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ORIGINAL ARTICLE

Endotoxemia, nutrition, and cardiometabolic disorders

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Abstract
Aims Circulating lipopolysaccharides (LPSs), associated with both infection and inflammation, may arise from the gastrointestinal tract microbiota, and the levels may be affected by daily nutrition. We investigated whether nutrient intake affects the association of serum LPS activity with prevalent obesity, metabolic syndrome (MetS), diabetes, and coronary heart disease (CHD) and with the risk of incident CHD events.
Methods The nutrition cohort ($n = 2{,}452$, mean age ± SD, 52.2 ± 10.1 years) of the FINRISK 1997 Study was followed up for 10 years. Information on macronutrient intake at baseline was collected from 24-h dietary recall. Serum endotoxin activities were determined by the *Limulus* amebocyte lysate assay.
Results LPS activity was associated directly with the total energy intake and indirectly with carbohydrate intake in lean, healthy subjects. High LPS was significantly associated with prevalent obesity, MetS, diabetes, and CHD events, independently of established risk factors, CRP, and total energy or nutrient intake. The ORs (95 % CI) were 1.49 (1.21–1.85, $p < 0.001$, Q2–4 vs. Q1) for obesity, 2.56 (1.97–3.32, $p < 0.001$, Q2–4 vs. Q1) for MetS, 1.94 (1.06–3.52, $p = 0.031$, Q2–4 vs. Q1) for CHD, and 1.01 (1.00–1.01, $p = 0.032$, LPS unit) for diabetes. In the follow-up, high LPS was significantly associated with the risk of CHD events with a hazard ratio of 1.88 (1.13–3.12, $p = 0.013$, Q2–4 vs. Q1). This association was independent of baseline established risk factors, diet, obesity, MetS, and diabetes.
Conclusion A high serum LPS activity is strongly associated with cardiometabolic disorders, which supports the role of bacterial infections and immune response in their etiology.

Keywords Lipopolysaccharide · Obesity · Metabolic syndrome · Diabetes · Cardiovascular disease

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Introduction

Lipopolysaccharide (LPS), often referred to as endotoxin, is a large glycolipid that consists of lipid and polysaccharide moieties joined by a covalent bond. This important virulence factor of Gram-negative bacteria is found in the outer bacterial membrane. In humans, increased systemic levels of endotoxins promote the pro-inflammatory response of the innate immune system.

"Metabolic endotoxemia" is a twofold to threefold increase in serum LPS level, which is detectable also in apparently healthy subjects and may result in low-grade inflammation [1–3]. The long-term effects of subclinical endotoxemia are deleterious, since endotoxemia associates with the risk of incident cardiovascular disease (CVD) events [4, 5] and diabetes [6]. Endotoxemia also associates with components and presence of metabolic syndrome (MetS) [6, 7], insulin resistance, dyslipidemia, obesity, and chronic inflammation [4, 7]. LPS may arise from various sources: bacterial infections, diet, and commensal microbiota. Lifestyle, dietary habits, and the use of antimicrobial agents may affect the variety of bacterial species and the microbial load. These modulations in the composition of commensal microbiota may be important contributors to the host metabolism affecting energy homeostasis.

Human gastrointestinal tracts, including the oral cavity and the gut, are colonized by Gram-negative bacteria. For example, the adult human intestine is inhabited by 10^{13}–10^{14} microorganisms [8]. In healthy conditions, intestinal alkaline phosphatase may maintain normal gut homeostasis by neutralizing LPS toxicity [9] and the intestinal epithelium defends itself from LPS translocation. Of note, fat-containing diet may promote intestinal transport of fat-soluble LPS molecules via chylomicrons [10]. In addition, it has been suggested that LPS may be absorbed by intestinal cells [11] and expression of genes involved in the barrier function in host epithelial cells may be modulated by bacteria [12].

The previous studies on humans suggest that endotoxemia may be associated with energy, fat, and carbohydrate intake, and furthermore, that postprandial endotoxin absorption is affected by the metabolic disease state. These studies are interventions with relatively small study populations, while large population-based studies with information on nutrient intake collected from dietary recalls do not exist. High energy intake composed of saturated fat or carbohydrate-rich meal may lead to acute low-grade endotoxemia [2, 13–15]. A high-fat meal has been shown to elevate circulating endotoxin levels both in healthy, lean subjects [2, 14] and in subjects with metabolic disorders, i.e., obesity, type 2 diabetes, or impaired glucose tolerance [15, 16]. Indeed, LPS appears to be a molecular link between high-fat diet, microbiota, and inflammation, which has been shown in a mouse model for the time being [3]. We investigated in a population-based nutrition cohort the association of serum LPS activity with prevalent obesity, MetS, diabetes, and coronary heart disease (CHD) and with the risk of incident CHD events, taking into account data on the individual energy and macronutrient intake registered in a dietary 24-h recall.

Materials and methods

Study population

The National FINRISK 1997 Study is a population-based risk factor survey with 8,444 participants. It was conducted in five geographical areas in Finland, and the age range was 25 to 74 years [17]. The survey methods follow the WHO MONICA protocol [18]. The National FINDIET 1997 Survey ($n = 2{,}452$) is a nutrition subsample of the FINRISK 1997. The study included a self-administered questionnaire and a clinical examination with weight, height, and blood pressure measurements, and blood samples. The study was approved by the ethics committee of the National Public Health Institute, and it was conducted according to the Declaration of Helsinki. All subjects gave an informed consent.

