels, hypertension and insulin resistance [29]. In particular, subjects with a combination of these features have been shown to have a five-fold increased risk of developing diabetes [30]. Cardiovascular diseases, and in particular obesity-related type 2 diabetes, are also characterised by a low-grade but chronic inflammatory state [31, 32]. This may be reflected in an increased production of pro-inflammatory cytokines and also in higher levels of acute-phase response proteins, coagulation factors, macrophages and immune cells and lower levels of adiponectin, the anti-inflammatory adipokine [32, 33].

In our study it was noted that pre-diabetic individuals categorised by both assays demonstrated a stronger association with cardiometabolic feature clustering and displayed a more pro-inflammatory, pro-atherogenic, hypertensive and insulin resistant profile. Though few prospective studies have comprehensively identified features related to pre-diabetes development, it has been suggested that risk factors for pre-diabetes mirror those for type 2 diabetes [34]. Consequently, on the basis of the similar risk profiles noted in this study between pre-diabetes (defined using both HbA_{1c} and FPG) and type 2 diabetes (classified by either method), these findings also indicate that combined use of both assays may be clinically useful for detecting individuals at highest odds of developing diabetes.

Although HbA_{1c} has long been used as a marker for glycaemic control, its diagnostic performance for type 2 diabetes is still questioned [35–37]. Though a more expensive assay, when compared with FPG, HbA_{1c} has advantages including convenience, greater pre-analytical stability, lower biological variability and increasing international standardisation [7, 37]. Moreover, HbA_{1c} has been shown to correlate with cardiovascular disease and all-cause mortality [38]. However, as diabetes is clinically defined by elevated blood glucose, and not by glycation of proteins, there is concern that using HbA_{1c} to classify type 2 diabetes may lead to major changes in the pathophysiological paradigm that defines the condition [7]. Although a report from the United States inferred that diagnosis by HbA_{1c}, rather than FPG, would not significantly alter type 2 diabetes prevalence, and that categorisation would remain unchanged in as many as 97.7% of subjects [39], evidence is still equivocal [40].

Notably, within our sample, a higher prevalence of diabetes was determined using HbA_{1c} (7.3%, 95% CI: 6.3%-8.6%) compared to FPG (4.3%, 95% CI: 3.5%-5.2%). However, a similar type 2 diabetes prevalence rate in middle-aged Irish adults, defined by HbA_{1c}, was recently reported using data from the nationally representative 2007 Survey of Lifestyle, Attitudes and Nutrition (7.1%, 95% CI: 5.2%-9.0%) [40, 41]. It was also noted that diabetic subjects identified by HbA_{1c} or FPG within the present study displayed markedly similar cardiometabolic profiles. In addition, HbA_{1c} demonstrated high predictive ability for type 2 diabetes diagnosed by FPG $\geq$7.0 mmol/l levels. Conversely, HbA_{1c} showed poor discriminatory capacity for pre-diabetes defined by impaired FPG.

As HbA_{1c} reflects long-term glycaemic exposure, including postprandial glucose spikes, rather than the acute dysglycaemia indicated by FPG, it is rational to assume that each assay may identify different individuals. Our results suggest that HbA_{1c} may provide greater sensitivity for diagnosing type 2 diabetes within this sample. However, the limited overlap and substantially varied cardiometabolic profiles in subjects diagnosed with pre-diabetes, by either HbA_{1c} or FPG, imply that HbA_{1c} alone may lack specificity to accurately detect individuals at risk of diabetes development. It was also noted that metabolic risk profiles in pre-diabetic subjects, classified by impaired FPG levels only, were also considerably increased. This indicates that a percentage of high-risk individuals would be missed if HbA_{1c} was employed as a sole diagnostic criterion.

This study has several strengths, including a high participation rate (67%). As far as we are aware, ours is the first to compare pre-diabetes and type 2 diabetes prevalence, defined using both HbA_{1c} and FPG criteria, in a middle-aged Irish population. Additionally, few studies have compared a broad range of metabolic risk features and biomarkers with pre-diabetes and type 2 diabetes diagnosed by both assays. Our results are of potential clinical significance in terms of screening and the use of HbA_{1c} as a method for diagnosing diabetes and determining cardiometabolic risk. Accurate estimates of progression rates to type 2 diabetes are needed for efficient allocation of resources and to optimise public health prevention strategies [42]. Importantly, our findings indicate that caution should be taken with regard to how risk is defined as inexact methods may overestimate future diabetes burden [43, 44].

Notwithstanding these strengths, several limitations can be identified. These include single measurements of HbA_{1c} and FPG and that we did not have OGTT results as a comparison test. Although use of a third assay would have allowed a more thorough evaluation of HbA_{1c} and FPG, as discussed by Bonora et al. [7] comparisons between diagnostic methods for pre-diabetes and type 2 diabetes are ambiguous, as a true gold standard test is unavailable. Also, cross-sectional data precludes examination of temporal relationships. Consequently, though results from our research suggest associations between variables, they do not demonstrate an ability to predict type 2 diabetes or future cardiovascular events.

Equally of concern is that our data were derived from a single primary care based sample. Although results from the Cork and Kerry Diabetes and Heart Disease Study demonstrate prevalence rates for obesity and cardiovascular outcomes similar to those observed in other nationally representative Irish studies [40, 41, 45], the possibility that this sample is not representative of the source population must be acknowledged. However, previous research suggests that approximately 98% of Irish adults are registered with a GP and that, even in the absence of a universal patient registration system, it is possible to perform population-based epidemiological studies that are representative using these methods [46]. In addition, Ireland presents a generally ethnically homogeneous population [47]. Thus, the associations we observed between cardiometabolic features and HbA_{1c} and FPG may be comparable to other middle-aged Irish adults. As random sampling of subjects and the use of validated methods for data collection ensured internal sample validity, it is equally possible that the relationships described may be generalisable to a similar middle-aged, Caucasian-European population. Nevertheless, future studies utilising longitudinal data in different samples will be needed to confirm these findings. In particular, it will be necessary to determine whether risk stratification, using both assays, is clinically useful as a method for predicting type 2 diabetes.

Conclusions

In summary, our results suggest that in middle-aged Caucasian-Europeans, when using ADA-recommended cut-points, HbA_{1c} alone is a poor indicator of diabetes risk, but is appropriate for type 2 diabetes diagnosis. Furthermore, combined use of HbA_{1c} and FPG identifies subjects at substantially increased cardiometabolic risk. Although the efficacy and cost-effectiveness of routine screening for diabetes in primary care has not been established [48–50], in light of the increasing prevalence of type 2 diabetes worldwide, there is a need to identify high-risk subjects. Dual screening, utilising both HbA_{1c} and FPG, may provide a more accurate method for predicting cardiometabolic events. Earlier diagnosis could enable earlier targeted interventions or therapies, thus attenuating development of type 2 diabetes and associated cardiovascular complications.

Supporting Information

S1 File. The Cork and Kerry Diabetes and Heart Disease Study (Phase II) Dataset.
(ZIP)

Author Contributions

Conceived and designed the experiments: SRM IJP CMP. Performed the experiments: SRM IJP CMP. Analyzed the data: SRM. Contributed reagents/materials/analysis tools: SRM IJP CMP. Wrote the paper: SRM IJP CMP.

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RESEARCH ARTICLE

Optimal Central Obesity Measurement Site for Assessing Cardiometabolic and Type 2 Diabetes Risk in Middle-Aged Adults

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Abstract

Objectives

Despite recommendations that central obesity assessment should be employed as a marker of cardiometabolic health, no consensus exists regarding measurement protocol. This study examined a range of anthropometric variables and their relationships with cardiometabolic features and type 2 diabetes in order to ascertain whether measurement site influences discriminatory accuracy. In particular, we compared waist circumference (WC) measured at two sites: (1) immediately below the lowest rib (WC rib) and (2) between the lowest rib and iliac crest (WC midway), which has been recommended by the World Health Organisation and International Diabetes Federation.

Materials and Methods

This was a cross-sectional study involving a random sample of 2,002 men and women aged 46-73 years. Metabolic profiles and WC, hip circumference, pelvic width and body mass index (BMI) were determined. Correlation, logistic regression and area under the receiver operating characteristic curve analyses were used to evaluate obesity measurement relationships with metabolic risk phenotypes and type 2 diabetes.

Results

WC rib measures displayed the strongest associations with non-optimal lipid and lipoprotein levels, high blood pressure, insulin resistance, impaired fasting glucose, a clustering of metabolic risk features and type 2 diabetes, in both genders. Rib-derived indices improved discrimination of type 2 diabetes by 3-7% compared to BMI and 2-6% compared to WC midway (in men) and 5-7% compared to BMI and 4-6% compared to WC midway (in women). A prediction model including BMI and central obesity displayed a significantly higher area under the curve for WC rib (0.78, P=0.003), Rib/height ratio (0.80, P<0.001), Rib/pelvis ratio (0.79, P<0.001), but not for WC midway (0.75, P=0.127), when compared to one with BMI alone (0.74).

Competing Interests: The authors declare that no competing interests exist.

Conclusions

WC rib is easier to assess and our data suggest that it is a better method for determining obesity-related cardiometabolic risk than WC midway. The clinical utility of rib-derived indices, or alternative WC measurements, deserves further investigation.

Introduction

Obesity is associated with dyslipidaemia, hypertension, insulin resistance and the development of metabolic syndrome and type 2 diabetes [1], leading to a greater likelihood of premature death. However, not all obese subjects are at increased cardiometabolic risk as a proportion are considered to be metabolically healthy [2]. The prevalence of obesity has escalated in many world populations [3]. Thus, there is an increasing need for inexpensive and non-invasive methods for use in clinical practice to identify overweight and obese individuals at highest odds of developing metabolic abnormalities and type 2 diabetes.

Body mass index (BMI) has traditionally been the chosen surrogate method used to determine excess body fat, but because it is a weight-for-height measure, BMI is unable to distinguish between fat and lean mass. Recent research has indicated that general obesity categorisation based on BMI might be inadequate [4,5], and studies have shown that BMI may misclassify adiposity [6–8].

Increasing evidence suggests that central obesity is a more important cardiometabolic risk factor [9,10] and waist circumference (WC) measurement has been recommended as a method for central obesity assessment. However, partly due to a lack of agreement on a universal measurement protocol, its clinical usefulness and superiority over BMI in the prediction of cardiometabolic events has been questioned [11,12]. Various transformations of WC have also been used, such as the waist/height ratio (WHtR) [13] and waist/hip ratio (WHR) [14]. Although extensive research has attempted to quantify relationships between different adiposity measures and morbidity [11], considerable controversy still exists as to which measurement site or index most accurately defines non-optimal body fat distribution [15].

In this study we examined a range of anthropometric variables and their relationships with metabolic risk phenotypes, including lipid and lipoprotein levels, high blood pressure, insulin resistance, impaired fasting glucose, a clustering of metabolic risk features and type 2 diabetes, in a random sample of 2,002 middle-aged men and women. In particular, we compared the discriminatory performance of WC measured at two locations (immediately below the lowest rib, and between the lowest rib and iliac crest), and variations of these measures, to address the hypothesis that the measurement site for central obesity affects its accuracy as a predictor of cardiometabolic risk.

Materials and Methods

Study population

The Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000, with a mix of urban and rural residents. Stratified sampling by age and sex was employed to recruit equal numbers of men and women from all registered attending patients in the 50–69 year age group. In total, 3,807 individuals were selected from the practice

list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3,051 were invited to participate in the study and of these, 2,047 (49.2% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1%). Details regarding the study design, sampling procedures and methods of data collection have been reported previously [16].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

Clinical and laboratory measurements

All study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, physician-diagnosed type 2 diabetes and prescription (Rx) medication use were gathered through a self-completed General Health Questionnaire. Triglyceride and high density lipoprotein cholesterol (HDL-C) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica GmbH, Hamburg, Germany). Fasting plasma glucose concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and fasting serum insulin was calculated using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium]. Three independent measurements of systolic and diastolic blood pressure (BP) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP.

Anthropometric variables

Anthropometric measurements were taken by researchers who were thoroughly trained according to the study research protocols [16]. The weight and height of each subject were measured to the nearest 0.1 kg and 0.1 cm respectively. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was assessed using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and BMI was calculated as weight divided by the square of height. Midway WC (WC midway) was measured between the lowest rib and iliac crest on bare skin. Participants were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. Rib WC (WC rib) was measured immediately below the lowest rib at the mid-axillary line and hip circumference was determined at the maximum perimeter of the hips. Pelvic width was calculated as the diameter between the right and left iliac crests using callipers. For each central obesity measure, the mean of two independent readings was used in analysis. Height, hip circumference and pelvic width were divided into WC midway and WC rib measurements deriving six variables: (1) *Midway/height ratio*, (2) *Midway/hip ratio*, (3) *Midway/pelvis ratio* and (4) *Rib/height ratio*, (5) *Rib/hip ratio*, (6) *Rib/pelvis ratio*.

