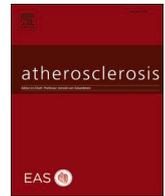


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Protective lifestyle behaviours and lipoprotein particle subclass profiles in a middle-to older-aged population

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ABSTRACT

Background and aims: Lipoprotein particle size is associated with increased atherosclerosis and cardiovascular disease risk. Certain lifestyle behaviours may be cardioprotective. We examined lipoprotein particle size and concentration relationships with a protective lifestyle behaviour (PLB) score.

Methods: This was a cross-sectional analysis of 2045 middle-to older-aged adults. Lipoprotein particle subclass size and concentrations were determined using nuclear magnetic resonance spectroscopy. Five protective behaviours included never smoking, moderate alcohol intake, moderate to vigorous physical activity, a high-quality diet (upper 40% Dietary Approaches to Stop Hypertension score) and a normal body mass index (BMI) (18.5–24.9 kg/m²). Linear and logistic regression analyses tested individual protective behaviour and PLB score associations with lipoprotein subclasses.

Results: Individual behaviour associations varied according to lipoprotein subclass, with normal BMI showing the greatest number of significant relationships. Logistic regression analyses revealed that subjects with the fewest number of protective behaviours had 1.4–2.8 increased odds of having less favourable lipoprotein profiles defined as above or below median level lipoprotein particle subclass size or concentration. Following additional adjustment for BMI, significant trend relationships were observed between the PLB score and large and medium very low-density lipoprotein ($p = 0.001$ and $p < 0.001$), total and smaller low-density lipoprotein (LDL) concentrations ($p = 0.008$ and $p < 0.001$), LDL size ($p = 0.003$) and a lipoprotein insulin resistance score ($p = 0.003$). **Conclusions:** Results show a cumulative protective effect of healthy lifestyle behaviours against an unfavourable potentially pro-atherogenic lipoprotein profile in middle-to older-aged adults, highlighting the importance of lifestyle promotion in healthy ageing.

1. Introduction

Chronic non-communicable diseases are reaching epidemic proportions worldwide [1] and are a major public health concern. The causal role of high cholesterol concentrations in the pathogenesis of chronic conditions is well established. Obesity and insulin resistance are linked to alterations in the lipoprotein particle profile and this may influence type 2 diabetes and cardiovascular disease (CVD) risk [2–4]. Traditional lipid tests quantify the cholesterol or triglyceride content of lipoproteins, and the amount of cholesterol carried by lipoprotein particles is thought to be an important parameter for estimating disease risk [5]. However, traditional cholesterol levels may not accurately reflect the true atherogenicity of plasma lipid profiles.

Nuclear magnetic resonance (NMR) spectroscopy simultaneously

quantifies the number and size of lipoprotein particles [6]. Lipoprotein particle size, in particular smaller low-density lipoprotein (LDL) and high-density lipoprotein (HDL) particles and large very low-density lipoprotein (VLDL) particles, has been shown to be associated with increased risk for atherosclerosis and premature CVD [2,7,8]. We have also shown increased likelihood of being metabolically healthy among obese and non-obese adults with favourable lipoprotein profiles characterised by less large VLDL and smaller LDL and more large LDL and HDL particles [9].

Research studies have demonstrated relationships between modifiable health behaviours and morbidity and mortality [10–13]. Obesity, high levels of alcohol consumption and smoking have been shown to increase the risk of morbidity [14,15], whereas moderate-to-high levels of physical activity and healthy diet have been shown to reduce the risk

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[16–18]. Since increased or decreased levels of these factors provide protection and prevent the likelihood of negative outcomes they have also been termed as protective lifestyle behaviours (PLB) [19].

A recent large study in the United States revealed that adhering to five low-risk behaviours may prolong life expectancy by 12.2 and 14 years for men and women, respectively – in comparison to those with no protective behaviours [20]. However, the mechanism for this association remains unclear. Although a number of studies have examined relationships between individual protective behaviours and lipoprotein concentrations, the focus on lipoprotein profiling in this context has been restricted to a narrow range of markers. In addition, no research to date has examined the cumulative effect of protective behaviours on lipoprotein subclasses.

Therefore, the main objective of this study was to fully examine lipoprotein particle size and concentration relationships with a 5-component PLB score (never smoking, moderate alcohol intake, moderate or high physical activity levels, healthy diet and healthy weight), using a cross-sectional sample of 2045 middle-to older-aged men and women, to determine whether the number of protective behaviours is related to lipoprotein subclass measures.

2. Materials and methods

2.1. Study population and setting

The Cork and Kerry Diabetes and Heart Disease Study (Phase II – Mitchelstown Cohort) was a single centre study conducted between 2010 and 2011. A random sample was recruited from a large primary care centre in Mitchelstown, County Cork, Ireland. The Living Health 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, 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% male) completed the questionnaire and physical examination components of the baseline assessment (response rate: 67%). Data on protective lifestyle behaviours were available for 2045 subjects. Details regarding the study design, sampling procedures and methods of data collection have been reported previously [21].

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. Clinical procedures

Study participants attended the clinic in the morning after an overnight fast and blood samples were taken on arrival. Fasting glucose and glycated haemoglobin A_{1c} (HbA_{1c}) concentrations were measured by Cork University Hospital Biochemistry Laboratory using standardised procedures and fresh samples. 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].

Anthropometric measurements were performed by trained researchers with reference to a standard operating procedures manual. Height was measured with a portable Seca Leicester height/length stadiometer (Seca, Birmingham, UK) and weight was measured using a portable electronic Tanita WB-100MA weighing scale (Tanita Corp, IL, USA). The weighing scale was placed on a firm flat surface and was

calibrated weekly. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

2.3. Lipoprotein profiling

Lipoprotein subclass particle concentrations and average VLDL, LDL and HDL particle diameters were measured on serum specimens by NMR spectroscopy at LipoScience, Inc (Raleigh, NC). VLDL, LDL and HDL subclasses were quantified based on the amplitudes of their spectroscopically-distinct lipid methyl group NMR signals [6]. Weighted-average VLDL, LDL and HDL particle sizes (in nanometre diameter units) were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its NMR signal. The following subclass categories were investigated: large VLDL (including chylomicrons, if present) (>60 nm), medium VLDL (42–60 nm), small VLDL (29–42 nm), intermediate-density lipoprotein [IDL] (25–35 nm), large LDL (20.5–23 nm), small LDL (18–20.5 nm), large HDL (9.4–14 nm), medium HDL (8.2–9.4 nm) and small HDL (7.3–8.2 nm). Particle concentrations are expressed as nanomoles per litre (VLDL and LDL) and micromoles per litre (HDL). A Lipoprotein Insulin Resistance score (LP-IR), ranging from 0 (least) to 100 (most) insulin resistant, which is a weighted combination of the six lipoprotein subclass and size parameters most closely associated with insulin resistance, was calculated [22].