Laboratory analysis

Subjects were asked to fast 4 h and to avoid heavier meals prior to blood sampling. The median fasting time was 5 (interquartile range 3–7) h. Lipids and γ-glutamyltransferase (GGT) measurements were taken from fresh serum samples. The rest of the serum and plasma biomarkers were determined from samples stored at −70 °C. Ultrasensitive C-reactive protein (CRP) was determined with Architect c8000 analyzer (Abbott Laboratories, Abbott Park, IL). Serum endotoxin activities were determined by a *Limulus* amebocyte lysate assay coupled with a chromogenic substrate (HyCult Biotechnology B.V., Uden, the Netherlands), and the interassay coefficient of variation was 9.2 % ($n = 75$). The laboratory analyses have been described earlier in detail [6].

Determination of risk factors and diseases

Obesity was defined as a body mass index (BMI)-based classification of adult overweight (≥25 kg/m^2) and obesity (≥30 kg/m^2) according to the World Health Organization [19].

The subjects were classified into those with and without *MetS* according to the International Diabetes Federation definition for Europids [20].

Prevalent *diabetes* and *CVD events* were defined as a doctor-diagnosed disease using the questionnaire, and the register data either as an intake of related drugs or as hospitalizations with the disease. CHD events included subjects with the history of myocardial infarction, revascularizations, or percutaneous transluminal coronary angioplasty. Additionally, history of stroke (excluding subarachnoid hemorrhage) was included in the prevalent CVD. Follow-up for incident CHD events was performed

for 10 years with the use of record linkage of the FINRISK data with three data sources: (1) National Hospital Discharge Register; (2) National Causes of Death Register; and (3) National Drug Reimbursement Register. The high validity of the data on CHD events has been shown previously [21].

Blood pressure measurements have been described in detail earlier [17]. *Hypertension* was determined according to the criteria of the American Heart Association [22] as blood pressure ≥140 mmHg systolic or ≥90 mmHg diastolic or the use of any antihypertensive drug.

Smoking was assessed by a self-administered questionnaire. Classification was performed into three categories: (1) current smokers (who had smoked regularly for ≥1 year and still smoked or had quit smoking <6 months ago); (2) former smokers (who used to smoke regularly but had quit ≥6 months before the survey); and (3) non-smokers (who had never smoked regularly).

Information on *diet* was collected from the interviewed 24-h dietary recall. A picture booklet of food portions was used to estimate portion sizes [23]. The average daily intakes of energy, energy-yielding nutrients, and fiber were calculated by the national food composition database Fineli®, using an in-house software [24]. We used standard energy densities in the analysis: 37 kJ/g for fat, 17 kJ/g for protein and carbohydrates, and 8 kJ/g for fiber (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF).

Statistical analysis

The statistical significance of differences between the subjects with and without cardiometabolic disorders was tested with t test or chi-square test. The values with skewed distribution (serum triglyceride, GGT, and CRP) were logarithmically transformed before comparisons. Smoking habit was used in the categories never, former, and current smokers. Subjects were excluded from the study if the reported total energy intakes were less ($n = 17$) or greater ($n = 0$) than 3xSD from the mean energy intake of the population [25]. Association between LPS and total energy and nutrient intake was analyzed by linear regression model, first unadjusted, followed by a multivariate model including age, sex, education years, BMI, current smoking, and serum GGT, CRP, and cholesterol concentrations, and the use of hypertension medication. The differences in LPS concentrations between lean (BMI < 25 kg/m^2), overweight (BMI ≥ 25 kg/m^2), and obese (≥30 kg/m^2) subjects were determined by the one-way analysis of variance (ANOVA). The association of prevalent cardiometabolic disorders with the LPS activity was analyzed using a logistic regression model. In the models where the dependent variables were obesity, MetS, diabetes, or CHD, the covariates included age, sex, education years, current smoking, hypertension (except the MetS model), cholesterol and CRP concentrations, and energy intake. The logistic regression models were repeated adjusting for protein, fat, and fiber intake instead of total energy. Those with prevalent CVD ($n = 151$) were excluded from the prospective analyses. The possible effect of hypertension medication to the relation between LPS and total energy and nutrient intake was tested by interaction terms in all regression models; the associations were not statistically significant.

The association of incident CHD events with the LPS activity was analyzed using seven different Cox regression models. All models were adjusted for age, sex, years of education, current smoking, hypertension (except the MetS models), cholesterol, and CRP concentrations. Model 2 was further adjusted for MetS, model 3 for MetS and energy (418.7 kJ or 100 kcal), model 4 for obesity, model 5 for obesity and energy, and finally Model 6 for diabetes and model 7 for diabetes and energy.

The statistical analyses were executed with the IBM SPSS Statistics 21 Statistical Package for Social Sciences.

