Elevated high density cholesterol and low grade systemic inflammation is associated with increased gut permeability in normoglycemic men

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ABSTRACT

Background & Aims: Serum lipids and lipoproteins are established biomarkers of cardiovascular disease risk that could be influenced by impaired gut barrier function via effects on the absorption of dietary and biliary cholesterol. The aim of this study was to examine the potential relationship between gut barrier function (gut permeability) and concentration of serum lipids and lipoproteins, in an ancillary analysis of serum samples taken from a previous study.

Methods and Results: Serum lipids, lipoproteins and functional gut permeability, as assessed by the percentage of the urinary recovery of ^{51}Cr-labelled EDTA absorbed within 24h, were measured in a group of 30 healthy men. Serum lipopolysaccharide, high sensitivity C-reactive protein and interleukin-6 were also measured as markers of low-grade inflammation. The group expressed a 5-fold variation in total gut permeability (1.11 - 5.03%). Gut permeability was unrelated to the concentration of both serum total and low density lipoprotein (LDL)-cholesterol, but was positively associated with serum high density lipoprotein (HDL)-cholesterol (r=0.434, *P*=0.015). Serum HDL-cholesterol was also positively associated with serum endotoxaemia (r=0.415, p=0.023).

Conclusion: The significant association between increased gut permeability and elevated serum HDL-cholesterol is consistent with the role of HDL as an acute phase reactant, and in this situation, potentially dysfunctional lipoprotein. This finding may have negative implications for the putative role of HDL as a cardio-protective lipoprotein.

INTRODUCTION

Impaired gut barrier function resulting from increased gut permeability ('leaky gut'), is a condition in which the intestinal barrier is physically breached by insults that disrupt tight junctions, causing a translocation of luminal contents, including bacteria and bacterial endotoxins e.g. lipopolysaccharides (LPS), that can produce a low grade systemic inflammation (1, 2). While these phenomena have been described almost exclusively in animal models (3), they have been reported by us in patients with type-2 diabetes (4), and by others in obesity, metabolic syndrome and the pathogenesis of other human diseases (5, 6).

Raised serum LDL-cholesterol and low serum HDL-cholesterol (< 1mmol/L), are established biomarkers of increased CVD risk (7), though whether raising HDL reduces CVD risk is still not clear, since the cardio-protective role of HDL depends on its functional capacity to prevent atherosclerosis, and not on its cholesterol content (8). The concentration of serum LDL-cholesterol is regulated, in part, by a reciprocal cross-talk between the intestinal absorption of dietary and biliary cholesterol, and hepatic biosynthesis of cholesterol (9). The absorption of cholesterol in the gut lumen is regulated by transport proteins in the apical cell membrane of enterocytes that control the influx and efflux of cholesterol. This mechanism is sensitive to the quantity of cholesterol in the gut lumen, and is down-regulated as the content of cholesterol in the gut lumen increases (10, 11). One possible effect of this mechanism is to limit the supply of cholesterol, chiefly to the liver, which could upregulate the cellular uptake of LDL, and lower serum LDL-cholesterol.

We hypothesised that increased intestinal paracellular permeability could disrupt cholesterol homeostasis by interfering with the feedback suppression of dietary and biliary cholesterol absorption in the gut, which normally serves as a mechanism to prevent an oversupply of cholesterol for lipoprotein synthesis. It is also possible that local inflammation could affect the balance of cholesterol transport into and out of enterocytes via the down-regulation of cholesterol efflux proteins (ABCA1, ABC G1), as seen in macrophages treated with bacterial endotoxins (12). These effects could manifest as an increase in serum LDL-cholesterol in response to the altered absorption of cholesterol in the gut lumen. Low grade systemic inflammation associated with bacterial LPS-induced endotoxaemia has

also been linked to reduced serum HDL-cholesterol and the production of dysfunctional HDL, in metabolic syndrome, type-2 diabetes, and chronic arthritis (13-15). The purpose of this study was to determine whether variation in gut barrier function is related to the concentration of serum lipids and lipoproteins in normal healthy men, at low risk of CVD.