Classification of biochemical and blood pressure measurements

Lipid, lipoprotein, glucose and BP measurements were categorised according to National Cholesterol Education Program Adult Treatment Panel III criteria [17]. Abnormal metabolic risks were defined as high triglyceride levels ≥1.7 mmol/l, low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females) and impaired fasting glucose levels 5.6–6.9 mmol/l. High BP was classified as systolic BP ≥130 mmHg and/or diastolic BP ≥85 mmHg or Rx anti-hypertensive medication use. The Homeostasis Model Assessment Index of Insulin Resistance (HOMA-IR) [18] was derived from fasting glucose and insulin concentrations as [(fasting plasma glucose x fasting serum insulin)/22.5], and insulin resistance was defined as a level equal to or above the 75th percentile in the study population. Having three or more cardiometabolic risk features was characterised as any combination of these variables. According to American Diabetes Association guidelines, type 2 diabetes was defined as HbA_{1c} ≥6.5% (≥48 mmol/mol) or fasting plasma glucose ≥7.0 mmol/l [19]. Individuals on insulin therapy and subjects indicating a diagnosis of diabetes (either self-reported physician diagnosis or Rx diabetes medication use), but who did not have positive HbA_{1c} or fasting plasma glucose test results, were excluded from analysis (N = 45).

Statistical analysis

The distribution of each metabolic characteristic was assessed using Shapiro-Wilk and Kolmogorov-Smirnov statistics. Categorical features are presented as percentages and continuous data are shown as a mean, plus or minus one standard deviation, or a median and interquartile range. Gender differences were evaluated using chi-square tests, independent *t*-tests or a Mann-Whitney U for skewed data. Relationships between anthropometric measurements and continuous cardiometabolic variables were investigated using partial correlations. Variables presenting a non-normal distribution were log-transformed. All obesity measures were gender-standardised and separate and stratified binary logistic regression models were used to compare index associations with cardiometabolic risk features and type 2 diabetes, adjusting for age.

The ability of selected indices to discriminate three or more cardiometabolic risk features and type 2 diabetes was measured using receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) provides a scale from 0.5 to 1.0 (with 0.5 representing random chance and 1.0 indicating perfect discrimination) by which to appraise the capacity of an obesity index to detect a positive result [20]. A higher AUC generally indicates greater diagnostic accuracy. Covariate-adjusted analysis [21] was performed to account for the potential confounding influence of both age and gender (full cohort) or age alone in stratified models. The AUC values were compared for statistical differences and were further evaluated by determining false positive rates at specific points on the curve corresponding to 90%, 80%, 70% and 60% sensitivities.

To further judge the ability of central obesity to discriminate type 2 diabetes, we compared a logistic regression prediction model containing BMI to models which included both BMI and selected central obesity measures. The accuracy of each model was assessed using the ROC curve. We additionally evaluated discrimination using Integrated Discrimination Improvement (IDI) analysis, which indicates the magnitude of improvement in the performance of a model by adding another variable [22]. To assess goodness-of-fit, the likelihood ratio (LR) chi-square statistics were examined by comparing models with or without an additional anthropometric measure. Calibration was measured using the Hosmer-Lemeshow (HL) test.

Data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) and Stata SE Version 13 (Stata Corporation, College Station, TX, USA) for

Windows. Seven subjects had missing anthropometric values. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance.

Results

Descriptive characteristics

Characteristics of the study population are presented in Table 1. According to BMI classification recommended by the World Health Organisation (WHO) [23], 1,550 (77.7%) participants were either overweight or obese, with 835 (85.6%) male subjects having a BMI $\geq$25 kg/m^2 compared to 715 (70.2%) females (P for difference <0.001). Mean WC and pelvic width measurements were also significantly increased in men while hip circumference levels were greater in women. Distinctions between WC midway and WC rib were observed in both genders, with average midway values being higher. With consideration to metabolic risk factors, male subjects were significantly more likely to have abnormal triglyceride levels, high BP, insulin

Table 1. Characteristics of the study population.

Feature	Males (N = 981)	Females (N = 1021)	P value
Age	59 (55–64)	59.0 (54–64)	0.791
Weight (kg)	87.38 ± 13.8	71.58 ± 13.6	<0.001
Height (m)	1.73 ± 0.1	1.60 ± 0.1	<0.001
BMI (kg/m2)	29.12 ± 4.2	28.02 ± 5.2	<0.001
WC midway (cm)	102.61 ± 11.1	91.37 ± 12.7	<0.001
WC rib (cm)	99.88 ± 10.1	85.10 ± 12.2	<0.001
Hip circumference (cm)	98.96 ± 8.7	101.79 ± 10.7	<0.001
Pelvic width (cm)	32.96 ± 2.4	31.97 ± 2.7	<0.001
Triglycerides (mmol/l)	1.32 (0.9–1.9)	1.10 (0.8–1.5)	<0.001
High triglycerides[1]	313 (32.9)	164 (16.5)	<0.001
HDL-C (mmol/l)	1.28 ± 0.3	1.62 ± 0.4	<0.001
Low HDL-C[2]	166 (17.3)	169 (16.8)	0.676
Average systolic BP (mmHg)	130.83 ± 15.6	128.44 ±17.9	0.001
Average diastolic BP (mmHg)	79.94 ± 9.6	80.42 ± 9.9	0.339
High blood pressure[3]	628 (64.3)	593 (58.3)	0.006
HOMA-IR	3.27 (1.3–3.8)	2.32 (1.0–2.7)	<0.001
Insulin resistance[4]	301 (32.0)	179 (18.2)	<0.001
Fasting plasma glucose (mmol/l)[5]	5.00 (4.7–5.4)	4.80 (4.5–5.2)	<0.001
Impaired fasting glucose[5,6]	150 (17.3)	80 (8.5)	<0.001
Three or more cardiometabolic risk features[5]	178 (20.0)	106 (10.9)	<0.001
Type 2 diabetes	92 (9.5)	50 (5.0)	<0.001

Mean and ± standard deviation are shown for continuous variables, P value calculated with a Student's *t*-test. Age, triglycerides, HOMA-IR, HbA_{1c} and fasting plasma glucose are shown as a median (interquartile range) with a P value according to a Mann-Whitney U. % are shown for categorical values with x^2 for difference in proportions, numbers and (%) may vary as some variables have missing values.

[1]Triglycerides $\geq$1.7 mmol/l.

[2]HDL-C <1.03 mmol/l (males) or HDL-C <1.29 mmol/l (females).

[3]BP $\geq$130/85 mmHg or on Rx for hypertension.

[4]HOMA-IR 75th percentile.

[5]Excluding subjects with type 2 diabetes.

[6]Fasting plasma glucose $\geq$5.6 mmol/l.

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Table 2. Partial correlations[1] between anthropometric measurements and cardiometabolic variables, stratified by gender.

Cardiometabolic feature	Weight	Height	BMI	WC midway	WC rib	Hip circumference	Pelvic width
MALES							
Triglycerides[2]	0.249	-0.062	0.306	0.296	**0.319**	0.257	0.162
HDL-C	-0.347	0.063[3]	-0.350	-0.345	**-0.354**	-0.327	-0.295
Systolic BP	0.189	-0.002[3]	0.205	0.175	**0.218**	0.168	0.138
Diastolic BP	0.220	0.012[3]	**0.230**	0.198	**0.228**	0.187	0.168
HbA1c[2]	0.178	-0.044[3]	0.218	0.249	**0.261**	0.214	0.123
HOMA-IR[2]	0.497	-0.005[3]	0.557	0.570	**0.572**	0.517	0.362
Glucose[2]	0.187	-0.093	0.254	0.260	**0.267**	0.219	0.122
FEMALES							
Triglycerides[2]	0.306	-0.033[3]	0.326	0.342	**0.404**	0.281	0.205
HDL-C	-0.283	0.074	-0.314	-0.301	**-0.364**	-0.265	-0.172
Systolic BP	0.148	-0.030[3]	**0.163**	0.135	0.161	0.126	0.078
Diastolic BP	0.172	-0.019[3]	**0.186**	0.136	0.170	0.149	0.081
HbA1c[2]	0.202	-0.029[3]	0.220	0.208	**0.256**	0.177	0.103
HOMA-IR[2]	0.516	-0.052[3]	0.550	0.493	**0.574**	0.462	0.288
Glucose[2]	0.281	-0.017[3]	0.298	0.303	**0.347**	0.268	0.183

[1]Adjusted for age.

[2]nLog transformed.

All correlation coefficients are significant (P<0.05) except: [3]P>0.05. The index associated with the highest correlative strength to the variable in the same row is highlighted.

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resistance, impaired fasting glucose, a clustering of cardiometabolic risk features and type 2 diabetes.

Partial correlations between anthropometric measurements and cardiometabolic variables

After adjustment for age, positive correlations for triglycerides, systolic BP, diastolic BP, HbA_{1c}, glucose, HOMA-IR, and negative correlations for HDL-C, were observed with weight, BMI and measurements of central obesity (Table 2). Significant inverse relationships were also noted for height with triglyceride and glucose concentrations in men, while HDL-C was positively correlated with height in women. Relationships were stronger between WC rib and a majority of metabolic variables, with triglycerides, HDL-C and HOMA-IR showing the highest correlative strengths. Nevertheless, metabolic variable correlations with BMI and WC midway, although reduced, were of a similar magnitude in men.

Associations between obesity measures, cardiometabolic risk features and type 2 diabetes

The results from regression models examining adiposity variable associations with individual metabolic risk factors (S1 Fig), three or more cardiometabolic risk features (Fig 1) and type 2 diabetes (Fig 2) are shown. Results are adjusted for age and odds ratios represent the odds associated with a one standard deviation increase in each obesity measure. Although the strength of relationship varied according index type, WC rib or rib-derived indices displayed, without exception, stronger associations with individual cardiometabolic risk factors, metabolic feature clustering and type 2 diabetes, in both genders. In general, stronger relationships with

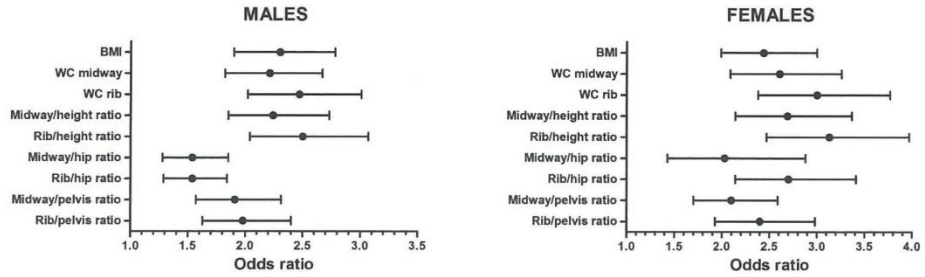

Fig 1. Odds ratios (95% CI) of having three or more cardiometabolic risk features for a one standard deviation increase in each obesity measure. Results are stratified by gender and adjusted for age. All models exclude subjects with type 2 diabetes.

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cardiometabolic variables were noted in women, with differences between BMI and central obesity being less pronounced in male subjects.

Receiver operating characteristic curve analysis

In ROC analysis, both WC rib and Rib/height ratio demonstrated a significantly higher AUC to detect three or more cardiometabolic risk features compared to WC midway in male subjects (Fig 3). In females, significant differences in the AUC were observed when compared to both WC midway and BMI. For type 2 diabetes (Fig 4), WC rib measures showed a higher discriminatory capacity in both genders, with the exception of the Rib/hip ratio in men. Rib-derived indices improved discrimination by 3–7% compared to BMI and 2–6% compared to WC midway (in men) and 5–7% compared to BMI and 4–6% compared to WC midway (in women). Rib measures also displayed greater specificity across a range of sensitivities (Fig 5). At higher sensitivities classification accuracy was improved by as much as 10% or more. However, false positive rates for the Rib/hip ratio were noticeably increased when compared to other adiposity variables in men.