Results

Characteristics of the subjects with or without cardiometabolic disorders are summarized in Table 1. In the FINRISK 1997 nutrition cohort at baseline, 65.8 % were overweight or obese, 30.1 % had MetS, 7.1 % diabetes, and 6.2 % CHD. Most of the cardiometabolic risk factors differed significantly between the counterparts. Males were more frequently obese, while females had more MetS, diabetes, or CHD. In total, 481 of the study subjects were using antihypertensive medication. Online Resource 1 summarizes the information on macronutrient intake from the 24-h dietary recall.

LPS activity did not correlate significantly with total energy, carbohydrate, or fat intake in univariate linear regression models. However, in a multivariate model, LPS activity was associated directly with the total energy intake with unstandardized regression coefficients/418 kJ (100 kcal) (SE) 0.90 (0.43, $p = 0.037$) and indirectly with the carbohydrates available, −0.055 (0.02, $p = 0.018$) (Table 2). The association of LPS and energy or carbohydrate intake arose only from lean, healthy subjects; no significant associations were observed in subjects with CHD, obesity, MetS, or DM (Table 2). Corresponding results were also obtained when cardiometabolically healthy subjects ($n = 763$) were compared to those with at least one of the listed disorders.

The mean (SD) endotoxin activity was higher in the subjects with prevalent cardiometabolic disorder compared

Table 1 Baseline risk factors of the subjects with and without cardiometabolic disorders

	Obesity[a]		*p* value	MetS[b]		*p* value	Diabetes[c]		*p* value	CHD[c]		*p* value
	No ($n = 829$)	Yes ($n = 1,614$)		No ($n = 1,706$)	Yes ($n = 739$)		No ($n = 2,278$)	Yes ($n = 174$)		No ($n = 2,301$)	Yes ($n = 151$)	
Age (years)	49.2 (10.2)[d]	53.7 (9.8)	**<0.001**	50.7 (10.1)	55.5 (9.5)	**<0.001**	51.7 (10.0)	58.7 (9.7)	**<0.001**	51.6 (10.0)	62.7 (7.7)	**<0.001**
Education (years)	11.8 (3.8)	10.5 (3.8)	**<0.001**	11.3 (3.8)	10.1 (3.9)	**<0.001**	11.0 (3.8)	10.3 (4.6)	**0.016**	11.0 (3.9)	8.7 (3.7)	**<0.001**
Cholesterol (mmol/l)	5.47 (1.02)	5.81 (1.04)	**<0.001**	5.58 (1.01)	5.95 (1.09)	**<0.001**	5.70 (1.04)	5.67 (1.10)	0.764	5.70 (1.05)	5.42 (0.84)	**0.009**
HDL cholesterol (mmol/l)	1.53 (0.36)	1.31 (0.35)	**<0.001**	1.49 (0.34)	1.14 (0.29)	**<0.001**	1.39 (0.37)	1.28 (0.37)	**<0.001**	1.40 (0.37)	1.13 (0.28)	**<0.001**
Triglycerides (mmol/l)[e]	1.17 (0.68)	1.77 (1.06)	**<0.001**	1.22 (0.64)	2.36 (1.18)	**<0.001**	1.53 (0.95)	2.09 (1.31)	**<0.001**	1.55 (0.99)	1.95 (0.87)	**<0.001**
GGT (U/l)[e]	28.5 (45.2)	45.7 (68.7)	**<0.001**	33.1 (43.9)	54.8 (87.5)	**<0.001**	39.4 (63.2)	46.3 (47.1)	**<0.001**	39.5 (62.7)	47.6 (48.1)	**0.002**
Glucose (mmol/l)	5.06 (0.84)	5.24 (1.29)	**0.002**	4.99 (0.72)	5.59 (1.72)	**<0.001**	5.06 (0.68)	6.89 (3.32)	**<0.001**	5.16 (1.15)	5.56 (1.34)	**0.006**
CRP (mg/l)[e]	1.66 (3.66)	3.06 (5.51)	**<0.001**	2.05 (4.63)	3.82 (5.56)	**<0.001**	2.52 (4.98)	3.49 (5.20)	**<0.001**	2.57 (5.06)	3.19 (3.32)	**0.001**
BMI (kg/m2)	22.7 (1.6)	29.5 (3.8)	**<0.001**	25.7 (3.9)	30.7 (4.2)	**<0.001**	27.0 (4.5)	29.6 (5.1)	**<0.001**	27.1 (4.6)	28.9 (4.1)	**<0.001**
Systolic blood pressure (mmHg)	132 (19)	142 (19)	**<0.001**	135 (19)	147 (19)	**<0.001**	138 (19)	147 (21)	**<0.001**	138 (20)	149 (21)	**<0.001**
Diastolic blood pressure (mmHg)	80 (10)	87 (10)	**<0.001**	82 (10)	89 (10)	**<0.001**	84 (11)	85 (10)	0.584	84 (11)	85 (12)	0.523
	N (%)											
Sex (male)	316 (38.1)	877 (54.3)	**<0.001**	767 (45.0)	422 (59.8)	**<0.001**	1,100 (48.3)	95 (54.6)	0.108	1,125 (48.9)	70 (46.4)	**<0.001**
Smoking			**<0.001**			**<0.001**			**0.044**			**0.002**
No	456 (55.0)	838 (51.9)	–	933 (54.7)	361 (48.8)	–	1,213 (49.5)	85 (48.9)	–	1,262 (54.8)	36 (23.8)	–
Former	146 (17.6)	438 (27.1)	–	367 (21.5)	218 (29.5)	–	532 (23.4)	55 (31.6)	–	551 (23.9)	36 (23.8)	–
Current	227 (27.4)	338 (20.9)	–	406 (23.8)	106 (14.3)	–	533 (23.4)	34 (19.5)	–	546 (23.7)	21 (13.9)	–
Prevalent diabetes	27 (3.3)	146 (9.0)	**<0.001**	69 (4.0)	104 (14.1)	**<0.001**	–	–	–	156 (6.8)	18 (11.9)	**<0.001**
Prevalent CHD	14 (1.7)	79 (4.9)	**<0.001**	44 (2.3)	49 (6.6)	**<0.001**	75 (3.3)	18 (10.3)	**<0.001**	–	–	–
Prevalent MetS	30 (3.6)	708 (43.9)	**<0.001**	–	–	–	635 (27.9)	104 (61.5)	**<0.001**	690 (30.0)	49 (32.5)	**<0.001**
Treated hypertension (AHA)	331 (39.9)	1,052 (65.2)	**<0.001**	494 (29.0)	590 (79.8)	**<0.001**	1,260 (55.3)	128 (73.6)	**<0.001**	1,321 (57.4)	67 (44.4)	**0.002**
Hypertension medication	78 (9.4)	401 (24.8)	**<0.001**	234 (13.7)	245 (33.2)	**<0.001**	401 (17.6)	80 (46.0)	**<0.001**	444 (19.3)	37 (24.5)	**<0.001**