2.4. Data collection

A general health and lifestyle questionnaire assessed demographic variables, lifestyle behaviours and morbidity. Information on age, sex, education, medication use, smoking status, alcohol drinking habits and presence of type 2 diabetes was provided by participants. Physical activity levels were measured using the validated International Physical Activity Questionnaire (IPAQ) [23].

A validated Food Frequency Questionnaire (FFQ) consisting of 150 different food items was used for dietary assessment [24,25]. The average medium serving of each food item consumed by participants over the last 12 months was converted into quantities using standard portion sizes. Food item quantity was expressed as (gm/d) and beverages as (ml/d). Based on the FFQ, the Dietary Approaches to Stop Hypertension (DASH) diet score was constructed [26]. DASH is a dietary pattern rich in fruits, vegetables, whole grains and low-fat dairy foods and is limited in sugar-sweetened foods and beverages, red meat and added fats. This diet has been promoted by the National Heart, Lung and Blood Institute (part of the National Institutes of Health, a United States government organisation) to prevent and control hypertension. DASH diet scores ranged from 11 to 42. Lower scores represent poorer and higher scores represent better quality diet [17]. Dietary fat (percent energy intake) was calculated from food frequency questionnaire responses.

2.5. Classification and scoring of variables

2.5.1. Exposure: protective behaviours

We classified protective behaviours according to the same methodology as used previously in research by our group [27]. Smoking status was defined as follows: (i) never smoked, i.e. having never smoked at least 100 cigarettes (5 packs) in their entire life; (ii) former smoker, i.e. having smoked 100 cigarettes in their entire life and do not smoke at present; and (iii) current smoker, i.e. smoking at present. These definitions were the same as those used in the SLÁN National Health and Lifestyle Survey [28]. A binary variable was then created: ‘never/former smoker’ or ‘current smoker’. For the purpose of the present analysis, ‘never/former smoker’ was compared with ‘current smoker’, with the former being defined as a protective behaviour.

Alcohol consumption was measured in units of alcohol consumed on a weekly basis and was categorised into the following levels: (i) non-

drinker, i.e. <1 drink per week; (ii) moderate drinker, i.e. between 1 and 14 drinks per week; and (iii) heavy drinker, i.e. >14 drinks per week. Moderate drinker was defined on the basis of previous work from the European Prospective Investigation into Cancer and Nutrition (EPIC) in the United Kingdom by Khaw et al. [29]. For the current analysis, these were then re-categorised as ‘moderate/non-drinker’ or ‘heavy drinker’, with the former being defined as a healthy lifestyle behaviour.

Physical activity was categorised as low, moderate and high levels of activity using the IPAQ. This was then recoded as a dichotomous variable: ‘moderate/high’ or ‘low’ physical activity, with ‘moderate/high’ levels of physical activity being defined as a protective behaviour. We classified a healthy diet as a DASH diet score in the top 40% for the study sample. Normal body weight was defined as a BMI in the range 18.5–24.9 kg/m².

A protective lifestyle behaviour (PLB) score was constructed using the same method as described in recent research by Li et al. [20]. For each low-risk behaviour, the participant received a score of 1 if they met the criterion for low risk. If the participant did not meet the criterion, they were classified as high risk for that factor and received a score of 0. The sum of these five scores provided a total number of low-risk behaviours of 0, 1, 2, 3, 4 or 5, with higher scores indicating a healthier lifestyle. The PLB score was then re-coded into a four-category variable: ‘0 or 1’; ‘2’; ‘3’; and ‘4 or 5’ protective behaviours.

2.5.2. Confounding variables

Based on the literature, potential confounders considered included age, sex, morbidity (type 2 diabetes) and education. Type 2 diabetes was determined as a fasting glucose level ≥ 7.0 mmol/l or a HbA_{1c} level $\geq 6.5\%$ (≥ 48 mmol/mol) [30] or by self-reported diagnosis. Categories of education included ‘some primary (not complete)’, ‘primary or equivalent’, ‘intermediate/group certificate or equivalent’, ‘leaving certificate or equivalent’, ‘diploma/certificate’, ‘primary university degree’ and ‘postgraduate/higher degree’. These were collapsed and recoded into a dichotomous variable: ‘primary education only’ and ‘intermediate or higher’.

Statin use and energy intake were also considered as potential confounders. However, neither variable demonstrated an association with the PLB score and adjustment for these variables in analyses did not change our results.

2.5.3. Statistical analysis

Descriptive characteristics were examined according to sex and by the number of protective behaviours. Categorical 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. Differences between protective behaviour groups were analysed using an ANOVA or Jonckheere trend test for continuous variables or with a linear-by-linear chi-square for categorical features. The relationships between a PLB score and lipoprotein concentrations were examined using Spearman’s rank-order correlation.

Skewed data were log-transformed and linear regression was utilised to determine individual protective behaviour and PLB score associations with lipoprotein subclasses. Two models were run: the first model was adjusted for age, sex, education and type 2 diabetes. Models which examined individual protective behaviours were additionally adjusted for each other. To correct for the multiple testing performed, we calculated false discovery rate adjusted *p* values via the Romano-Wolf multiple hypothesis correction method using the *rwolf* command in Stata [31].

Logistic regression was used to determine associations between the number of protective behaviours and lipoprotein status (categorised as above or below median level for each lipoprotein subclass) [9]. The odds ratio (OR) for each model with a 95% confidence interval (CI) is reported.