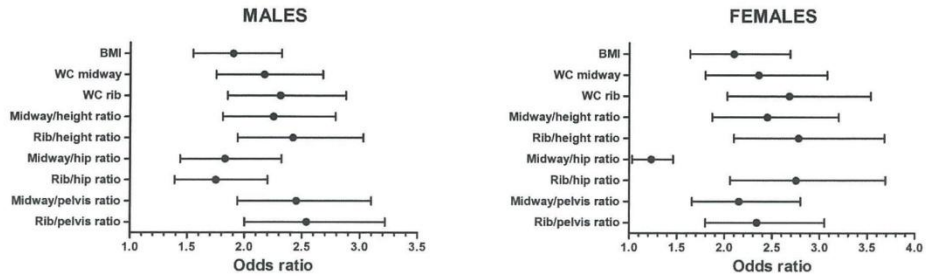

Fig 2. Odds ratios (95% CI) of having type 2 diabetes for a one standard deviation increase in each obesity measure. Results are stratified by gender and adjusted for age.

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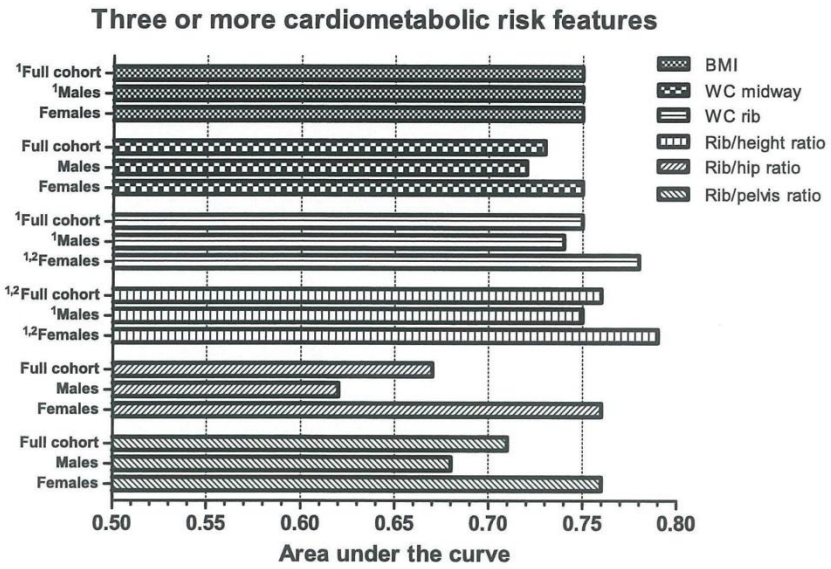

Fig 3. Adjusted area under the receiver operating characteristic curve values for selected obesity measures to discriminate subjects with three or more cardiometabolic risk features. Bars represent AUC values. All models exclude subjects with type 2 diabetes. Statistical differences in the AUC values are shown in superscript Arabic numbers as: [1]P<0.05 compared to WC midway; [2]P<0.05 compared to BMI.

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Evaluation of prediction models

As presented in Table 3, we examined prediction models for type 2 diabetes which included BMI and an additional central obesity measure. The HL test showed P values that were non-significant, suggesting that model fits were acceptable. Additionally, the LR chi-squares were reduced in models including central obesity variables, indicating improved goodness-of-fit. Using the IDI statistic, a significant but marginal increase in discrimination was observed for WC midway, with a small and non-significant increase in the AUC (0.75, P = 0.127) (Fig 6). In contrast, a prediction model including BMI and WC rib measures displayed a significantly higher AUC (Figs 7–9) for WC rib (0.78, P = 0.003), Rib/height ratio (0.80, P<0.001) and Rib/pelvis ratio (0.79, P<0.001) when compared to a model with BMI alone (0.74).

Discussion

Both the WHO and International Diabetes Federation (IDF) have suggested midway WC measurement as the preferred method for central obesity assessment [12,24]. In contrast, the United States National Institutes of Health (NIH) recommends measuring WC at the superior border of the iliac crest [25]. However, there is a lack of scientific rationale to support either of these measurement protocols [26]. Although previous studies have compared these two criteria, to the best of our knowledge, this is the first to comprehensively evaluate rib WC measures and both WC midway and BMI as predictors of cardiometabolic risk and type 2 diabetes. Our findings suggest that WC rib, rather than WC midway, is a better indicator of central obesity as it improves discrimination of type 2 diabetes within our population. One possible explanation

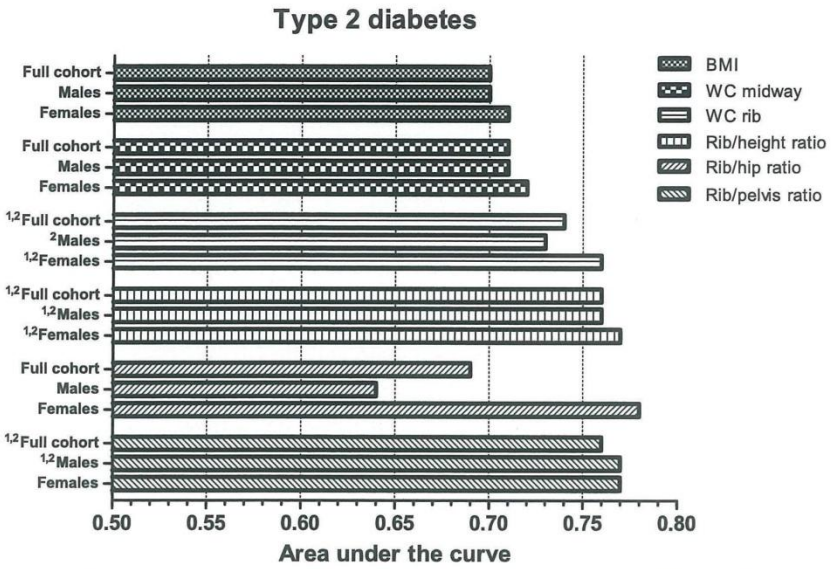

Fig 4. Adjusted area under the receiver operating characteristic curve values for selected obesity measures to discriminate subjects with type 2 diabetes. Bars represent AUC values. Statistical differences in the AUC values are shown in superscript Arabic numbers as: [1]P<0.05 compared to WC midway; [2]P<0.05 compared to BMI.

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for this relationship may be that rib-level measurement is less influenced by inter-individual variables such as body posture or elasticity of the abdominal wall, which are partly unrelated to actual body adiposity.

The results from previous research investigating different WC measurement criteria are conflicting. A systematic review of 120 studies [27] concluded that the measurement procedure

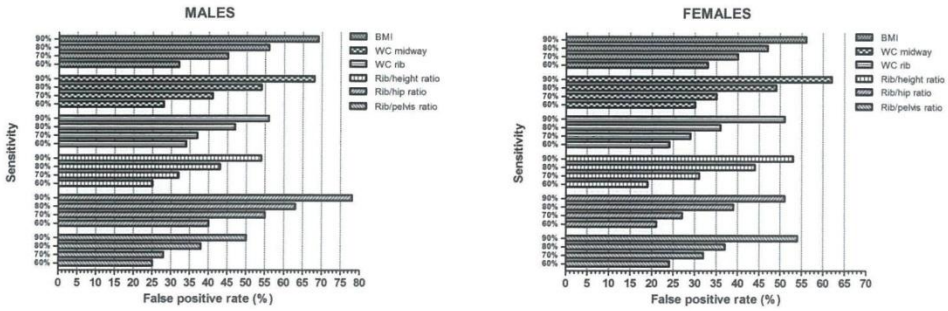

Fig 5. False positive rates corresponding to 90%, 80%, 70% and 60% sensitivities for selected obesity measures to classify subjects with type 2 diabetes. Results are stratified by gender and adjusted for age. Bars represent false positive rates (percentages).

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Table 3. Tests of calibration, goodness-of-fit and discrimination for prediction models to identify subjects with type 2 diabetes.

Model[1]	HL x^2 (P value)	LR x^2 (P value)	AUC (95% CI)	IDI (95% CI)
BMI alone	4.39 (0.82)	919.38 (<0.001)	0.74 (0.70–0.78)	-
BMI and WC midway	2.32 (0.97)	900.78 (<0.001)	0.75 (0.71–0.79)[2]	0.0177 (0.002–0.0334)
BMI and WC rib	5.01 (0.76)	877.54 (<0.001)	0.78 (0.74–0.82)[3]	0.0283 (0.0111–0.0455)
BMI and Rib/height ratio	5.34 (0.72)	858.75 (<0.001)	0.80 (0.76–0.84)[4]	0.0364 (0.0162–0.0566)
BMI and Rib/pelvis ratio	6.58 (0.58)	860.73 (<0.001)	0.79 (0.75–0.82)[5]	0.0290 (0.0135–0.0445)

[1]All models include age and gender.

[2]P value = 0.127 compared to model with BMI alone.

[3]P value = 0.003 compared to model with BMI alone.

[4]P value<0.001 compared to model with BMI alone.

[5]P value<0.001 compared to model with BMI alone.

doi:10.1371/journal.pone.0129088.t003

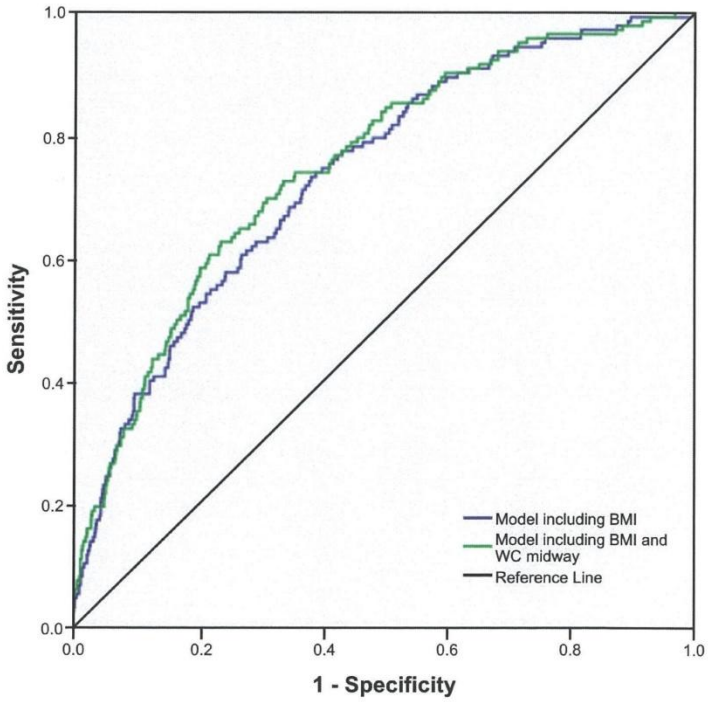

Fig 6. Receiver operating characteristic curves for prediction models to discriminate subjects with type 2 diabetes. Figures show ROC curves for a model including BMI and a model including BMI and WC midway. All models include age and gender.

doi:10.1371/journal.pone.0129088.g006

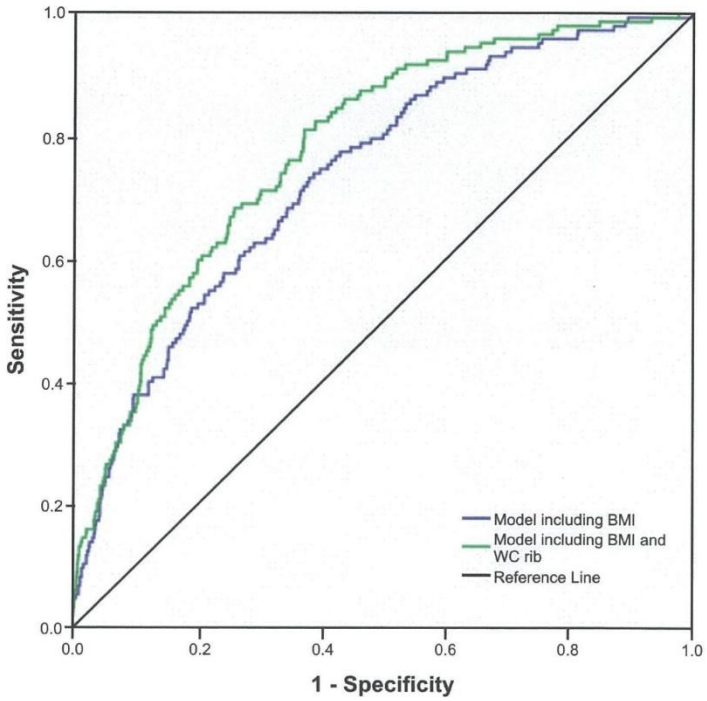

Fig 7. Receiver operating characteristic curves for prediction models to discriminate subjects with type 2 diabetes. Figures show ROC curves for a model including BMI and a model including BMI and WC rib. All models include age and gender.