[a] Obesity is defined according to the WHO BMI-based classification of adult overweight (≥25 kg/m^2) and obesity (≥30 kg/m^2). Lean subjects are compared with overweight and obese subjects

[b] MetS is defined according to International Diabetes Federation guidelines

[c] Data on CHD events and diabetes were collected from hospital discharge register and drug reimbursement records

[d] Values are means and standard deviations

[e] Values log-transformed before statistical analysis

p values were obtained by *t* test or chi-square test

Significant *p* values are in bold face

Table 2 Multivariate linear regression models for the LPS activity

	Obesity[a]		MetS[b]		Diabetes[c]		CHD[c]	
	No (n = 829)	Yes (n = 1,614)	No (n = 1,706)	Yes (n = 739)	No (n = 2,278)	Yes (n = 174)	No (n = 2,301)	Yes (n = 151)
Age (years)	−0.12 (0.11), 0.253[d]	−0.29 (0.10), **0.004**	−0.06 (0.08), 0.437	−0.46 (0.17), **0.010**	−0.23 (0.08), **0.003**	−0.21 (0.32), 0.499	−0.23 (0.08), **0.003**	−0.52 (0.48), 0.285
Sex	7.69 (2.40), **0.01**	8.97 (2.09), **< 0.001**	7.71 (1.75), **< 0.001**	7.33 (3.25), **0.024**	8.40 (1.64), **< 0.001**	6.55 (6.04), 0.280	7.89 (1.62), **< 0.001**	23.4 (7.87), **0.004**
Education (years)	0.14 (0.26), 0.533	−0.02 (0.25), 0.934	0.09 (0.20), 0.645	−0.04 (0.40), 0.915	0.11 (0.20), 0.577	−0.35 (0.67), 0.600	0.08 (0.19), 0.712	0.01 (0.96), 0.988
Current smoking	−2.28 (2.15), 0.291	−6.98 (2.21), **0.002**	−2.65 (1.67), 0.115	−10.7 (3.61), **0.003**	−4.58 (1.64), **0.005**	−7.39 (7.40), 0.320	−4.71 (1.63), **0.004**	−13.6 (8.39), 0.108
Cholesterol (mmol/l)	3.40 (1.02), **0.001**	3.64 (0.89), **< 0.001**	2.71 (0.76), **< 0.001**	4.53 (1.38), **0.001**	3.80 (0.71), **< 0.001**	0.35 (2.56), 0.893	3.28 (0.70), **< 0.001**	13.7 (4.26), **0.002**
Triglycerides (mmol/l)[e]	26.5 (2.31), **< 0.001**	39.5 (1.94), **< 0.001**	27.6 (1.81), **< 0.001**	54.0 (3.50), **< 0.001**	34.8 (1.58), **< 0.001**	43.0 (5.78), **< 0.001**	35.9 (1.54), **< 0.001**	40.4 (9.16), **< 0.001**
GGT (U/l)[e]	3.47 (1.84), 0.060	2.40 (1.46), 0.100	2.67 (1.28), **0.038**	1.91 (2.31), 0.409	2.82 (1.20), **0.019**	0.10 (4.33), 0.982	2.54 (1.19), **0.032**	2.40 (5.08), 0.638
CRP (mg/l)[e]	0.86 (0.93), 0.355	0.24 (0.94), 0.797	0.34 (0.73), 0.641	1.04 (1.50), 0.490	0.48 (0.71), 0.504	0.62 (2.91), 0.832	0.33 (0.70), 0.637	3.78 (3.38), 0.267
BMI (kg/m^2)	1.42 (0.60), **0.018**	0.38 (0.25), 0.138	0.324 (0.20), 0.104	0.49 (0.36), 0.177	0.28 (0.18), 0.117	0.60 (0.67), 0.373	0.29 (0.18), 0.103	0.81 (0.88), 0.357
Energy (418 kJ or 100 kcal)	1.64 (0.65), **0.012**	0.59 (0.55), 0.283	1.48 (0.48), **0.002**	−0.58 (0.98), 0.553	1.02 (0.44), **0.021**	−0.67 (1.87), 0.721	0.95 (0.44), **0.031**	1.50 (2.26), 0.509
Carbohydrate (g)	−0.11 (0.03), **0.002**	−0.03 (0.03), 0.299	−0.08 (0.03), **0.001**	0.02 (0.05), 0.742	−0.06 (0.02), **0.011**	0.03 (0.12), 0.818	−0.06 (0.02), **0.010**	−0.07 (0.13), 0.600
Fat (g)	−0.12 (0.08), 0.116	−0.06 (0.07), 0.357	−0.14 (0.06), **0.011**	0.07 (0.12), 0.544	−0.10 (0.06), 0.074	0.15 (0.23), 0.515	−0.08 (0.05), 0.125	−0.18 (0.28), 0.534
Hypertension medication	−3.27 (3.28), 0.319	−5.22 (2.12), **0.014**	−2.85 (2.09), 0.173	−6.95 (3.19), **0.030**	−4.33 (1.87), **0.021**	−8.97 (5.81), 0.125	−3.80 (1.82), **0.036**	−21.6 (7.40), **0.005**