Data analysis was conducted using Stata SE Version 13 (Stata Corporation, College Station, TX, USA) for Windows. For all analyses, a *p*

value (two-tailed) of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Descriptive characteristics of the cohort

Characteristics of the study population according to sex are presented in Table 1. Significant differences between the sexes were noted for education, type 2 diabetes, levels of alcohol consumption and physical activity, diet quality and BMI, with male participants having fewer protective behaviours than females. Sex differences were also observed for all lipoprotein subclasses with the exception of IDL concentrations.

Table 1
Descriptive characteristics of the study population by sex.

Feature	Males (n = 1007)	Females (n = 1038)	<i>p</i>
General			
Age (median)	59.0 (55.0–64.0)	59.0 (54.0–64.0)	0.895
Primary education only (%)	310 (32.7)	227 (23.6)	<0.001
On cholesterol-lowering medications (%)	340 (33.8)	371 (35.7)	0.348
Type 2 diabetes (%)	118 (11.7)	66 (6.4)	<0.001
Energy intake, kcal (mean)	2034.7 ± 831.9	1994.9 ± 836.1	0.284
Protective behaviours			
Never or former smoker (%)	841 (85.4)	875 (85.5)	0.923
Non or moderate drinker (%)	515 (74.6)	518 (96.3)	<0.001
Moderate or high physical activity (%)	528 (57.3)	448 (46.0)	<0.001
DASH diet, upper 40th percentile (%)	226 (24.6)	488 (51.1)	<0.001
BMI, 18.5–24.9 kg/m ² (%)	135 (13.5)	288 (27.8)	<0.001
Number of protective behaviours:			<0.001
0 or 1 (%)	243 (24.1)	198 (19.1)	
2 (%)	371 (36.8)	324 (31.2)	
3 (%)	279 (27.7)	300 (28.9)	
4 or 5 (%)	114 (11.3)	216 (20.8)	
Lipoprotein particle concentration			
Total TRL, nmol/l (median)	66.5 (40.4–103.2)	50.2 (30.6–79.6)	<0.001
Large VLDL, nmol/l (median)	1.4 (0.5–4.3)	0.7 (0.4–2.0)	<0.001
Medium VLDL, nmol/l (median)	24.9 (12.3–45.1)	18.1 (8.4–32.4)	<0.001
Small VLDL, nmol/l (median)	33.3 (18.8–52.4)	27.9 (15.3–46.2)	<0.001
Total LDL, nmol/l (mean)	1307.0 ± 403.5	1220.8 ± 403.4	<0.001
IDL, nmol/l (median)	91.0 (48.0–165.0)	93.0 (52.0–153.0)	0.965
Large LDL, nmol/l (mean)	480.3 ± 266.2	705.5 ± 290.6	<0.001
Small LDL, nmol/l (mean)	710.8 ± 392.8	403.8 ± 376.8	<0.001
Total HDL, μ mol/l (mean)	36.6 ± 5.8	39.9 ± 6.0	<0.001
Large HDL, μ mol/l (median)	4.5 (2.8–6.6)	8.2 (5.6–11.6)	<0.001
Medium HDL, μ mol/l (mean)	12.4 ± 5.6	14.6 ± 6.4	<0.001
Small HDL, μ mol/l (mean)	19.2 ± 5.4	16.5 ± 6.1	<0.001
Lipoprotein particle size			
VLDL size, nm (mean)	46.0 ± 6.6	44.1 ± 5.2	<0.001
LDL size, nm (mean)	20.6 ± 0.5	21.1 ± 0.5	<0.001
HDL size, nm (mean)	9.1 ± 0.4	9.5 ± 0.5	<0.001
LP-IR score (median)	44.0 (28.0–59.0)	21.0 (8.0–38.0)	<0.001

TRL: triglyceride-rich lipoprotein; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; IDL: intermediate-density lipoprotein; HDL: high-density lipoprotein; LP-IR: lipoprotein insulin resistance.

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

p values determined from a Mann Whitney U, Pearson’s chi-square or *t*-test.

3.2. Lipoprotein profiles according to protective lifestyle behaviours

Table 2 shows characteristics of the study population according to the number of protective behaviours. A significant linear association was noted between the number of PLB score components and lipoprotein particle subclass size and concentration, with mean or median levels decreasing or increasing (in the case of large LDL, total HDL, large HDL, LDL size and HDL size) with the greater number of protective behaviours. For example, the percentage difference in lipoprotein concentrations between having 4 or 5 protective behaviours and 0 or 1 was 150%, 58.3%, 12.4% and 110.5% for large VLDL, small LDL, small HDL and the LP-IR score, respectively. No significant associations were observed between the number of protective components and small VLDL and medium HDL. Significant linear associations were also observed for the age, education and morbidity variables.

Correlation analysis (Table 3) revealed significant inverse associations between the PLB score and concentrations of total TRL, large VLDL, medium VLDL, total LDL, IDL, small LDL, small HDL, VLDL size and the LP-IR score. Large LDL, total HDL, large HDL, LDL size and HDL size were positively correlated with the PLB score. Correlations between the PLB score and lipoprotein subclasses were stronger for total TRL, large LDL and small LDL, with large VLDL, medium VLDL, LDL size and the LP-IR score showing the highest correlative strengths.

3.3. PLB score and lipoprotein profile

A linear regression analysis exhibiting associations between a PLB score and lipoprotein particle subclasses is shown in Table 4. In analyses adjusted for age, sex, education and type 2 diabetes, the PLB score remained significantly associated with each examined lipoprotein subclass with the exception of small VLDL, total HDL and medium HDL. In analyses which adjusted for each PLB score component, smoking status was significantly inversely related to total TRL, medium VLDL and small

Table 3

Spearman correlation coefficients between a PLB score and lipoprotein subclasses.