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had no substantial influence on WC relationships with morbidity and mortality, leading the authors to recommend the NIH protocol as it may be more readily adapted by health practitioners and is more suitable for self-measurement by the general public. However, effect sizes and discriminatory differences between WC sites were not compared. In contrast, Ma et al. [28] found WC midway to be slightly better than NIH-recommended iliac measurement to predict hypertension, metabolic syndrome and diabetes. Nevertheless, WC rib was not assessed in this study. Bosy-Westphal et al. [26] also observed lower associations between the iliac site and metabolic characteristics and visceral adipose tissue (VAT) in females. Relationships between cardiometabolic variables and WC midway and rib were similar in men, while WC rib was more strongly correlated with VAT in women.

Regardless of controversies surrounding WC measurement protocol, both advantages and disadvantages exist regarding the general application of central obesity assessment within clinical practice. Although some studies have suggested WC to be the simplest and best overall method for cardiometabolic health appraisal [29], as metabolic risk cut-points for WC are different between genders, and vary between ethnic groups [12,30], the practical usability of WC measurement is still uncertain [11].

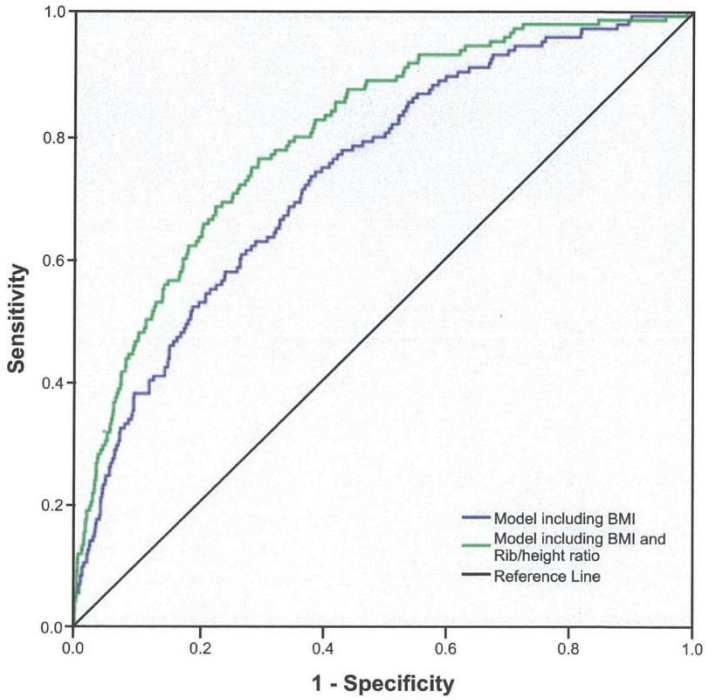

Fig 8. Receiver operating characteristic curves for prediction models to discriminate subjects with type 2 diabetes. Figures show ROC curves for a model including BMI and a model including BMI and Rib/height ratio. All models include age and gender.

doi:10.1371/journal.pone.0129088.g008

In keeping with other findings [13,31], our results imply that transformations of WC may improve discrimination of cardiometabolic outcomes. The use of a ratio to define central obesity is also potentially beneficial as it might allow uniform diagnostic thresholds to be used (between ethnicities, genders or both), making it attractive from a public health perspective [32,33]. Notably, however, the WHR was a markedly inferior discriminator of risk in male subjects within this sample. Reduced associations for WHR were also observed by Schneider et al. [34], who theorised that as both WC and hip circumference exhibit strong relationships with cardiometabolic features, a ratio of the two may show less. Additionally, both measures may increase or decrease proportionally in an individual [35]. It could be that sex differences observed for WHR are due to gender variations in body composition, and that changes in hip circumference, relative to WC, are more pronounced in middle-aged men than in women.

Although WC rib measures demonstrated stronger relationships with metabolic variables, consistent with previous research [11], our study also revealed that anthropometric associations with a majority of cardiometabolic risk factors and type 2 diabetes were reduced in men. One possible explanation for this finding is the greater prevalence of overweight and obesity amongst males within this population, perhaps minimising associations and predictive

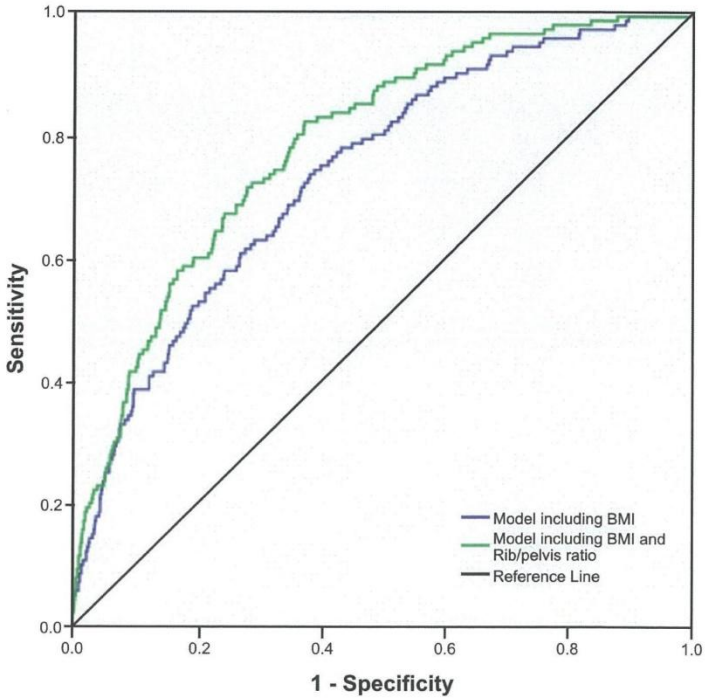

Fig 9. Receiver operating characteristic curves for prediction models to discriminate subjects with type 2 diabetes. Figures show ROC curves for a model including BMI and a model including BMI and Rib/pelvis ratio. All models include age and gender.

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abilities. It was also noted that discriminatory differences between central obesity and BMI were greater when detecting type 2 diabetes compared to a clustering of metabolic variables, in both genders. A reason for this may be that central adiposity independently predicts type 2 diabetes, beyond traditionally assessed cardiometabolic disease markers [36].

Compared with BMI, central obesity is thought to be more strongly correlated with VAT [10]. Research has implied that fatty acids released from VAT drain into the liver and skeletal muscle causing metabolic dysfunction within these organs [37]. Adipokines secreted from VAT may also contribute to cardiometabolic disease through inflammation of vascular tissue [9]. Increased VAT has been shown to be associated with increased risk of dyslipidaemia, hypertension and type 2 diabetes [38,39]. Consequently, observed differences in discrimination for cardiometabolic disease mediators and type 2 diabetes suggest that central obesity should be independently evaluated as a cardiometabolic risk factor, and that its inclusion as a mandatory component of the metabolic syndrome may be appropriate [24].

However, the findings from previous studies which have contrasted central obesity (either WC, WHtR or WHR) with BMI to discriminate cardiometabolic conditions have been inconclusive [11,15]. Possible reasons for variations between studies may include different WC

measurement protocols or dissimilar methods for classifying cardiometabolic outcomes. Although AUC values for central obesity measures are frequently reported to be larger when compared to BMI for predicting type 2 diabetes [40], as the AUC lacks clinical relevance, there is argument against using it as a summary statistic of the ROC curve as similar AUC values may have different diagnostic properties [21]. Though other studies have reported metabolic risk thresholds for obesity indices based on maximum sensitivity, optimal sensitivity and specificity or the shortest distance to the y axis [12], cut-points are necessarily arbitrary, and may vary between populations.

Central obesity measures have been proposed as stand-alone, pre-screening tools [33] for use in high-risk populations to enable clinicians to detect those who might benefit from further diagnostic or therapeutic procedures [41,42]. In this scenario it is desirable to optimise sensitivity (the percentage of people with or at risk of a condition, who would be correctly identified), in order to rule out healthy subjects. Importantly, by comparing false positive rates (the proportion of healthy individuals who would be misclassified) across a range of sensitivities for multiple indices, our results demonstrate WC rib measures to be more accurate classifiers, at higher sensitivities, compared to WC midway and BMI.

Nevertheless, debate exists regarding the clinical efficacy of central obesity measurement. To some extent this is due to a lack of evidence regarding how much of an increase in predictive accuracy central obesity measures might add over traditionally assessed cardiometabolic risk indicators [11]. Though our findings suggest that central obesity variables may provide additional prognostic information, these results also indicate that the degree of improvement is significantly influenced by measurement procedure.

While only requiring a flexible measuring tape, midway WC is difficult to obtain as it requires the identification of two bony landmarks, a computed distance between the two, and a circumference evaluation—essentially four separate measurements. As central obesity assessment competes for the limited time available during patient appraisal, and necessitates specific training to ensure reliable data are obtained [10], a simpler measurement protocol is desirable. WC rib is more easily determined and offers a more practical method for use within healthcare practice and epidemiological research, and would be equally suitable for self-assessment. Furthermore, Bosy-Westphal et al. [26] and Wang et al. [43] also concluded that WC rib had a higher reproducibility. As measurement error may limit the minimal detectable difference in a parameter [26], it is possible that the higher discriminatory accuracy we observed may be due to greater measurement precision.

Though our findings are of potential public health and clinical significance, several limitations should be considered. Given the modest number of outcomes within our sample we did not adjust for multiple factors in analyses. Our primary aim was to compare general and central obesity relationships, rather than to determine overall strengths of association. Nevertheless, the possibility that confounding features may influence adiposity variables in different ways cannot be discounted and future studies with larger samples might find different relationships. Also, as cross-sectional data precludes examination of the temporal relationship between obesity measures and cardiometabolic disease, our results may suggest associations, but they do not demonstrate an ability to predict type 2 diabetes.

Equally of concern is that we did not have other WC measurement sites to contrast and that our data were derived from a single primary care based sample. However, Ireland represents a generally ethnically homogeneous population [44]. Consequently, random sampling of subjects and the use of validated methods for data collection ensured internal sample validity and the relationships described may be generalisable to a similar middle-aged, Caucasian-European population. Nonetheless, future studies utilising longitudinal data in different samples will be needed to evaluate the validity and reliability of alternative WC measurements. In particular, it

will be necessary to determine whether risk stratification, using central obesity, is clinically useful and superior to currently recommended BMI classification [45].

Conclusions

In summary, our results indicate that measurement protocol for WC may be important for determining central obesity and assessing cardiometabolic health. Rib-level measures were more strongly related to cardiometabolic risk factors and demonstrated improved discrimination of type 2 diabetes. In light of the increasing prevalence of obesity and cardiometabolic disease worldwide, effective methods that help assess the probability of diabetes development are needed [46,47]. The clinical utility of WC measured at the lowest rib, rib-derived indices or alternative WC measurements as potentially more accurate predictors of metabolic risk and type 2 diabetes, compared to WHO and IDF-recommended WC midway measurement or BMI, deserves further investigation.

Supporting Information

S1 Figs. Odds ratios (95% CI) of having non-optimal cardiometabolic risk features for a one standard deviation increase in each obesity measure. Results are stratified by gender and adjusted for age. Figures show odds ratios (95% CI) regarding obesity measurement associations with high triglycerides, low HDL-C, high blood pressure, insulin resistance and impaired fasting glucose. Models examining impaired fasting glucose exclude subjects with type 2 diabetes.
(PDF)

Acknowledgments

The work on central obesity measurement procedure was led by the late Professor Jan Van den Broeck and this paper is dedicated to his memory. Jan was a Senior Research Fellow in the HRB Centre for Health and Diet Research at University College Cork before taking up the post of Professor of Global Nutrition at the Centre for International Health, University of Bergen. He was an outstanding epidemiologist and an inspiring friend and colleague.

Author Contributions

Conceived and designed the experiments: SRM JVB. Analyzed the data: SRM. Contributed reagents/materials/analysis tools: SRM IJP. Wrote the paper: SRM IJP JVB CMP.

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DIABETOLOGY & METABOLIC SYNDROME

RESEARCH Open Access

Assessing cardiometabolic risk in middle-aged adults using body mass index and waist–height ratio: are two indices better than one? A cross-sectional study

Seán R. Millar*, Ivan J. Perry and Catherine M. Phillips

Abstract

Background: A novel obesity classification method has been proposed using body mass index (BMI) and waist–height ratio (WHtR) together. However, the utility of this approach is unclear. In this study we compare the metabolic profiles in subjects defined as overweight or obese by both measures. We examine a range of metabolic syndrome features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell counts to determine whether a combination of both indices more accurately identifies individuals at increased obesity-related cardiometabolic risk.