Serum LPS activity is used as a continuous variable

[a] Obesity is defined according to the WHO BMI-based classification of adult overweight (≥25 kg/m^2) and obesity (≥30 kg/m^2). Lean subjects are compared with overweight and obese subjects

[b] MetS is defined according to International Diabetes Federation guidelines

[c] Data on CHD events and diabetes were collected from hospital discharge register and drug reimbursement records

[d] Values are unstandardized regression coefficients, standard errors, and p values

[e] Values log-transformed before statistical analysis

Significant p values are in bold face

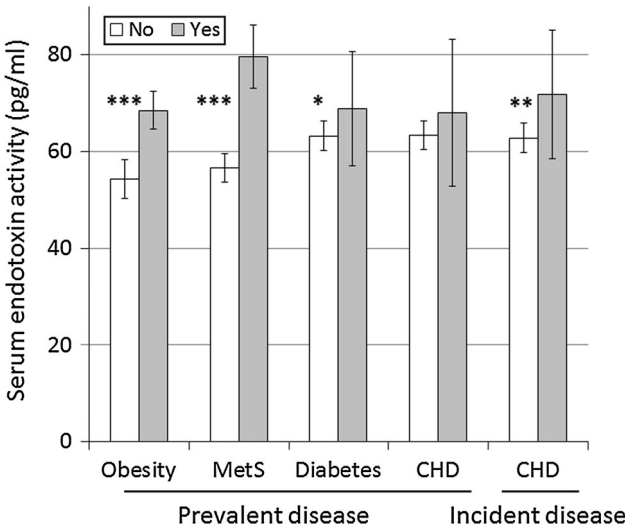

Fig. 1 Serum endotoxin activities in the cardiometabolic disorders. LPS activity (pg/ml) was determined by *Limulus* amebocyte lysate assay. Mean endotoxin activities with 95 % CI are shown. Subjects without and with a cardiometabolic disorder are indicated by *white* and *gray bars*, respectively. The statistical significance of differences in endotoxin activities was tested with *t* test; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$

to subjects without: 68.5 (39.8) versus 54.2 (29.4) pg/ml, ($p < 0.001$) for obesity, 79.6 (44.8) versus 56.6 (31.0) pg/ml ($p < 0.001$) for MetS, and 68.9 (39.7) versus 63.2 (37.0) pg/ml ($p = 0.05$) for diabetes (Fig. 1). The difference between those with and without prevalent CHD, 68.0 (37.2) versus 63.4 (37.3) pg/ml, was not significant ($p = 0.243$). In addition, LPS increased with increasing BMI. The mean activities were 54.2 (29.4), 65.8 (37.5), and 73.7 (37.3) pg/ml, ($p < 0.001$) in lean, overweight, and obese subjects, respectively.

In logistic regression models, high LPS was significantly associated with prevalent obesity, MetS, diabetes, and CHD. These associations were independent of cardiometabolic risk factors, CRP, and energy-yielding nutrients and fiber, or total energy intake. In the second–fourth quartiles (Q2–4) compared to the first quartile (Q1) of the LPS activity when adjusted for total energy, the ORs (95 % CI) were 1.49 (1.21–1.85, $p < 0.001$) for obesity, 2.56 (1.97–3.32, $p < 0.001$) for MetS, and 1.94 (1.06–3.52, $p = 0.031$) for CHD. The OR for diabetes was 1.01 (1.00–1.01, $p = 0.032$) per pg/ml increase in the LPS activity. The regression models are presented in Table 3. The ORs did not change notably when, instead of total energy intake, the models were adjusted for intake of macronutrients, protein, fat, and fiber (data not shown).