Lipoprotein particle	Correlation coefficients	<i>p</i>
Total TRL, nmol/l	−0.140	<0.001
Large VLDL, nmol/l	−0.192	<0.001
Medium VLDL, nmol/l	−0.204	<0.001
Small VLDL, nmol/l	−0.037	0.104
Total LDL, nmol/l	−0.091	<0.001
IDL, nmol/l	−0.072	0.001
Large LDL, nmol/l	0.150	<0.001
Small LDL, nmol/l	−0.177	<0.001
Total HDL, μ mol/l	0.057	0.011
Large HDL, μ mol/l	0.191	<0.001
Medium HDL, μ mol/l	0.016	0.472
Small HDL, μ mol/l	−0.102	<0.001
VLDL size, nm	−0.129	<0.001
LDL size, nm	0.190	<0.001
HDL size, nm	0.167	<0.001
LP-IR score	−0.231	<0.001

TRL: triglyceride-rich lipoprotein; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; IDL: intermediate-density lipoprotein; HDL: high-density lipoprotein; LP-IR: lipoprotein insulin resistance.

VLDL concentrations. Non or moderate alcohol use was inversely related to large VLDL, IDL, total HDL, large HDL, medium HDL, VLDL and HDL size, and was positively associated with total TRL and small VLDL. Physical activity levels were inversely related to medium VLDL particle concentrations while high diet quality was associated with large VLDL, small VLDL, IDL, small HDL, LDL size and the LP-IR score. A normal BMI demonstrated the greatest number of significant relationships with lipoprotein subclasses after adjustment for other protective behaviours.

Table 5 presents results from logistic regression analyses displaying associations between the number of protective behaviours and lipoprotein particle subclasses. In adjusted models, a dose-response

Table 2

Characteristics of the study population according to the number of protective behaviours.

Feature	Number of protective behaviours				<i>p</i> trend
	0 or 1 (n = 441)	2 (n = 695)	3 (n = 579)	4 or 5 (n = 330)	
Age (median)	59.0 (55.0–64.0)	60.0 (55.0–64.0)	59.0 (54.0–63.0)	58.0 (54.0–63.0)	0.003
Primary education only (%)	137 (35.0)	230 (35.2)	124 (22.3)	46 (14.9)	<0.001
On cholesterol-lowering medications (%)	142 (32.2)	258 (37.1)	202 (34.9)	109 (33.0)	0.979
Type 2 diabetes (%)	58 (13.2)	85 (12.2)	32 (5.5)	9 (2.7)	<0.001
Energy intake, kcal (mean)	1913.8 \pm 873.9	2040.0 \pm 859.9	2069.3 \pm 843.3	1992.2 \pm 691.4	0.164
Never or former smoker (%)	257 (62.8)	596 (86.1)	542 (93.9)	321 (97.3)	<0.001
Non or moderate drinker (%)	51 (37.0)	303 (80.4)	382 (92.0)	297 (99.7)	<0.001
Moderate or high physical activity (%)	28 (7.7)	257 (38.7)	403 (71.6)	298 (90.6)	<0.001
DASH diet, upper 40th percentile (%)	15 (4.2)	133 (21.2)	296 (52.1)	270 (82.8)	<0.001
BMI, 18.5–24.9 kg/m ² (%)	26 (5.9)	101 (14.6)	114 (19.7)	182 (55.3)	<0.001
Total TRL, nmol/l (median)	64.9 (40.6–102.9)	58.0 (36.9–90.4)	55.1 (31.9–85.5)	46.7 (28.6–73.2)	<0.001
Large VLDL, nmol/l (median)	1.5 (0.6–3.9)	1.1 (0.5–3.5)	0.8 (0.4–2.5)	0.6 (0.3–1.5)	<0.001
Medium VLDL, nmol/l (median)	26.6 (14.9–46.9)	23.0 (11.5–38.6)	19.3 (8.5–34.7)	14.6 (6.4–28.0)	<0.001
Small VLDL, nmol/l (median)	32.8 (17.7–51.1)	30.3 (16.5–50.7)	30.4 (17.0–49.8)	28.0 (15.4–47.9)	0.107
Total LDL, nmol/l (mean)	1311.2 \pm 435.8	1278.8 \pm 408.4	1240.3 \pm 390.5	1205.6 \pm 374.9	<0.001
IDL, nmol/l (median)	99.0 (54.0–166.0)	95.0 (51.0–164.0)	88.0 (49.0–156.0)	86.0 (41.5–141.5)	0.002
Large LDL, nmol/l (mean)	544.9 \pm 300.1	571.6 \pm 294.1	602.6 \pm 295.4	696.7 \pm 300.9	<0.001
Small LDL, nmol/l (mean)	645.8 \pm 438.6	591.3 \pm 409.8	525.0 \pm 400.2	408.1 \pm 368.5	<0.001
Total HDL, μ mol/l (mean)	37.7 \pm 6.2	38.4 \pm 6.4	38.5 \pm 6.0	38.7 \pm 5.3	0.037
Large HDL, μ mol/l (median)	5.2 (3.4–8.0)	5.8 (3.7–8.7)	6.4 (3.9–9.9)	8.2 (5.1–14.8)	<0.001
Medium HDL, μ mol/l (mean)	13.5 \pm 6.0	13.5 \pm 6.5	13.4 \pm 6.2	13.7 \pm 5.6	0.483
Small HDL, μ mol/l (mean)	18.2 \pm 5.6	18.3 \pm 6.3	17.8 \pm 5.7	16.2 \pm 5.8	<0.001
VLDL size, nm (mean)	46.2 \pm 6.6	45.4 \pm 6.2	44.7 \pm 5.7	43.5 \pm 5.1	<0.001
LDL size, nm (mean)	20.7 \pm 0.6	20.8 \pm 0.5	20.9 \pm 0.6	21.1 \pm 0.5	<0.001
HDL size, nm (mean)	9.2 \pm 0.5	9.2 \pm 0.5	9.3 \pm 0.5	9.5 \pm 0.5	<0.001
LP-IR score (median)	40.0 (23.0–58.0)	35.0 (20.0–52.0)	29.0 (12.0–48.0)	19.0 (6.0–37.3)	<0.001

TRL: triglyceride-rich lipoprotein; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; IDL: intermediate-density lipoprotein; HDL: high-density lipoprotein; LP-IR: lipoprotein insulin resistance.

Mean \pm standard deviation or median (interquartile range) are shown for continuous variables. Numbers and % (in brackets) for categorical variables will vary in different analyses as some variables have missing values.

p for trend determined from a Jonckheere test, a linear-by-linear chi-square or an ANOVA.

Table 4

Linear regression analysis of the associations between a PLB score, individual protective behaviours and lipoprotein subclasses.