Methods: This was a cross-sectional study involving a random sample of 1856 men and women aged 46–73 years. Metabolic and anthropometric profiles were assessed. Linear and logistic regression analyses were used to compare lipid, lipoprotein, blood pressure, glycaemic and inflammatory biomarker levels between BMI and WHtR tertiles. Multinomial logistic regression was performed to determine cardiometabolic risk feature associations with BMI and WHtR groupings. Receiver operating characteristic curve analysis was used to evaluate index discriminatory ability.

Results: The combination of BMI and WHtR tertiles identified consistent metabolic variable differences relative to those characterised on the basis of one index. Similarly, odds ratios of having cardiometabolic risk features were noticeably increased in subjects classified as overweight or obese by both measures when compared to study participants categorised by either BMI or WHtR separately. Significant discriminatory improvement was observed for detecting individual cardiometabolic risk features and adverse biomarker levels. In a fully adjusted model, only individuals within the highest tertile for both indices displayed a significant and positive association with pre-diabetes, OR: 3.4 (95 % CI: 1.9, 6.0), $P < 0.001$.

Conclusions: These data provide evidence that the use of BMI and WHtR together may improve body fat classification. Risk stratification using a composite index may provide a more accurate method for identifying high and low-risk subjects.

Keywords: Body mass index, Waist–height ratio, Cardiometabolic risk, Screening

Background

Excess body fat has been shown to be associated with dyslipidaemia, hypertension, insulin resistance, chronic low-grade inflammation and the development of metabolic syndrome (MetS), type 2 diabetes and cardiovascular complications [1–4]. Numerous studies have also demonstrated a high mortality rate in subjects with a body mass index (BMI) $\geq$30 kg/m^2 [5]. But because it is a weight-for-height measure, BMI is unable to distinguish between fat and lean mass and elevated BMI may

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not always indicate increased adiposity or predict cardiometabolic events [6, 7].

Evidence suggests that central obesity is a more important metabolic risk factor and waist circumference (WC) measurement has been recommended as a method for central obesity assessment [8, 9]. However, as WC diagnostic thresholds are different for men and women, and may vary between ethnic groups [10], the practical utility of WC measurement has been questioned [11].

The waist–height ratio (WHtR) (WC divided by height) has been advocated as an alternative surrogate measure of central obesity [12]. As a ratio, this index may circumvent problematic issues relating to gender or population-specific risk cut-points [13, 14]. But results from studies which have compared BMI and WHtR discriminatory abilities have been inconclusive, with some showing WHtR to be only marginally superior to BMI for predicting cardiometabolic outcomes [15–17].

The prevalence of obesity has escalated in many world populations [18]. Thus, there is an increasing need to identify overweight and obese individuals at highest odds of developing cardiometabolic diseases. Recently, a new obesity classification method was proposed, utilising BMI in conjunction with WHtR [14]. Risk stratification using a composite index may provide a more effective method for identifying high and low-risk subjects. This could allow earlier diagnosis, thus attenuating metabolic complications and chronic morbidity development.

The aim of this study was to compare the metabolic profiles in subjects defined as overweight or obese, using BMI and WHtR, in a random sample of 1856 middle-aged men and women. In particular, we examined a range of MetS features, pro-inflammatory cytokines, acute-phase response proteins, coagulation factors and white blood cell (WBC) counts to determine whether a combination of BMI and WHtR more accurately identifies individuals at increased obesity-related cardiometabolic risk.

Methods

Study population

The Cork and Kerry Diabetes and Heart Disease Study (Phase II) was a single centre, cross-sectional study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Livinghealth Clinic serves a population of approximately 20,000, with a mix of urban and rural residents. Stratified sampling was employed to recruit equal numbers of men and women from all registered attending patients in the 46–73 year age group. In total, 3807 potential participants were selected from the practice list. Following the exclusion of duplicates, deaths, and subjects incapable of consenting or attending appointment, 3051 were invited to participate in the study and of these, 2047 (49.2 % male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67.1 %). Details regarding the study design, sampling procedures and methods of data collection have been reported previously [19].

Ethics committee approval conforming to the Declaration of Helsinki was obtained from the Clinical Research Ethics Committee of University College Cork. A letter signed by the contact GP in the clinic was sent out to all selected participants with a reply slip indicating acceptance or refusal. All subjects gave signed informed consent, including permission to use their data for research purposes.

Clinical and laboratory procedures

All study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Data on age, gender, morbidity, prescription (Rx) medication use and smoking and alcohol behaviours were gathered through a self-completed General Health Questionnaire (GHQ). Physical activity levels were assessed using the validated International Physical Activity Questionnaire (IPAQ) [20]. Three independent measurements of systolic and diastolic blood pressure (BP) were obtained with the subject in a seated position using an Omron M7 digital sphygmomanometer (Omron Healthcare Co. Ltd., Japan). The mean of the second and third readings was considered to be a subject's BP.

Triglyceride and high density lipoprotein cholesterol (HDL-C) levels were measured by Cork University Hospital Biochemistry Laboratory on Olympus 5400 biochemistry analysers with Olympus reagents using standardised procedures and fresh samples (Olympus Diagnostica GmbH, Hamburg, Germany). Glucose concentrations were determined using a glucose hexokinase assay (Olympus Life and Material Science Europa Ltd., Lismeehan, Co. Clare, Ireland) and glycated haemoglobin A_{1c} (HbA_{1c}) levels were measured in the haematology laboratory on an automated high-pressure liquid chromatography instrument Tosoh G7 [Tosoh HLC-723 (G7), Tosoh Europe N.V, Tessenderlo, Belgium]. Serum insulin, c-reactive protein (CRP), tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6), adiponectin, leptin, resistin and plasminogen activator inhibitor-1 (PAI-1) were assessed using a biochip array system (Evidence Investigator; Randox Laboratories, UK). Complement component 3 (C3) was measured by immunoturbidimetric assay (RX Daytona; Randox Laboratories). White blood cell counts were determined by flow cytometry technology as part of a full blood count.

Classification of biochemical and blood pressure measurements

Patients with type 2 diabetes indicated by either HbA_{1c} levels ≥6.5 % (≥48 mmol/mol) or FPG levels ≥7.0 mmol/l, a self-reported physician diagnosis, Rx diabetes medication use, or those who were on insulin therapy, were excluded (N = 184). Lipid, lipoprotein and BP measurements were classified according to National Cholesterol Education Program Adult Treatment Panel III guidelines [21]. Abnormal metabolic risks were defined as high triglycerides ≥1.7 mmol/l and low HDL-C (<1.03 mmol/l in males or <1.29 mmol/l in females). Dyslipidaemia was determined according to both high triglyceride and low HDL-C levels. High BP was classified as systolic BP ≥130 mmHg and/or diastolic BP ≥85 mmHg or Rx anti-hypertensive medication use. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) [22] was derived from fasting glucose and insulin concentrations as [(fasting plasma glucose x fasting serum insulin)/22.5] and insulin resistance was defined as a level equal to or above the 75th percentile in the study sample. Having three or more MetS risk features (≥3 metabolic features) was characterised as any combination of the following: high triglycerides, low HDL-C levels, high BP and insulin resistance. Subjects were classified as having pre-diabetes if they had both elevated HbA_{1c} levels ≥5.7 % (≥39 mmol/mol) and impaired fasting plasma glucose levels ≥5.6 mmol/l [23]. As internationally recognised risk cut-points for the examined biomarkers have not been established, we classified inflammation and raised immune activation as a level equal to or above the 75th percentile for each biomarker (C3, CRP, IL-6, TNF-α, leptin, resistin, PAI-1 and WBC) with the exception of adiponectin (equal to or below the 25th percentile).

Anthropometric variables

The weight and height of each participant were measured to the nearest 0.1 kg and 0.1 cm respectively. Portable electronic Tanita WB-100MA weighing scales (Tanita Corporation, IL, USA) were placed on a firm, flat surface and were calibrated weekly to ensure accuracy. Height was measured using a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and BMI was calculated as weight divided by the square of height. Waist circumference was measured immediately below the lowest rib at the mid-axillary line on bare skin. Subjects were instructed to breathe in, and then out, and to hold their breath while measurement was made to the nearest 0.1 cm using a Seca 200 measuring tape. The WHtR was calculated as WC divided by height. Two independent readings were taken for WC and the mean of the two was used in analysis.

Both BMI and WHtR were divided into equal tertiles. Subjects were categorised on the basis of their BMI or WHtR percentiles as *normal weight* (<33 %), *overweight* (33–66 %) and *obese* (>66 %). In our sample these cut-points corresponded to <26.2, 26.2–29.7, >29.7 for BMI and <0.52, 0.52–0.58, >0.58 for WHtR. The BMI and WHtR groups were combined to form a 5-category variable: (1) *normal weight by both*, (2) *overweight by either*, (3) *overweight by both*, (4) *obese by either* and (5) *obese by both*. Overweight subjects classified as obese by either index were assigned to the higher category. Seven subjects had missing anthropometric values and were excluded from statistical analysis.

Lifestyle data

Lifestyle variables utilised from the IPAQ [20] and GHQ included physical activity level, smoking status and alcohol use. Self-reported physical activity within the previous 6 months was collapsed into two categories: *high or moderate* ($N = 1324$) and *no physical exercise* ($N = 312$). Subjects were considered to be current smokers if they smoked cigarettes during the recruitment phase of the study ($N = 257$). Alcohol use was assessed by asking study participants how often they consumed alcohol on a monthly or weekly basis, and was dichotomised as follows: 'never or less than once a month' and '2–4 times monthly'—*occasional drinker* ($N = 1165$), and 'twice or more weekly'—*regular drinker* ($N = 614$).

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics Version 20 (IBM Corp., Armonk, NY, USA) and Stata SE Version 13 (Stata Corporation, College Station, TX, USA) for Windows. Descriptive characteristics were examined according to normal weight, overweight and obese defined by BMI and WHtR tertiles. Dichotomous features are presented as percentages and continuous variables are shown as a mean (plus or minus one standard deviation) or a median and interquartile range for skewed data. Linear and logistic regression (adjusting for gender) were used to examine continuous and dichotomous metabolic variable differences between overweight and obese categories. Skewed continuous data were $\log_{10}$ transformed. Multinomial logistic regression was performed to determine cardiometabolic risk feature associations with each BMI and WHtR tertile combination. Subjects classified as normal weight by both indices were used as the reference category. All multinomial regression models were adjusted using age, gender, physical activity, smoking status and alcohol use as independent covariates.

The discriminatory ability of BMI, WHtR, and both BMI and WHtR used together, was assessed using

receiver operating characteristic curve (ROC) analysis. The area under the curve (AUC) provides a scale from 0.5 to 1.0 (with 0.5 representing random chance and 1.0 indicating perfect discrimination) by which to appraise the capacity of an obesity index to detect a positive result. Three separate analyses were performed. The first analysis assessed each anthropometric measure as a continuous variable. The second analysis explored cardiometabolic risk feature discrimination using index tertiles. A final analysis examined the 5-category BMI/WHtR combination variable used in previous regression models. Significant differences between AUC values were determined. For all analyses, a P value (two-tailed) of less than 0.05 was considered to indicate statistical significance.

Results

Descriptive characteristics

The characteristics of the study population were summarised according to BMI and WHtR tertiles (Table 1). A higher tertile level was related to an increased cardiometabolic risk profile as defined by lipid/lipoprotein, BP, glycaemic indicator and biomarker levels, with obese groups showing the highest proportion of cardiometabolic risk factors. In general, cardiometabolic profiles were broadly similar across BMI and WHtR overweight and obese categories, with the percentage of subjects with dyslipidaemia, high BP, insulin resistance, ≥3 metabolic features and pre-diabetes showing little variation according to classification by either index.

Cardiometabolic profiles according to classification of normal weight, overweight and obese

The levels of agreement between normal weight, overweight and obese tertiles are shown in Fig. 1. Kappa statistics were similar for normal and obese classifications (Kappa: 0.66, SE: 0.02 for normal weight vs. Kappa: 0.68, SE: 0.02 for obese) with marginal overlap between subjects defined as overweight (Kappa: 0.38, SE: 0.02). In both overweight and obese groups (Table 2), the combination of BMI and WHtR tertiles identified consistent and significant metabolic variable differences relative to those characterised on the basis of one index. Subjects that were classified as overweight or obese by both indices displayed higher mean BMI, WC and median triglyceride levels, reduced HDL-C and adiponectin concentrations, and a higher percentage had adverse biomarker levels, insulin resistance, metabolic feature clustering and pre-diabetes.