During the follow-up of 10 years, among subjects with no history of CVD at baseline ($n = 2{,}301$) altogether 137 incident CHD events appeared. In the multivariate analysis, high LPS was significantly associated with CHD events with a hazard ratio 1.90 (1.15–3.16, $p = 0.013$) (Table 4). The hazard ratio was not substantially changed when the model was adjusted for total energy intake (Fig. 2) or macronutrients (data not shown), or further, for prevalent obesity, MetS, or diabetes (Table 4).

Discussion

This large population-based study shows that endotoxemia is associated with prevalent cardiometabolic disorders, i.e.,

Table 3 Associations between risk factors and prevalent cardiometabolic disorders

	Obesity		MetS		Diabetes		CHD	
	OR (95 % CI)	*p* value	OR (95 % CI)	*p* value	OR (95 % CI)	*p* value	OR (95 % CI)	*p* value
Age (year)	1.02 (1.01–1.03)	<0.001	1.03 (1.02–1.04)	0.001	1.07 (1.05–1.09)	<0.001	1.12 (1.09–1.16)	<0.001
Male	2.11 (1.71–2.60)	<0.001	2.03 (1.65–2.51)	<0.001	1.37 (0.96–1.97)	0.084	3.33 (1.94–5.71)	<0.001
Education (year)	0.97 (0.95–0.99)	0.025	0.96 (0.94–0.99)	0.008	1.02 (0.98–1.07)	0.278	0.94 (0.87–1.0)	0.049
Current smoking	0.64 (0.52–0.80)	<0.001	0.88 (0.70–1.11)	0.281	0.95 (0.63–1.45)	0.817	1.29 (0.75–2.21)	0.360
Hypertension	2.04 (1.68–2.47)	<0.001	–	–	1.54 (1.05–2.25)	0.025	1.10 (0.67–1.81)	0.719
Cholesterol (mmol/l)	1.19 (1.08–1.30)	0.001	1.23 (1.13–1.35)	<0.001	0.83 (0.71–0.99)	0.034	0.63 (0.49–0.79)	<0.001
CRP (mg/l)	1.10 (1.06–1.15)	<0.001	1.07 (1.04–1.09)	<0.001	1.01 (0.98–1.04)	0.472	0.99 (0.95–1.03)	0.557
Energy (418 kJ or 100 kcal)	0.99 (0.98–1.01)	0.313	0.98 (0.96–0.99)	0.005	0.98 (0.96–1.01)	0.151	0.98 (0.95–1.02)	0.371
High LPS*	**1.49 (1.21–1.85)**	**<0.001**	**2.56 (1.97–3.32)**	**<0.001**	**1.01 (1.00–1.01)**	**0.032**	**1.94 (1.06–3.52)**	**0.031**

Logistic regression model

Obesity is defined according to the WHO BMI-based classification of adult overweight (≥25 kg/m^2) and obesity (≥30 kg/m^2). Lean subjects are compared with overweight and obese subjects

MetS is defined according to International Diabetes Federation guidelines

Data on CHD events and diabetes were collected from hospital discharge register and drug reimbursement records

Associations between high LPS and prevalent cardiometabolic disorders are in bold

* High LPS (LPS Q2–4 vs. Q1) in obesity, MetS, and CHD groups; per unit increase in LPS (pg/ml) in diabetes group

Table 4 Associations between risk factors and incident coronary heart disease events