Feature	Log total TRL		Log large VLDL		Log medium VLDL		Log small VLDL	
	$\beta \pm$ S.E.	<i>p</i>						
PLB score								
Model 1	-0.07 ± 0.02	0.002	-0.20 ± 0.03	0.002	-0.18 ± 0.03	0.002	-0.01 ± 0.02	.627
Never or former smoker								
Model 1	-0.14 ± 0.04	0.004	-0.16 ± 0.09	.052	-0.15 ± 0.07	0.03	-0.16 ± 0.06	0.016
Model 2	-0.21 ± 0.06	0.002	-0.13 ± 0.11	.246	-0.25 ± 0.09	0.006	-0.27 ± 0.08	0.004
Non or moderate drinker								
Model 1	0.10 ± 0.06	0.1	-0.49 ± 0.11	0.002	-0.16 ± 0.09	0.052	0.44 ± 0.08	0.002
Model 2	0.15 ± 0.06	0.02	-0.43 ± 0.11	0.002	-0.13 ± 0.09	0.106	0.50 ± 0.09	0.002
Moderate or high physical activity								
Model 1	-0.08 ± 0.03	0.008	-0.19 ± 0.06	0.002	-0.17 ± 0.05	0.004	-0.02 ± 0.05	0.683
Model 2	-0.07 ± 0.04	0.094	-0.08 ± 0.08	0.357	-0.14 ± 0.06	0.024	-0.04 ± 0.06	0.487
DASH diet [upper 40th percentile]								
Model 1	-0.03 ± 0.04	0.315	-0.19 ± 0.07	0.002	-0.15 ± 0.05	0.006	0.06 ± 0.05	0.234
Model 2	0.01 ± 0.05	0.735	-0.30 ± 0.09	0.002	-0.09 ± 0.07	0.18	0.13 ± 0.06	0.034
BMI [18.5–24.9 kg/m ²]								
Model 1	-0.29 ± 0.04	0.002	-0.67 ± 0.07	0.002	-0.62 ± 0.06	0.002	-0.16 ± 0.06	0.01
Model 2	-0.31 ± 0.05	0.002	-0.81 ± 0.10	0.002	-0.66 ± 0.08	0.002	-0.18 ± 0.07	0.05
Feature	Total LDL		Log IDL		Large LDL		Small LDL	
	$\beta \pm$ S.E.	<i>p</i>						
PLB score								
Model 1	-41.71 ± 9.7	0.002	-0.10 ± 0.03	0.002	20.92 ± 6.6	0.008	-55.07 ± 9.2	0.002
Never or former smoker								
Model 1	-4.04 ± 21.7	0.898	0.08 ± 0.07	0.254	8.49 ± 18.3	0.661	-17.17 ± 25.9	0.575
Model 2	-3.74 ± 35.3	0.93	0.13 ± 0.10	0.144	21.39 ± 23.0	0.375	-36.38 ± 32.7	0.331
Non or moderate drinker								
Model 1	-4.68 ± 33.9	0.9	-0.26 ± 0.09	0.004	-14.40 ± 22.7	0.581	35.59 ± 32.4	0.287
Model 2	-16.00 ± 36.4	0.695	-0.27 ± 0.10	0.012	-37.04 ± 23.7	0.132	47.28 ± 33.7	0.192
Moderate or high physical activity								
Model 1	-38.77 ± 19.5	0.04	-0.15 ± 0.05	0.006	7.72 ± 13.24	0.565	-34.38 ± 18.6	0.04
Model 2	-24.47 ± 25.6	0.339	-0.11 ± 0.07	0.116	4.14 ± 16.69	0.796	-19.05 ± 23.8	0.427
DASH diet [upper 40th percentile]								
Model 1	-22.56 ± 21.1	0.295	-0.17 ± 0.06	0.004	14.98 ± 14.1	0.303	-26.27 ± 20.1	0.198
Model 2	-35.61 ± 27.6	0.186	-0.19 ± 0.07	0.022	16.59 ± 17.96	0.363	-36.92 ± 25.6	0.108
BMI [18.5–24.9 kg/m ²]								
Model 1	-86.24 ± 23.7	0.004	-0.17 ± 0.06	0.008	123.38 ± 15.8	0.002	-193.16 ± 22.3	0.002
Model 2	-80.32 ± 31.6	0.008	-0.15 ± 0.09	0.078	155.43 ± 20.6	0.002	-217.31 ± 29.2	0.002
Feature	Total HDL		Log large HDL		Medium HDL		Small HDL	
	$\beta \pm$ S.E.	<i>p</i>						
PLB score								
Model 1	-0.04 ± 0.14	0.778	0.07 ± 0.01	0.002	-0.23 ± 0.15	0.096	-0.38 ± 0.14	0.008
Never or former smoker								
Model 1	-0.86 ± 0.39	0.07	0.02 ± 0.04	0.689	-1.14 ± 0.40	0.014	0.15 ± 0.39	0.707
Model 2	-0.76 ± 0.51	0.224	0.07 ± 0.05	0.214	-0.88 ± 0.55	0.118	-0.35 ± 0.51	0.475
Non or moderate drinker								
Model 1	-4.44 ± 0.49	0.002	-0.16 ± 0.05	0.004	-3.85 ± 0.52	0.002	0.26 ± 0.50	0.633
Model 2	-4.63 ± 0.53	0.002	-0.19 ± 0.05	0.002	-4.03 ± 0.56	0.002	0.40 ± 0.53	0.467
Moderate or high physical activity								
Model 1	0.25 ± 0.29	0.443	0.05 ± 0.03	0.076	-0.30 ± 0.29	0.331	0.22 ± 0.28	0.463
Model 2	0.31 ± 0.37	0.447	-0.06 ± 0.04	0.856	-0.25 ± 0.40	0.543	0.55 ± 0.37	0.162
DASH diet [upper 40th percentile]								
Model 1	-0.84 ± 0.31	0.004	0.003 ± 0.03	0.906	-0.17 ± 0.32	0.561	-0.73 ± 0.30	0.018
Model 2	-0.71 ± 0.40	0.078	0.004 ± 0.04	0.91	0.26 ± 0.43	0.595	-0.99 ± 0.40	0.016
BMI [18.5–24.9 kg/m ²]								
Model 1	0.41 ± 0.35	0.264	0.35 ± 0.03	0.002	-0.25 ± 0.35	0.485	-1.90 ± 0.33	0.002
Model 2	0.52 ± 0.46	0.254	0.39 ± 0.05	0.002	-0.03 ± 0.49	0.952	-2.26 ± 0.46	0.002
Feature	VLDL size		LDL size		HDL size		Log LP-IR score	
	$\beta \pm$ S.E.	<i>p</i>						
PLB score								
Model 1	-0.61 ± 0.16	.002	0.06 ± 0.01	.002	0.06 ± 0.01	.002	-0.16 ± 0.02	.002
Never or former smoker								
Model 1	-0.16 ± 0.43	0.731	0.01 ± 0.03	0.822	0.02 ± 0.03	0.591	-0.04 ± 0.06	0.499
Model 2	0.15 ± 0.56	0.824	0.04 ± 0.04	0.377	0.07 ± 0.04	0.074	-0.08 ± 0.07	0.266
Non or moderate drinker								
Model 1	-3.13 ± 0.55	0.002	-0.04 ± 0.04	0.323	-0.08 ± 0.04	0.03	-0.08 ± 0.07	0.238
Model 2	-2.78 ± 0.56	0.002	-0.08 ± 0.04	0.08	-0.09 ± 0.04	0.024	-0.04 ± 0.07	0.595
Moderate or high physical activity								
Model 1	-0.89 ± 0.31	0.004	0.02 ± 0.02	0.501	0.03 ± 0.02	0.204	-0.12 ± 0.04	0.006
Model 2	-0.46 ± 0.40	0.256	-0.07 ± 0.03	0.838	-0.02 ± 0.03	0.493	-0.03 ± 0.05	0.607