Associations between cardiometabolic risk features and BMI/WHtR combinations

Table 3 presents results from multinomial logistic regression models examining each BMI and WHtR tertile combination. A clear dose–response association was noted, with odds ratios of having cardiometabolic risk features being noticeably increased in subjects classified by both indices. In univariate analysis (not shown), odds ratios of having pre-diabetes were 0.6 (95 % CI: 0.3, 1.5), 1.9 (95 % CI: 1.1, 3.4), 1.8 (95 % CI: 1.0, 3.3) and 4.1 (95 % CI: 2.5, 6.7) for subjects categorised as *overweight by either*, *overweight by both*, *obese by either* and *obese by both* measures respectively. In a fully adjusted model, only patients within the highest BMI and WHtR tertile displayed a significant and positive association with pre-diabetes defined by both HbA_{1c} and fasting plasma glucose levels, OR: 3.4 (95 % CI: 1.9, 6.0), P < 0.001.

Discrimination of cardiometabolic risk features

In ROC analysis (Table 4), when used as a continuous variable, significantly higher AUC values for WHtR were found to discriminate high triglycerides, ≥3 metabolic features, elevated C3 and WBC levels when compared to BMI. BMI displayed a significantly higher AUC for detecting increased leptin levels compared to WHtR. A combination of both measures displayed significantly higher discriminatory accuracy for high triglycerides, metabolic feature clustering, C3 and CRP compared to BMI, and for leptin compared to WHtR. Significant improvement for detecting insulin resistance and high WBC levels were noted compared to when either BMI or WHtR were used independently.

When indices were examined as tertiles, significant differences between BMI and WHtR remained for discriminating high triglyceride, leptin and WBC concentrations. The BMI/WHtR 5-category variable was a significantly better discriminator of high triglycerides, low HDL-C, pre-diabetes, high C3, CRP, IL-6, TNF-α and WBC levels compared to BMI, and of leptin compared to WHtR. Significantly higher AUC values for detecting insulin resistance and ≥3 metabolic features were also found compared to when either measure were used alone.

Discussion

The aim of this study was to determine whether risk stratification using BMI and WHtR together more accurately identifies individuals at increased obesity-related cardiometabolic risk. Our findings indicate that both measures classify different subjects, particularly within the overweight range. These results also demonstrate that individuals defined as overweight or obese, by both BMI and WHtR, exhibit different cardiometabolic profiles compared to subjects categorised by either index separately. Participants identified by both measures demonstrated stronger associations with individual cardiometabolic risk factors, metabolic feature clustering and displayed a more pro-inflammatory, pro-antherogenic

Table 1 Characteristics of the study population

Feature	Normal weight		Overweight		Obese	
	BMI (N = 619)	WHtR (N = 619)	BMI (N = 618)	WHtR (N = 618)	BMI (N = 619)	WHtR (N = 619)
Male	212 (34.2)	145 (23.4)	346 (56.0)	349 (56.5)	327 (52.8)	391 (63.2)
Age	58 (54, 63)	57 (54, 62)	59 (54, 63)	59 (54, 64)	60 (55, 64)	60 (55, 64)
Weight (kg)	64.6 ± 8.5	65.6 ± 9.4	78.3 ± 9.1	78.6 ± 10.2	92.5 ± 12.6	91.2 ± 13.5
BMI (kg/m^2)	23.7 ± 1.8	24.3 ± 2.5	27.9 ± 1.0	27.9 ± 2.2	33.3 ± 3.5	32.7 ± 4.0
WC (cm)	79.7 ± 8.9	77.7 ± 7.2	91.8 ± 8.0	92.2 ± 5.8	102.7 ± 10.0	104.3 ± 8.4
WHtR	0.48 ± 0.04	0.47 ± 0.03	0.55 ± 0.04	0.55 ± 0.02	0.62 ± 0.05	0.62 ± 0.04
Triglycerides (mmol/l)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	1.2 (0.9, 1.7)	1.2 (0.9, 1.7)	1.4 (1.0, 2.0)	1.5 (1.1, 2.0)
High triglycerides[a]	67 (11.0)	49 (8.0)	147 (24.5)	152 (25.5)	195 (33.3)	208 (35.1)
HDL-C (mmol/l)	1.7 ± 0.4	1.7 ± 0.4	1.4 ± 0.3	1.4 ± 0.3	1.3 ± 0.3	1.3 ± 0.3
Low HDL-C[b]	46 (7.6)	39 (6.4)	74 (12.2)	80 (13.2)	142 (23.8)	143 (24.0)
Dyslipidaemia	12 (2.0)	10 (1.6)	37 (6.1)	37 (6.1)	69 (11.5)	71 (11.9)
Systolic BP (mmHg)	124.9 ± 17.4	124.8 ± 17.2	129.5 ± 15.5	129.2 ± 15.5	133.0 ± 15.9	133.4 ± 16.0
Diastolic BP (mmHg)	77.2 ± 9.6	77.8 ± 9.6	80.7 ± 8.8	80.3 ± 9.4	82.5 ± 9.9	82.3 ± 9.5
High BP[c]	271 (43.8)	263 (42.5)	359 (58.2)	366 (59.3)	471 (76.3)	472 (76.5)
Glucose (mmol/l)	4.8 (4.5, 5.0)	4.7 (4.5, 5.0)	4.9 (4.6, 5.2)	4.9 (4.7, 5.2)	5.1 (4.7, 5.4)	5.1 (4.8, 5.5)
Insulin (μU/ml)	5.3 (3.8, 7.9)	5.3 (3.8, 7.9)	8.4 (5.7, 12.0)	8.3 (5.6, 12.2)	12.9 (8.2, 18.4)	12.9 (8.4, 18.4)
HOMA-IR	1.1 (0.8, 1.7)	1.1 (0.8, 1.7)	1.8 (1.2, 2.7)	1.8 (1.2, 2.7)	2.9 (1.8, 4.3)	2.9 (1.9, 4.3)
Insulin resistance[d]	31 (5.2)	29 (4.9)	121 (20.2)	122 (20.4)	293 (49.4)	294 (49.6)
≥3 metabolic features	16 (2.6)	13 (2.1)	63 (10.2)	59 (9.5)	163 (26.3)	170 (27.5)
HbA_{1c} (%)	5.6 (5.4, 4.8)	5.6 (5.4, 5.8)	5.7 (5.5, 5.8)	5.7 (5.5, 5.9)	5.7 (5.5, 6.0)	5.7 (5.5, 6.0)
Pre-diabetes[e]	27 (4.4)	26 (4.3)	49 (8.1)	41 (6.7)	86 (14.2)	95 (15.7)
C3 (mg/dl)	125.7 ± 22.9	125.2 ± 22.4	134.2 ± 22.7	135.2 ± 22.8	144.5 ± 22.9	144.0 ± 22.8
High C3[f]	79 (13.2)	77 (12.8)	133 (22.0)	137 (22.6)	239 (39.8)	237 (39.6)
CRP (ng/ml)	1.1 (0.8, 1.6)	1.1 (0.8, 1.6)	1.3 (1.0, 1.9)	1.3 (1.0, 2.0)	1.7 (1.2, 3.1)	1.8 (1.2, 3.0)
High CRP[f]	91 (15.1)	85 (14.1)	124 (20.5)	136 (22.4)	236 (39.4)	230 (38.5)
IL-6 (pg/ml)	1.4 (1.0, 2.3)	1.4 (1.0, 2.1)	1.6 (1.2, 2.5)	1.7 (1.2, 2.5)	2.1 (1.5, 3.3)	2.2 (1.5, 3.4)
High IL-6[f]	118 (19.5)	102 (16.9)	126 (20.9)	129 (21.3)	207 (34.5)	220 (36.8)
TNF-α (pg/ml)	5.6 (4.6, 6.9)	5.5 (4.5, 6.6)	5.9 (4.9, 7.2)	5.8 (4.9, 7.1)	6.3 (5.2, 7.5)	6.4 (5.3, 7.7)
High TNF-α[f]	117 (19.4)	110 (18.2)	153 (25.4)	140 (23.1)	181 (30.2)	201 (33.6)
Adiponectin (ng/ml)	6.6 (4.2, 9.8)	6.9 (4.7, 10.2)	4.6 (2.9, 6.9)	4.6 (2.9, 6.9)	4.1 (2.6, 6.3)	3.8 (2.5, 5.5)
Low adiponectin[f]	78 (12.9)	65 (10.8)	176 (29.1)	167 (27.6)	199 (33.2)	221 (36.9)
Leptin (ng/ml)	1.3 (0.6, 2.0)	1.4 (0.8, 2.1)	1.8 (1.0, 2.7)	1.8 (1.0, 2.8)	3.1 (1.9, 5.1)	2.7 (1.6, 4.7)
High leptin[f]	39 (6.5)	67 (11.1)	109 (18.0)	122 (20.1)	303 (50.5)	262 (43.7)
Resistin (ng/ml)	4.8 (3.9, 6.4)	4.9 (3.8, 6.4)	4.9 (3.7, 6.5)	4.9 (3.8, 6.6)	5.3 (4.0, 7.0)	5.2 (4.0, 6.7)
High resistin[f]	133 (22.0)	136 (22.5)	141 (23.3)	152 (25.1)	178 (29.7)	164 (27.4)
PAI-1 (ng/ml)	24.3 ± 10.3	24.0 ± 10.4	28.1 ± 13.6	27.5 ± 11.7	28.7 ± 12.0	29.7 ± 13.7
High PAI-1[f]	100 (16.6)	94 (15.6)	164 (27.2)	161 (26.6)	187 (31.2)	196 (32.8)
WBC (10^9/l)	5.7 ± 2.4	5.5 ± 1.6	5.8 ± 1.6	5.9 ± 2.3	6.1 ± 1.5	6.3 ± 1.5
High WBC[f]	125 (20.7)	103 (17.0)	149 (24.5)	153 (25.3)	177 (29.7)	195 (32.7)

Mean and ±standard deviation are shown for continuous variables. Age, triglycerides, glucose, insulin, HOMA-IR, HbA_{1c}, CRP, IL-6, TNF-α, adiponectin, leptin and resistin are shown as a median (interquartile range). % (in brackets) for dichotomous variables will vary as some variables have missing values

[a] Triglycerides ≥1.7

[b] HDL-C <1.03 (males) or HDL-C <1.29 (females)

[c] Systolic BP ≥130 and/or diastolic BP ≥85 or use of Rx anti-hypertensives

[d] HOMA-IR ≥2.96

[e] Both HbA_{1c} levels ≥5.7 % and fasting plasma glucose levels ≥5.6

[f] Threshold: C3 ≥148; CRP ≥2.25; IL-6 ≥2.72; TNF-α ≥7.2; adiponectin ≤3.1; leptin ≥3.07; resistin ≥6.6; PAI-1 ≥33.66; WBC ≥6.6

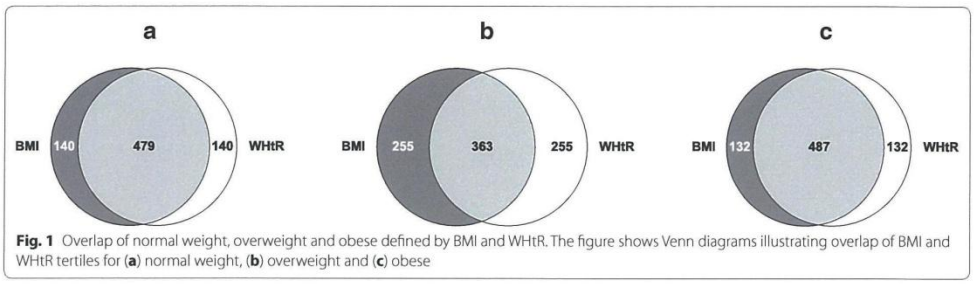

Fig. 1 Overlap of normal weight, overweight and obese defined by BMI and WHtR. The figure shows Venn diagrams illustrating overlap of BMI and WHtR tertiles for (**a**) normal weight, (**b**) overweight and (**c**) obese

and insulin resistant profile. Use of both indices also significantly improved discrimination of cardiometabolic risk features. These results suggest that joint use of BMI and WHtR may be clinically useful as a method to detect individuals at risk of cardiometabolic disorders.