	Incident CHD events													
	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	HR[a] (95 %CI)	*p* value	HR (95 %CI)	*p* value	HR (95 %CI)	*p* value	HR (95 %CI)	*p* value	HR (95 %CI)	*p* value	HR (95 %CI)	*p* value	HR (95 %CI)	*p* value
Age (year)	1.08 (1.06–1.11)	<0.001	1.08 (1.06–1.10)	<0.001	1.08 (1.06–1.10)	<0.001	1.08 (1.06–1.11)	<0.001	1.08 (1.06–1.10)	<0.001	1.08 (1.05–1.10)	<0.001	1.08 (1.05–1.10)	<0.001
Sex	2.54 (1.74–3.72)	<0.001	2.48 (1.70–3.62)	<0.001	2.61 (1.74–3.90)	<0.001	2.43 (1.66–3.57)	<0.001	2.64 (1.75–3.97)	<0.001	2.53 (1.73–3.70)	<0.001	2.73 (1.82–4.09)	<0.001
Education (year)	0.92 (0.87–0.98)	0.006	0.93 (0.88–0.98)	0.010	0.93 (0.88–0.98)	0.010	0.93 (0.88–0.98)	0.008	0.93 (0.88–0.98)	0.008	0.93 (0.88–0.98)	0.007	0.93 (0.88–0.98)	0.008
Current smoking	1.62 (1.1–2.39)	0.015	1.74 (1.18–2.58)	0.006	1.75 (1.18–2.58)	0.005	1.79 (1.21–2.66)	0.004	1.79 (1.21–2.66)	0.004	1.72 (1.16–2.54)	0.007	1.72 (1.17–2.55)	0.006
Hypertension[b]	1.37 (0.91–2.07)	0.132	–	–	–	–	1.21 (0.79–1.83)	0.382	1.20 (0.79–1.82)	0.390	1.34 (0.89–2.02)	0.167	1.33 (0.88–2.01)	0.171
Cholesterol (mmol/l)	1.01 (0.86–1.20)	0.901	0.99 (0.84–1.17)	0.917	0.99 (0.84–1.17)	0.939	1.00 (0.85–1.19)	0.980	1.01 (0.85–1.19)	0.943	1.02 (0.87–1.21)	0.806	1.72 (1.17–1.21)	0.803
CRP (mg/l)	1.03 (1.01–1.06)	0.009	1.03 (1.00–1.05)	0.058	1.03 (1.00–1.05)	0.057	1.03 (1.00–1.05)	0.034	1.03 (1.00–1.05)	0.037	1.03 (1.01–1.06)	0.007	1.03 (1.01–1.06)	0.007
High LPS[c]	**1.90 (1.15–3.16)**	**0.013**	**1.69 (1.01–2.81)**	**0.045**	**1.68 (1.01–2.80)**	**0.047**	**1.81 (1.09–3.00)**	**0.022**	**1.79 (1.07–2.97)**	**0.026**	**1.82 (1.10–3.03)**	**0.020**	**1.81 (1.09–3.02)**	**0.022**
Energy (418 kJ or 100 kcal)	–	–	–	–	0.99 (0.96–1.02)	0.494	–	–	0.98 (0.96–1.01)	0.276	–	–	0.99 (0.96–1.01)	0.302
MetS[d]	–	–	2.24 (1.56–3.21)	<0.001	2.20 (1.53–3.17)	<0.001	–	–	–	–	–	–	–	–
Diabetes	–	–	–	–	–	–	–	–	–	–	2.65 (1.70–4.14)	<0.001	2.60 (1.66–4.07)	<0.001
Overweight[e]	–	–	–	–	–	–	1.85 (1.10–3.14)	0.021	1.83 (1.08–3.10)	0.024	–	–	–	–
Obese[e]	–	–	–	–	–	–	2.36 (1.36 (4.12)	0.002	2.32 (1.33–4.04)	0.003	–	–	–	–

Associations between high LPS and incident coronary heart disease events are in bold

[a] Cox regression model. During the follow-up of 10 years, among subjects with no history of CVD at baseline ($n = 2{,}301$) altogether 137 incident CHD events appeared

[b] Treated hypertension combined with systemic blood pressure

[c] The highest three LPS quartiles compared with the lowest quartile

[d] MetS is defined according to International Diabetes Federation guidelines

[e] The WHO BMI-based classification of adult overweight ($\geq$25 kg/m^2) and obesity ($\geq$30 kg/m^2)

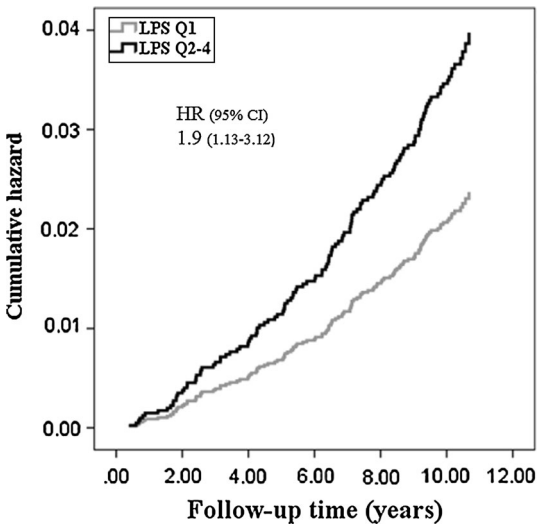

Fig. 2 Cumulative hazard for incident CHD events. The cumulative hazard in LPS quartiles (Q1 in *gray* vs. Q2–4 in *black*) was analyzed by Cox regression model adjusted for age, sex, education years, current smoking, cholesterol and CRP concentrations, hypertension, and energy intake in the follow-up of 10 years. The CHD events included subjects with myocardial infarction, coronary death, coronary bypass surgery, or percutaneous transluminal coronary angioplasty

obesity, MetS, diabetes, and CHD independently of established cardiometabolic risk factors and factors known to affect serum endotoxin activity (age, sex, cholesterol, BMI, CRP). Interestingly, we showed that the association is also independent of energy or macronutrients. In addition, high LPS activity is associated with an increased risk of incident CHD events independently of baseline cardiometabolic disorders, risk factors, or macronutrients. Previously, we have reported in the population-based FINRISK 1997 Study that endotoxemia is associated with increased risk for incident diabetes [6]. In concordance, the present nutrition subsample of the FINRISK 1997 Study showed also a direct association between high LPS levels and incident diabetes independently of nutrient intake.