(continued on next page)

Table 4 (continued)

Feature	VLDL size		LDL size		HDL size		Log LP-IR score	
	$\beta \pm$ S.E.	<i>p</i>						
DASH diet [upper 40th percentile]								
Model 1	-0.40 \pm 0.33	0.224	0.05 \pm 0.03	0.018	0.02 \pm 0.02	.519	-0.09 \pm 0.05	0.049
Model 2	-0.67 \pm 0.43	0.11	0.06 \pm 0.03	0.046	0.01 \pm 0.03	0.792	-0.16 \pm 0.05	0.002
BMI [18.5–24.9 kg/m ²]								
Model 1	-2.12 \pm 0.39	0.002	0.22 \pm 0.03	0.002	0.29 \pm 0.03	0.002	-0.70 \pm 0.05	0.002
Model 2	-2.87 \pm 0.52	0.002	0.27 \pm 0.04	0.002	0.32 \pm 0.03	0.002	-0.83 \pm 0.06	0.002

TRL: triglyceride-rich lipoprotein; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; IDL: intermediate-density lipoprotein; HDL: high-density lipoprotein; LP-IR: lipoprotein insulin resistance.

Beta (β) coefficients \pm standard errors (S.E.) are shown.

Model 1: adjusted for age, sex, education and type 2 diabetes.

Model 2: further adjusted for each protective behaviour. Significant *p* highlighted in bold.

relationship was observed; subjects with the fewest number of protective behaviours had 1.4–2.8 increased odds of having less favourable lipoprotein profiles, defined as above or below median level lipoprotein particle subclass size or concentration, when compared to individuals with 4 or 5. In a final multivariable analysis, which additionally adjusted for BMI, significant trend relationships were observed between the number of protective behaviours and large VLDL ($p = 0.001$), medium VLDL ($p < 0.001$), total LDL ($p = 0.008$), IDL ($p = 0.031$), small LDL ($p < 0.001$) concentrations, LDL size ($p = 0.003$) and the LP-IR score ($p = 0.003$).

4. Discussion

The present study of 2045 middle-to older-aged men and women is the first to examine relationships between a multicomponent lifestyle score and NMR-derived lipoprotein profiles. Our findings show that subjects with the fewest number of protective behaviours (0–1) had 1.4–2.8 increased odds of having less favourable lipoprotein profiles, characterised by more large and medium VLDL particles, more total and small LDL particles, smaller LDL size and a greater lipoprotein insulin resistance score, when compared to individuals with 4 or 5 protective behaviours. Most associations between our five-component PLB score and lipoprotein subclass measures remained significant in linear regression analyses when adjusted for age, sex, education and type 2 diabetes. A dose-response relationship between the number of protective behaviours and above or below median level lipoprotein particle subclass size or concentration was also observed in multivariable logistic regression models. These results demonstrate that adopting a healthy lifestyle may be an effective approach to improve lipoprotein profiles defined by higher or lower subclass concentrations [9,32], attenuate atherogenesis, prevent CVD and chronic disease risk and promote healthy ageing.

In our analysis, a greater number of protective behaviours was associated with a more favourable lipoprotein profile characterised by less large VLDL and smaller LDL particles. Large VLDL and smaller LDL particles have been linked to increased risk for atherosclerosis [2,7]. Large VLDL particles may be particularly important in terms of CVD risk, as they are associated with the pro-atherogenic small dense LDL phenotype [2]. Relative to LDL particles, these large lipid-enriched VLDL particles are more efficiently hydrolysed by lipoprotein lipase, have greater capacity to penetrate the endothelial wall and be preferentially retained in the arterial intima [33]. Hepatic over-production of large triglyceride-rich VLDL is a hallmark of dyslipidaemia in obesity and insulin resistance, which may initiate diabetic dyslipidaemia [34, 35]. Furthermore, we report lower LP-IR scores among subjects with a greater number of protective behaviours. The LP-IR score is an alternative means of assessing a patient's insulin resistance status based on lipoprofile data [22].

Examination of individual PLB score components revealed varying associations according to lipoprotein subclass. Excluding normal BMI, non or moderate alcohol use and diet quality demonstrated the greatest

number of significant relationships in adjusted linear regression analyses. Noticeably, the association with alcohol use remained significant for nine of the 16 examined measures when additionally adjusted for each PLB score component. However, we noted complex relationships with alcohol use, with effects that differed according to lipoprotein subclass. Non or moderate alcohol use was positively associated with total TRL and small VLDL, and was inversely associated with total HDL, large HDL, medium HDL and HDL size, suggesting that heavier alcohol use is associated with a more favourable profile with regard to these lipoprotein subclasses.