Although it is straightforward to assess, and easy to calculate, limitations regarding the use of BMI as a sole method for adiposity appraisal have been widely acknowledged [7, 11]. Though frequently employed within epidemiological research and healthcare practice, BMI does not discriminate between fat and lean body mass, therefore persons of short stature or muscular build may be misidentified [24]. Recent research has indicated that general obesity categorisation based on BMI might be inadequate [25]. Importantly, the finding that approximately half of obese subjects are metabolically healthy when classified using dual-energy X-ray absorptiometry-derived body fat percentage, compared to approximately one-third by BMI [26], signals that caution should be exercised with regard to how obesity is defined [24].

Compared with BMI, WC is thought to be more strongly correlated with visceral adipose tissue (VAT) which has been shown to be associated with increased risk of dyslipidaemia, hypertension and type 2 diabetes [9, 27, 28]. Though the exact mechanism of association between VAT and metabolic risk is still poorly understood, research has implied that fatty acids released from VAT drain into the liver and skeletal muscle, causing metabolic dysfunction within these organs [29]. Adipokines secreted from VAT may also contribute to cardiometabolic disease through inflammation of vascular tissue [8, 30]. Although WC measurement has been recommended as a method for VAT and cardiometabolic risk assessment, controversy exists regarding its clinical efficacy. In particular, the need for gender and ethnic-specific risk cut-points, and the fact that WC does not take whole body fat distribution into account, indicate constraints regarding its practical application and usefulness within a clinical setting [11].

The WHtR is potentially advantageous as it may not require conversion to gender or population-specific cut-offs or percentiles [13]. It has been previously suggested that a WHtR $\geq$0.5 may serve as a useful boundary for increased cardiometabolic risk, with a WHtR $\geq$0.6 threshold indicating substantially increased risk [14]. Additionally, it has been shown that height has an inverse association with cardiovascular disease mortality and total mortality [31, 32], indicating that its use within an adiposity variable may be clinically important. In a recent meta-analysis of 31 prospective or cross-sectional studies, Ashwell et al. demonstrated WHtR to be a better discriminator of hypertension, metabolic syndrome, type 2 diabetes and cardiovascular disease when compared to BMI [15]. Pooled results showed that WHtR improved discrimination of all outcomes by 3–4 %. However, other studies have suggested that differences in predictive abilities are minimal, and have questioned the measurement of height in addition to WC [17].

The results from our research suggest that both BMI and WHtR provide important and independent information, and that joint measurement may help refine body fat risk classification. Within our sample it was noted that participants who were categorised as overweight on the basis of one index also displayed an increased cardiometabolic risk profile. As a percentage of these individuals might be considered normal weight if either measure were used alone, these results indicate that use of both indices may provide a more sensitive method for detecting patients at increased cardiometabolic risk. We also observed noticeably strong associations with cardiometabolic risk factors in subjects who were classified as overweight or obese by both BMI and WHtR together. This suggests that joint measurement may equally provide a more specific procedure for identifying high-risk subjects within overweight and obese categories. In particular, patients within the highest tertile for both indices were at a significantly higher risk compared to other obese subjects. In addition, a significant association with pre-diabetes was only observed within this tertile after

Table 2 Cardiometabolic profiles according to classification of normal weight, overweight and obese defined by either BMI, WHtR or both

Feature	Normal weight by both (N = 479)	Overweight by either (N = 263)	Overweight by both (N = 363)	P value[a]	Obese by either (N = 264)	Obese by both (N = 487)	P value[b]
Male[c]	122 (25.5)	105 (39.9)	231 (63.6)	<0.001	136 (51.5)	291 (59.8)	0.03
Age[d]	58 (54, 62)	58 (54, 65)	59 (54, 63)	0.885	59 (54, 64)	60 (55, 64)	0.15
Weight (kg)[e]	63.1 ± 8.1	71.9 ± 8.0	79.6 ± 9.2	<0.001	81.8 ± 9.5	94.6 ± 12.6	<0.001
BMI (kg/m^2)[e]	23.3 ± 1.8	26.2 ± 1.5	27.9 ± 1.0	<0.001	29.7 ± 1.9	33.8 ± 3.6	<0.001
WC (cm)[e]	76.6 ± 7.2	85.7 ± 6.7	93.0 ± 5.7	<0.001	95.1 ± 7.2	105.7 ± 8.5	<0.001
WHtR[e]	0.46 ± 0.03	0.52 ± 0.03	0.55 ± 0.02	<0.001	0.57 ± 0.03	0.63 ± 0.05	<0.001
Triglycerides (mmol/l)[e]	0.9 (0.7, 1.2)	1.1 (0.8, 1.5)	1.3 (0.9, 1.7)	0.005	1.3 (1.0, 1.8)	1.5 (1.1, 2.0)	0.034
High triglycerides[e]	36 (7.6)	43 (16.7)	93 (26.6)	0.031	71 (27.7)	166 (35.8)	0.06
HDL-C (mmol/l)[e]	1.7 ± 0.4	1.6 ± 0.4	1.4 ± 0.3	<0.001	1.4 ± 0.3	1.3 ± 0.3	<0.001
Low HDL-C[e]	31 (6.7)	23 (8.8)	46 (12.9)	0.038	39 (15.1)	123 (26.3)	0.001
Dyslipidaemia[e]	7 (1.5)	8 (3.1)	21 (5.9)	0.059	24 (9.3)	58 (12.3)	0.253
Systolic BP (mmHg)[e]	124.2 ± 17.5	126.4 ± 16.2	129.4 ± 14.7	0.027	132.5 ± 16.4	133.4 ± 15.8	0.52
Diastolic BP (mmHg)[e]	77.1 ± 9.6	78.8 ± 9.4	80.5 ± 8.8	0.003	82.2 ± 9.3	82.5 ± 9.8	0.61
High BP[e]	195 (40.7)	134 (51.0)	213 (58.8)	0.069	175 (66.3)	384 (79.2)	<0.001
Glucose (mmol/l)[e]	4.7 (4.5, 5.0)	4.8 (4.6, 5.1)	4.9 (4.7, 5.3)	0.038	4.9 (4.7, 5.2)	5.1 (4.8, 5.5)	<0.001
Insulin (μU/ml)[e]	5.1 (3.7, 7.5)	6.2 (4.3, 9.2)	8.8 (6.0, 12.1)	<0.001	10.2 (6.8, 14.3)	14.0 (9.0, 20.2)	<0.001
HOMA-IR[e]	1.1 (0.8, 1.6)	1.4 (0.9, 2.0)	2.0 (1.3, 2.7)	<0.001	2.2 (1.5, 3.2)	3.2 (2.0, 4.6)	<0.001
Insulin resistance[e]	20 (4.4)	20 (7.8)	72 (20.5)	<0.001	79 (31.1)	254 (54.5)	<0.001
≥3 metabolic features[e]	8 (1.7)	13 (4.9)	35 (9.6)	0.024	39 (14.8)	147 (30.2)	<0.001
HbA_{1c} (%)[e]	5.6 (5.4, 5.8)	5.7 (5.5, 5.9)	5.7 (5.4, 5.8)	0.54	5.7 (5.5, 5.8)	5.8 (5.6, 6.0)	0.002
Pre-diabetes[e]	22 (4.7)	8 (3.1)	31 (8.7)	0.018	21 (8.1)	80 (16.8)	0.002
C3 (mg/dl)[e]	124.0 ± 23.1	130.2 ± 20.4	134.7 ± 23.0	<0.001	139.2 ± 23.2	145.7 ± 22.9	<0.001
High C3[e]	56 (12.1)	42 (16.2)	77 (21.8)	0.013	76 (29.1)	200 (42.6)	<0.001
CRP (ng/ml)[e]	1.0 (0.8, 1.5)	1.3 (1.0, 2.0)	1.3 (1.0, 1.8)	0.976	1.5 (1.1, 2.5)	1.8 (1.2, 3.2)	<0.001
High CRP[e]	60 (12.9)	52 (20.1)	67 (18.9)	0.891	78 (30.1)	194 (41.4)	<0.001
IL-6 (pg/ml)[e]	1.3 (1.0, 2.1)	1.4 (1.1, 2.4)	1.7 (1.2, 2.4)	0.317	1.9 (1.3, 2.9)	2.3 (1.6, 3.5)	0.001
High IL-6[e]	82 (17.6)	49 (18.9)	70 (19.7)	0.99	73 (28.3)	177 (37.7)	0.01
TNF-α (pg/ml)[e]	5.5 (4.6, 6.7)	5.8 (4.6, 6.9)	5.9 (4.9, 7.2)	0.521	5.8 (5.0, 7.3)	6.4 (5.3, 7.0)	0.067
High TNF-α[e]	87 (18.7)	50 (19.4)	89 (25.1)	0.276	68 (26.4)	157 (33.4)	0.047
Adiponectin (ng/ml)[e]	7.0 (4.7, 10.3)	5.9 (3.8, 9.0)	4.2 (2.7, 6.3)	<0.001	4.8 (2.9, 6.7)	3.8 (2.5, 5.5)	0.023
Low adiponectin[e]	48 (10.3)	43 (16.6)	117 (33.0)	0.007	70 (27.0)	175 (37.2)	0.032
Leptin (ng/ml)[e]	1.3 (0.7, 2.0)	1.6 (1.0, 2.4)	1.7 (0.9, 2.7)	0.001	2.3 (1.3, 4.0)	3.2 (1.9, 5.2)	<0.001
High leptin[e]	30 (6.5)	39 (15.1)	56 (15.8)	0.009	87 (33.6)	239 (50.9)	<0.001
Resistin (ng/ml)[e]	4.9 (3.8, 6.4)	4.8 (3.8, 6.7)	4.9 (3.7, 6.4)	0.946	4.9 (4.0, 6.8)	5.3 (4.0, 7.0)	0.274
High resistin[e]	100 (21.5)	66 (25.5)	79 (22.3)	0.452	72 (27.9)	135 (28.7)	0.611
PAI-1 (ng/ml)[e]	23.8 ± 10.2	25.5 ± 10.9	28.1 ± 12.0	0.033	29.3 ± 15.6	29.2 ± 12.1	0.761
High PA1-1[e]	69 (14.8)	52 (20.2)	103 (29.0)	0.046	71 (27.5)	156 (33.2)	0.161
WBC (10^9/l)[e]	5.5 ± 1.7	5.8 ± 3.0	5.9 ± 1.6	0.92	6.1 ± 1.6	6.2 ± 1.4	0.345
High WBC[e]	84 (17.9)	56 (22.0)	88 (24.6)	0.832	74 (28.7)	149 (31.9)	0.418

Mean and ±standard deviation are shown for continuous variables. Age, triglycerides, glucose, insulin, HOMA-IR, HbA_{1c}, CRP, IL-6, TNF-α, adiponectin, leptin and resistin are shown as a median (interquartile range). % (in brackets) for dichotomous variables will vary as some variables have missing values

[a] P value for difference: overweight by either compared to overweight by both

[b] P value for difference: obese by either compared to obese by both

[c] x^2 for difference

[d] Mann Whitney U for difference

[e] P value for difference adjusted for gender. Overweight subjects classified as obese by either index were assigned to the higher category

Table 3 Odds ratios (95 % CI) of having cardiometabolic risk features according to classification of overweight and obese