In mice, feeding studies have shown an influence of the diet on circulating endotoxin levels [3]. In mice grown in germ-free environment, endotoxemia has been associated with the onset of insulin resistance, weight gain, and low-grade inflammation following high-fat diet. In humans, similar observations have been reported with relatively small intervention studies carried out in controlled environment. A Western-style high-fat diet seems to result in gut dysbiosis, an altered composition of the microbiota, which may disrupt the intestinal barrier leading to translocation of LPS into circulation via increased gut permeability or secretion with chylomicrons [3, 26]. Present lifestyle including a high-fat and energy-rich diet induces endotoxemia and furthermore low-grade inflammation compared with no meal or a meal rich in fruit and fiber [2, 14]. Both glucose intake and cream intake have been shown to induce inflammation and insulin resistance, while only intake of cream elevated plasma LPS concentrations [27]. In contrast, the intake of an equivalent amount of carbohydrate as orange juice caused neither inflammation nor endotoxemia in the same study. Indeed, the orange juice seems to neutralize the pro-inflammatory effect of a high-fat, high-carbohydrate meal and prevent endotoxemia and insulin resistance [28]. These observations suggest the concept of nutrition as a potential modulator of postprandial endotoxemia and inflammation. Large studies with data on nutrition, metabolic state, and endotoxemia are scarce. Therefore, in the present study, we focused to investigate the association of LPS with cardiometabolic disorders, taking into account data on the energy and macronutrient intake.

In the present study, we found that endotoxemia associated directly with daily energy but indirectly with carbohydrate intake. This is in agreement with previous studies [13], but deviating from the feeding trials [2, 14, 29], since no association was found between endotoxemia and fat intake either in univariate or in multivariate analyses. These associations were clearly dependent on the metabolic conditions, since in any of the cardiometabolic disorders studied separately no association between endotoxemia and energy or carbohydrate intake was observed. Harte et al. [15] showed that subjects with compromised metabolic state had a stronger acute endotoxemia response to high-fat diet than metabolically healthy subjects. A very recent study showed no significant impact of acute fat intake to endotoxin activity in healthy subjects or patients with type 1 diabetes, suggesting that metabolic endotoxemia may be more substantive in patients with chronic metabolic disorders [30]. However, the design in the feeding trials compared to the present one is totally different, because we have merely measured the fasting period endotoxemia and tried to avoid the effect of postprandial status. Despite of the results, the intake of energy or macronutrients did not affect the association of high LPS activity with the cardiometabolic disorders. The reported energy and macronutrients intake values between the cardiometabolic groups had relatively minor variation, although there were some statistically significant differences. The significant disparity might be due to the dietary counseling for patients diagnosed, e.g., for diabetes.

The FINRISK 1997 is an extensive study with a prospective design, well-determined risk factor data for multivariate adjustments, and long follow-up time. Yet, as a limitation, the information on macronutrient intake was collected from the 24-h dietary recall, which cannot provide information on long-term diet and may be affected by causal diet variations during the registration period. To

reduce misclassification, we excluded subjects if the reported total energy intakes were less or greater than 3xSD from the mean energy intake of the population [25].

The mean LPS activity of the study population (63.6 EU/ml) was on the same level as found in Finnish healthy blood donors (35.9 EU/ml) [31] and the middle-aged subjects (122.8 EU/ml) [4]. Again, mean LPS concentration of 6.7 pg/ml has been reported in the healthy elderly [32] and 850 pg/ml in patients with Gram-negative sepsis [33]. It is problematic to compare results of the endotoxin measurements between different studies due to the variety of units reported, assay kits, standardizations, and even among the assay lots available. The origin of serum endotoxin activity remains unknown, since the limulus assay is not specific to any bacterial species, and endotoxin activity diverges even between different bacterial clones. Generally, endotoxins from anaerobic bacteria, commonly found in the gut and oral cavity, are more reactive in the limulus assay than, for example, *E. coli* [34], which in most assay kits is used as a standard. Therefore, measuring endotoxemia with the limulus assay mirrors the exposure only to certain, but unknown, mixture of LPS.

Although metabolic endotoxemia probably plays a significant role in circulating LPS levels, the most likely sources of endotoxins are chronic infections by Gram-negative microbes, such as periodontal pathogens. Periodontitis is a common chronic oral infection of multiple, mostly Gram-negative anaerobic bacterial species, such as *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans,* and *Porphyromonas gingivalis*. It affects, especially the middle-aged and elderly, and gives rise to bacteremia and endotoxemia, which are more frequent than previously thought. LPS may travel to the bloodstream through inflamed periodontal tissue during daily routines, e.g., tooth brushing, or via saliva to gastrointestinal tract [35, 36]. Through binding to pathogen-sensing system, LPS induces release of a large number of inflammatory cytokines, which play an important role in metabolic processes. Clinical periodontitis itself has been associated with incident cardiovascular disease events and type 2 diabetes [37, 38]. Similarly as in the most studies exploring the relation between endotoxemia and diet, in the present study, the periodontal status of the subjects was not examined.

Altogether, our earlier studies [4, 6, 7] and the present data show that high serum LPS activity is strongly associated with cardiometabolic disorders and the risk of future CHD events. In addition to metabolic endotoxemia, the results support the role of bacterial infections and immune response in the etiology of cardiometabolic diseases. Although energy intake was correlated with endotoxemia in healthy subjects, the overall associations were independent of macronutrient intake.

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Conflict of interest Elisa Kallio, Katja Hätönen, Markku Lehto, Veikko Salomaa, Satu Männistö, and Pirkko Pussinen declare that they have no conflict of interest.

Human and animal rights disclosure All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975.

Informed consent disclosure Informed consent was obtained from all patients for being included in the study.

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