A previous study by Mukamal et al. [36] also found conflicting relationships between alcohol use and lipoproteins. In this research, increased alcohol intake was positively associated with large and medium HDL and with lower small VLDL – as our findings also suggest. Other studies have also observed greater alcohol consumption to be associated with higher HDL concentrations in a dose-response manner [37,38]. In a study of 2171 older adults, Muth et al. [39] found highest weekly alcohol consumption to be associated with the greatest total HDL size and greatest number of medium and large HDL particles, as well as higher total HDL concentrations; total small HDL did not differ. In our research, we defined alcohol use dichotomously, similar to a method employed in a recent research by Li et al. [20]. It is possible that defining non or moderate alcohol use with an alternative method may have yielded different relationships.

Our results demonstrating an association between healthy diet and lipoprotein profiles are also supported by the literature, although relationships are commonly based on a narrow selection of lipid markers [40]. We found healthy diet to be associated with large VLDL, small VLDL, IDL, small HDL, LDL size and the LP-IR score. Previous research by Phillips et al. [41] revealed a more favourable lipoprotein profile characterised by less smaller LDL and small HDL particles and less large VLDL particles among those with better diet quality. Importantly, diet quality is correlated with weight gain and the research by Phillips et al. also showed that risk of central obesity, defined by the waist-to-hip-ratio, was lower among subjects in the highest DASH quartile compared to the lowest quartile.

There are limited data available investigating the effects of smoking on lipoprotein subclasses. In our study, smoking status was independently associated with total TRL, medium VLDL and small VLDL. Using a sample of 612 subjects, Beauchamp et al. [42] found female smokers to have a more atherogenic lipoprotein profile than non-smokers; these results were consistent for both conventional lipid concentrations and NMR-determined lipoprotein profiles. However, some sex-specific smoking-related changes were observed among males. In a sample of 1504 participants, Gossett et al. [43] showed smoking to be associated with small increases in total cholesterol, LDL, triglycerides and small decreases in HDL particle concentrations. As this study also found a modest dose-response effect of smoking intensity on lipoprotein concentrations, the authors suggested that smoking reduction is unlikely to be an effective method for improving lipoproteins among smokers and that complete smoking cessation would be a more promising strategy.

Table 5

Logistic regression analysis of the associations between the number of protective behaviours and lipoprotein subclasses^a.

Number of protective behaviours	Total TRL		Large VLDL		Medium VLDL		Small VLDL	
	OR (95% CI)	<i>p</i> trend						
Model 1								
4 or 5	1 (Reference)	0.001	1 (Reference)	<0.001	1 (Reference)	<0.001	1 (Reference)	0.329
3	1.16 (0.86, 1.55)		1.72 (1.27, 2.34)		1.43 (1.06, 1.92)		1.00 (0.75, 1.33)	
2	1.30 (0.98, 1.74)		2.19 (1.62, 2.97)		1.99 (1.49, 2.67)		1.04 (0.79, 1.39)	
0 or 1	1.71 (1.24, 2.35)		2.70 (1.94, 3.76)		2.54 (1.84, 3.51)		1.16 (0.85, 1.59)	
Model 2								
4 or 5	1 (Reference)	0.141	1 (Reference)	0.001	1 (Reference)	<0.001	1 (Reference)	0.797
3	0.93 (0.68, 1.26)		1.22 (0.88, 1.69)		1.04 (0.76, 1.43)		0.92 (0.68, 1.24)	
2	1.02 (0.75, 1.39)		1.50 (1.08, 2.07)		1.40 (1.02, 1.93)		0.95 (0.70, 1.29)	
0 or 1	1.26 (0.90, 1.78)		1.70 (1.19, 2.43)		1.66 (1.17, 2.36)		1.03 (0.74, 1.45)	
Number of protective behaviours	Total LDL		IDL		Large LDL		Small LDL	
	OR (95% CI)	<i>p</i> trend						
Model 1								
4 or 5	1 (Reference)	<0.001	1 (Reference)	0.004	1 (Reference)	0.001	1 (Reference)	<0.001
3	1.21 (0.90, 1.61)		1.08 (0.81, 1.44)		1.49 (1.09, 2.03)		1.74 (1.27, 2.38)	
2	1.48 (1.11, 1.98)		1.34 (1.00, 1.78)		1.70 (1.25, 2.31)		2.23 (1.64, 3.04)	
0 or 1	1.73 (1.26, 2.38)		1.51 (1.10, 2.07)		1.71 (1.22, 2.39)		2.55 (1.81, 3.58)	
Model 2								
4 or 5	1 (Reference)	0.008	1 (Reference)	0.031	1 (Reference)	0.17	1 (Reference)	<0.001
3	1.09 (0.81, 1.49)		1.01 (0.75, 1.36)		1.18 (0.85, 1.63)		1.34 (0.96, 1.87)	
2	1.32 (0.98, 1.80)		1.24 (0.91, 1.67)		1.31 (0.95, 1.82)		1.68 (1.21, 2.34)	
0 or 1	1.51 (1.08, 2.23)		1.37 (0.98, 1.93)		1.25 (0.87, 1.79)		1.79 (1.24, 2.58)	
Number of protective behaviours	Total HDL		Large HDL		Medium HDL		Small HDL	
	OR (95% CI)	<i>p</i> trend						
Model 1								
4 or 5	1 (Reference)	0.537	1 (Reference)	<0.001	1 (Reference)	0.179	1 (Reference)	0.036
3	0.78 (0.73, 1.31)		1.42 (1.03, 1.96)		0.83 (0.62, 1.11)		1.49 (1.10, 2.00)	
2	0.92 (0.69, 1.23)		1.87 (1.36, 2.56)		0.93 (0.70, 1.25)		1.49 (1.11, 2.00)	
0 or 1	1.13 (0.82, 1.56)		2.21 (1.56, 3.12)		1.20 (0.88, 1.66)		1.43 (1.04, 1.98)	
Model 2								
4 or 5	1 (Reference)	0.762	1 (Reference)	0.069	1 (Reference)	0.342	1 (Reference)	0.514
3	0.94 (0.69, 1.28)		0.97 (0.68, 1.36)		0.80 (0.59, 1.08)		1.25 (0.91, 1.71)	
2	0.88 (0.65, 1.20)		1.22 (0.87, 1.72)		0.89 (0.66, 1.21)		1.23 (0.90, 1.68)	
0 or 1	1.08 (0.77, 1.53)		1.33 (0.91, 1.93)		1.14 (0.81, 1.61)		1.13 (0.80, 1.60)	
Number of protective behaviours	VLDL size		LDL size		HDL size		LP-IR score	
	OR (95% CI)	<i>p</i> trend						
Model 1								
4 or 5	1 (Reference)	0.027	1 (Reference)	<0.001	1 (Reference)	<0.001	1 (Reference)	<0.001
3	1.41 (1.02, 1.94)		1.64 (1.18, 2.29)		1.27 (0.93, 1.73)		1.53 (1.11, 2.11)	
2	1.37 (1.00, 1.88)		2.12 (1.53, 2.94)		1.68 (1.24, 2.28)		2.27 (1.65, 3.11)	
0 or 1	1.51 (1.07, 2.13)		2.39 (1.68, 3.42)		1.79 (1.28, 2.49)		2.79 (1.97, 3.95)	
Model 2								
4 or 5	1 (Reference)	0.854	1 (Reference)	0.003	1 (Reference)	0.279	1 (Reference)	0.003
3	1.09 (0.78, 1.54)		1.26 (0.89, 1.79)		0.90 (0.64, 1.25)		1.01 (0.71, 1.42)	
2	1.02 (0.73, 1.43)		1.59 (1.12, 2.25)		1.16 (0.83, 1.61)		1.44 (1.02, 2.03)	
0 or 1	1.06 (0.73, 1.54)		1.68 (1.15, 2.46)		1.13 (0.79, 1.62)		1.61 (1.11, 2.34)	