Feature	Odds ratios (95 % CI)[a]							
	Overweight compared to normal weight				Obese compared to normal weight			
	Either BMI or WHtR	P value	Both BMI and WHtR	P value	Either BMI or WHtR	P value	Both BMI and WHtR	P value
High triglycerides	2.1 (1.3, 3.5)	0.003	3.5 (2.3, 5.4)	<0.001	3.4 (2.1, 5.5)	<0.001	5.6 (3.7, 8.6)	<0.001
Low HDL-C	1.4 (0.8, 2.5)	0.3	2.1 (1.3, 3.7)	0.005	2.2 (1.2, 3.8)	0.008	5.8 (3.6, 9.2)	<0.001
Dyslipidaemia	1.8 (0.6, 5.3)	0.263	3.8 (1.5, 9.3)	0.004	4.6 (1.9, 11.6)	0.001	8.6 (3.7, 19.6)	<0.001
High BP	1.5 (1.1, 2.1)	0.02	2.1 (1.5, 2.8)	<0.001	3.0 (2.1, 4.2)	<0.001	5.7 (4.1, 7.9)	<0.001
Insulin resistance	1.8 (0.9, 3.7)	0.083	5.4 (3.1, 9.6)	<0.001	9.5 (5.3, 16.8)	<0.001	26.6 (15.5, 45.7)	<0.001
≥3 metabolic features	2.6 (1.0, 6.6)	0.043	5.4 (2.4, 12.0)	<0.001	7.8 (3.5, 17.5)	<0.001	22.2 (10.5, 47.0)	<0.001
Pre-diabetes	0.6 (0.2, 1.4)	0.227	1.6 (0.9, 3.1)	0.142	1.6 (0.8, 3.2)	0.218	3.4 (1.9, 6.0)	<0.001
High C3	1.3 (0.8, 2.1)	0.260	2.8 (1.9, 4.3)	<0.001	3.3 (2.1, 5.0)	<0.001	7.9 (5.4, 11.6)	<0.001
High CRP	1.6 (1.0, 2.6)	0.032	1.8 (1.2, 2.7)	0.007	3.6 (2.4, 5.5)	<0.001	6.1 (4.2, 8.9)	<0.001
High IL-6	1.0 (0.7, 1.6)	0.897	1.1 (0.8, 1.7)	0.541	1.7 (1.2, 2.6)	0.008	2.7 (1.9, 3.8)	<0.001
High TNF-α	1.1 (0.7, 1.6)	0.738	1.4 (1.0, 2.0)	0.089	1.2 (0.8, 1.9)	0.323	2.2 (1.5, 3.1)	<0.001
Low adiponectin	1.4 (0.8, 2.3)	0.204	2.7 (1.8, 4.2)	<0.001	2.6 (1.6, 4.2)	<0.001	3.9 (2.6, 6.0)	<0.001
High leptin	3.6 (2.1, 6.3)	<0.001	5.9 (3.5, 9.9)	<0.001	15.7 (9.3, 26.6)	<0.001	46.6 (27.9, 77.6)	<0.001
High resistin	1.3 (0.9, 1.9)	0.205	1.3 (0.9, 1.9)	0.194	1.5 (1.0, 2.2)	0.046	1.8 (1.2, 2.5)	0.001
High PAI-1	1.2 (0.8, 1.8)	0.460	2.0 (1.3, 2.9)	<0.001	1.9 (1.3, 2.9)	0.002	2.7 (1.9, 3.9)	<0.001
High WBC	1.5 (1.0, 2.4)	0.073	1.9 (1.2, 2.9)	0.003	2.7 (1.7, 4.2)	<0.001	3.2 (2.2, 4.8)	<0.001

[a] Multinomial logistic regression, reference category: normal weight by both BMI and WHtR. Overweight subjects classified as obese by either index were assigned to the higher category. All models adjusted for age, gender, physical activity, smoking and alcohol use

adjustment for other risk factors. This might imply that the relationship between obesity and diabetes is better indicated at this mode and level of adiposity.

Nevertheless, it was also noted that subjects classified by both indices were, on average, more overweight or obese, and thus would probably be identified if either BMI or WHtR were used alone. Additionally, discriminatory improvements for detecting individual cardiometabolic features were modest. However, cardiometabolic diseases are multifactorial, as it has been shown that subjects with a combination of features are at higher risk of cardiometabolic events [33, 34]. Also, a degree of measurement error is to be expected during any anthropometric assessment. We have recently shown that using BMI and WHtR together significantly improves discrimination of type 2 diabetes [30]. Cut-points on the ROC demonstrated greater sensitivity and specificity than when either index were used independently. As the sum of risk factors may be greater than the individual parts for predicting cardiometabolic events, and as measurement error may limit the minimal detectable difference in a cardiometabolic risk parameter [35], it could be that these findings are due to the greater measurement accuracy that joint BMI and WHtR assessment may provide.

Although it is hoped that public health programs may eventually reduce the prevalence of obesity-related metabolic disorders, current strategies to combat obesity are failing as overweight and obesity rates continue to increase worldwide [18]. As a percentage of obese subjects are considered to be metabolically healthy [24], there is an increasing need for cheap and non-invasive methods to detect overweight and obese individuals at highest odds of developing cardiometabolic diseases. In previous research we have shown that assessing both bioelectrical impedance-derived body fat percentage and BMI may help to discriminate individuals at greater cardiometabolic risk than BMI alone [36]. Those identified using both tools had a more metabolically unhealthy profile and were non-responsive to dietary changes. These findings suggest that stratification of obese individuals, based on their metabolic health phenotype, could be important in the early identification of those who should be prioritised for pharmacological and lifestyle interventions [24]. Joint use of BMI and WHtR may provide a convenient and inexpensive means for risk stratification. Such a method might be useful in resource-poor settings where blood sampling is unavailable, or in populations without regular access to primary health services.

As far as we are aware, our study is the first to comprehensively examine the joint use of BMI and WHtR in a middle-aged European population. Strengths include a high participation rate, the use of questionnaires to assess

Table 4 Area under the receiver operating characteristic curve values (95 % CI) for index models to discriminate cardiometabolic risk features

Feature	As a continuous variable						As a categorical variable (tertiles)				Overweight and obese by either or both[a]	
	BMI alone		WHtR alone		Both BMI and WHtR		BMI alone		WHtR alone			
	AUC	95 % CI	AUC	95 % CI	AUC	95 % CI	AUC	95 % CI	AUC	95 % CI	AUC	95 % CI
High triglycerides	0.68	0.65, 0.71	0.71[b]	0.68, 0.73	0.71[b]	0.68, 0.74	0.68	0.65, 0.71	0.70[d]	0.67, 0.73	0.70[d]	0.67, 0.73
Low HDL-C	0.67	0.63, 0.70	0.68	0.65, 0.72	0.68	0.65, 0.71	0.66	0.62, 0.69	0.67	0.64, 0.71	0.68[d]	0.64, 0.71
Dyslipidaemia	0.68	0.64, 0.73	0.70	0.65, 0.74	0.70	0.65, 0.74	0.69	0.64, 0.73	0.69	0.65, 0.73	0.70	0.66, 0.74
High BP	0.70	0.67, 0.72	0.70	0.67, 0.72	0.70	0.68, 0.72	0.69	0.67, 0.72	0.69	0.67, 0.71	0.70	0.67, 0.72
Insulin resistance	0.80	0.78, 0.82	0.80	0.78, 0.83	0.81[b,c]	0.79, 0.83	0.78	0.76, 0.80	0.77	0.75, 0.80	0.79[d,e]	0.77, 0.82
≥3 metabolic features	0.76	0.73, 0.79	0.78[b]	0.75, 0.81	0.78[b]	0.75, 0.81	0.75	0.72, 0.78	0.75	0.72, 0.78	0.77[d,e]	0.74, 0.80
Pre-diabetes	0.70	0.66, 0.74	0.70	0.66, 0.74	0.70	0.66, 0.74	0.67	0.63, 0.71	0.68	0.64, 0.72	0.69[d]	0.65, 0.73
High C3	0.70	0.67, 0.73	0.72[b]	0.69, 0.75	0.72[b]	0.69, 0.75	0.69	0.66, 0.71	0.70	0.67, 0.73	0.71[d]	0.68, 0.73
High CRP	0.69	0.66, 0.72	0.69	0.67, 0.72	0.70[b]	0.67, 0.72	0.67	0.64, 0.70	0.68	0.65, 0.71	0.69[d]	0.66, 0.72
High IL-6	0.66	0.63, 0.69	0.67	0.64, 0.69	0.67	0.64, 0.69	0.65	0.62, 0.68	0.66	0.63, 0.69	0.66[d]	0.63, 0.69
High TNF-α	0.63	0.60, 0.66	0.63	0.60, 0.66	0.63	0.60, 0.66	0.62	0.59, 0.65	0.63	0.61, 0.66	0.63[d]	0.60, 0.66
Low adiponectin	0.79	0.77, 0.81	0.79	0.77, 0.81	0.79	0.77, 0.81	0.79	0.77, 0.81	0.79	0.76, 0.81	0.79	0.77, 0.81
High leptin	0.86[c]	0.84, 0.87	0.84	0.82, 0.86	0.86[c]	0.84, 0.88	0.84[e]	0.82, 0.86	0.81	0.79, 0.83	0.84[e]	0.82, 0.86
High resistin	0.57	0.54, 0.60	0.56	0.53, 0.59	0.57	0.54, 0.60	0.57	0.54, 0.60	0.56	0.53, 0.59	0.56	0.53, 0.60
High PAI-1	0.61	0.58, 0.64	0.62	0.59, 0.65	0.62	0.59, 0.65	0.62	0.59, 0.64	0.62	0.59, 0.65	0.62	0.59, 0.65
High WBC	0.59	0.56, 0.62	0.61[b]	0.58, 0.64	0.63[b,c]	0.60, 0.66	0.58	0.55, 0.61	0.60[d]	0.57, 0.63	0.59[d]	0.56, 0.62

All models include age and gender

[a] 5-category variable: 1 normal weight by both, 2 overweight by either, 3 overweight by both, 4 obese by either and 5 obese by both. Overweight subjects classified as obese by either index were assigned to the higher category

[b] $P < 0.05$ compared to BMI (continuous)

[c] $P < 0.05$ compared to WHtR (continuous)

[d] $P < 0.05$ compared to BMI (categorical)

[e] $P < 0.05$ compared to WHtR (categorical)

lifestyle behaviours and inclusion of a wide range of metabolic variables to define cardiometabolic health. Our findings are of potential public health and clinical significance in terms of screening and the use of stratification based on obesity assessment as a method for determining cardiometabolic risk.

Notwithstanding these strengths, methodological limitations should be considered when examining results from this study. Given the modest number of outcomes within our sample, we did not stratify by gender in analysis. Although some studies have implied heterogeneous relationships between measures of adiposity and cardiometabolic outcomes relating to gender [11], previous work by our group has suggested that these may be explained by sex differences in obesity prevalence [30]. In addition, recommended risk cut-points for BMI and WHtR are the same for men and women, and the gender variable was accounted for in statistical analyses.

Also of concern is that we did not use established obesity index cut-offs and that our data were cross-sectional, as this precludes examination of temporal relationships. Although World Health Organisation cut-points for BMI are commonly used [5], and thresholds for WHtR have been recommended [14, 37], for the purposes of this research it was necessary to place both variables on the same scale. Future studies, utilising longitudinal data, will be needed to evaluate the applicability, validity and reliability of joint measurement [14] using established and recommended diagnostic cut-points. In particular, it will be necessary to determine whether risk stratification, using both BMI and WHtR, is clinically useful and superior to currently recommended BMI classification [38].

Finally, our data were derived from a single primary care based sample which may not be representative of the source population. However, Ireland presents a generally ethnically homogeneous group [39]. Thus, the relationships we observed are likely to be similar in other middle-aged Irish adults. In addition, random sampling of subjects and the use of validated methods for data collection ensured internal sample validity and the results from this research may be generalisable to a similar middle-aged Caucasian-European population.

Conclusions

In summary, our findings reveal that cardiometabolic risk profiles in individuals defined as overweight or obese, by both BMI and WHtR, are significantly increased when compared to subjects categorised by either index separately. Use of both measures also improved discrimination of individual cardiometabolic risk factors and identified a subset of at-risk individuals who might otherwise be missed. Although assessment of WC, in addition to BMI, competes for the limited time available during patient appraisal within clinical practice [9], obtaining two measurements (one for general obesity, and one for central obesity) does not entail any extra cost [14]. In light of the increasing prevalence of cardiometabolic diseases worldwide [40], effective methods that help to identify subjects at greatest risk are needed. Risk stratification utilising BMI and WHtR together may provide a simple, cost-effective and more accurate technique for predicting obesity-related cardiometabolic events. Earlier identification of individuals at risk could enable earlier targeted interventions or therapies, thus attenuating development of cardiovascular complications.

Abbreviations

AUC: area under the curve; BMI: body mass index; BP: blood pressure; CI: confidence interval; C3: complement component 3; CRP: c-reactive protein; GHQ: general health questionnaire; HbA_{1c}: glycated haemoglobin A_{1c}; HDL-C: high density lipoprotein cholesterol; IL-6: interleukin 6; IPAQ: international physical activity questionnaire; MetS: metabolic syndrome; OR: odds ratio; PAI-1: plasminogen activator inhibitor-1; ROC: receiver operating characteristic curve; Rx: prescription; TNF-α: tumour necrosis factor alpha; VAT: visceral adipose tissue; WBC: white blood cell; WC: waist circumference; WHtR: waist–height ratio.

Authors' contributions

SRM conceived of the study and performed the statistical analysis. SRM, IJP and CMP drafted the final manuscript. All authors read and approved the final manuscript.

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Competing interests

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