TRL: triglyceride-rich lipoprotein; VLDL: very low-density lipoprotein; LDL: low-density lipoprotein; IDL: intermediate-density lipoprotein; HDL: high-density lipoprotein; LP-IR: lipoprotein insulin resistance.

Odds ratios (OR) and 95% confidence intervals (CI) are shown.

Model 1: adjusted for age, sex, education and type 2 diabetes.

Model 2: further adjusted for normal BMI (18.5–24.9 kg/m²).

^a Above median except for large LDL, total HDL, large HDL, LDL size and HDL size (below median).

Interestingly, we observed independent associations between moderate or high physical activity levels and only one lipoprotein subclass (medium VLDL). As with diet quality, physical activity is correlated with body mass. Consequently, the main mechanism by which physical activity may improve lipoprotein profiles, and provide protection against chronic disease, may be through weight loss. Research by Chrichton et al. [44] found higher intensity physical activity to be associated with a more favourable lipid profile among normal weight, but not overweight or obese subjects. As a high percentage of subjects in our sample were overweight/obese (78%), this may partly account for the lack of statistically significant relationships with other lipoprotein subclasses observed in our study.

We noted that having a normal BMI demonstrated the greatest number of significant relationships with lipoprotein measures after adjustment for other PLB score components, indicating that adiposity is strongly related to pro-atherogenic alterations in the lipoprotein subclass profile – consistent with previous observations with BMI [3,9]. A normal BMI also showed a significant linear association across protective behaviour categories. This suggests that observed associations between a PLB score and non-communicable diseases and mortality [20], which are mediated by adverse lipoprotein levels, may also largely be explained by increased adiposity among subjects with fewer protective behaviours. Obesity has long been recognised as a major risk factor for CVD and other chronic conditions [45–47]. However, our results

demonstrate significant trend relationships between the number of protective behaviours and large VLDL, medium VLDL, total LDL, IDL, small LDL, LDL size and the LP-IR score. Furthermore, in multivariable logistic regression models adjusted for BMI, these findings show a cumulative protective effect against metabolic dysregulation that is independent of having a healthy body weight.

4.1. Strengths and limitations

Research on protective behaviours is of public health importance. Such investigation contributes to the knowledge base and may inform public health guidelines and policy. As far as we are aware, this research is the first to test a five-component PLB score (and individual PLB score component) associations with 15 lipoprotein subclasses and the LP-IR score. Other strengths include the large number of middle-to older-aged study participants, equal representation by sex (49% male) and the use of validated questionnaires to collect a wide range of diet, lifestyle and phenotypic data. With the elderly population growing [48], it is to be expected that the number of patients with non-communicable diseases will increase. Importantly, our findings suggest that modifications in certain lifestyle behaviours and adopting a healthier diet may have potential cardioprotective effects, which may be of particular importance to older adults.

Despite these strengths, several limitations should be noted. The cross-sectional study design limits inference with regards to causality and precludes drawing conclusions regarding the temporal direction of relationships. Moreover, the use of self-reported questionnaires is subject to potential inaccuracies, recall and reporting bias and residual confounding arising from imprecise measurement of variables should also be considered. Data were collected from a single primary care-based sample, which may not be representative of the general 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 [49].

4.2. Conclusions

In conclusion, our findings show that in middle-to older aged adults a combination of protective behaviours may potentially confer cardioprotective benefits, which could attenuate dyslipidaemia and related metabolic dysregulation. As an unfavourable lipoprotein profile may precede many non-communicable diseases, these data highlight the potential benefits of following a healthy lifestyle and maintaining a BMI in the normal range. Improving our understanding of the relationships between protective behaviours and biomarkers of health is warranted, with a view to informing public health planning and policy to improve and maintain optimal cardiometabolic health at a population level.

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CRedit authorship contribution statement

Seán R. Millar: Formal analysis, Writing - original draft, conceived design of the paper. All authors read and approved the final manuscript. **Janas M. Harrington:** All authors read and approved the final manuscript. **Ivan J. Perry:** All authors read and approved the final manuscript. All other authors provided critical revisions. **Catherine M. Phillips:** conceived design of the paper, All authors read and approved

the final manuscript.

Declaration of competing interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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