METHODS

Design: A cross-sectional study to evaluate possible links between gut permeability and serum lipids and lipoproteins, by the secondary analysis of serum samples from healthy men who acted as controls in a previous study in diabetes (16). The original protocol was approved by the Central London NRES Committee (REC reference no. 11/LO/1141) and the University of Surrey Ethics Committee, and was conducted according to the declaration of Helsinki.

Study Population: Thirty men, mean age 56 years (range 43-67 years) were recruited through primary care and volunteer databases at the University of Surrey. All subjects provided written informed consent. Exclusion criteria included use of antibiotics in the previous three months, use of anti-inflammatory medications, diuretics, proton-pump inhibitors, inflammatory bowel disease, Crohn's disease, coeliac disease, irritable bowel disease or a positive family history of type 2 diabetes. Prior renal function was assessed to ensure suitability for the administration of a radioisotope (eGFR > 60 mL/min/1.73 m^2) (17). The cohort included men who were obese (n=3), over weight (n=13) and normal body weight (n=14). The cardio-metabolic risk of participants was assessed by a scoring system based on a modified version of the Adult Treatment Panel III guidelines, as previously described for the 'RISCK' study in healthy men aged 30-70 years. The mean cardio-metabolic risk score, as a measure of predisposition to the development of metabolic syndrome (≥4), was 1.5 (range 0-6) (18).

Blood pressure, anthropometrics and biochemistry: Body weight, body mass index (BMI) and body composition were measured by bio-impedance (Tanita, Arlington Heights, IL, USA), following a 10 hour overnight fast and emptying of the bladder. Waist circumference was measured at the level of the umbilicus. After 5 minutes rest, blood pressure was determined as the mean of three readings (Omron MX3 Plus, Omron Healthcare Europe, Milton Keynes, United Kingdom). Blood was taken from an ante-cubital vein and collected into serum tubes containing EDTA and clotting activator, and pyrogen free tubes for measurement of serum biomarkers of inflammation, lipids and LPS. Blood samples were centrifuged at 3000 x g at 4°C for 10 minutes and serum and plasma stored at -20°C or -80°C as appropriate.

Dietary intakes: This was assessed by a 7-day diet diary. Subjects were instructed to avoid probiotic food items, fermented foods such as yogurt, soft cheese, kimchi, in addition to prebiotic/probiotic supplements for two weeks before the test day. Diet diaries were analysed by a single operator, using DietPlan6 (Forestfield Software Ltd, Horsham, UK).

Intestinal permeability: Intestinal permeability was functionally assessed by the 24h urinary excretion of orally administered ^{51}Cr-EDTA, as previously described (4, 16, 17, 19). The ^{51}Cr-EDTA tracer was administered after an overnight fast, after which, participants were asked to collect all of their urine for the next 24 hours. The urine was collected into one container for the first 6 hours and into a separate container between 6-24 hours. The percentage recovery ^{51}Cr-EDTA between 0-6h and 6-24h was taken as a measure of gut permeability in the small and large intestine, respectively. The total permeability of the gut was taken as the 0-6 and 6-24h samples combined.

Biochemical Analyses: Serum high sensitivity C-reactive protein (hsCRP) was measured by an accredited laboratory (The Surrey Pathology Partnership), interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) were measured in-house, using a Luminex platform and Biorad bio-plex kits and software. The limit of detection were 0.03mg/l for hsCRP, 5 pg/mL for TNF-α and 0.7pg/ml for IL-6 with average intra-assay CVs were 1.4%, 0.6% and 1.0%, respectively. Plasma triglycerides (TG), total cholesterol, HDL cholesterol, and non-esterified fatty acids (NEFA) were measured on an ILab650, using commercially available kits (Randox Laboratories, UK, and Instrumentation Laboratory, UK). Mean intra-assay CVs were 1.4%, 1.9%, 0.6% and 1.0% and inter-assay CVs were 1.9%, 3.0%, 1.1% and 1.8% for TG, total cholesterol, HDL-cholesterol, and NEFA, respectively. Serum LDL-cholesterol was calculated using the Friedewald formula. Serum LPS was measured in duplicate using Endosafe-MCS (Charles River Laboratories, Lyon, France), as previously described (20). Samples were diluted from 1/20 to 1/200 and validated for recovery using positive product control (PPC) samples to account for sample losses due to interfering factors. The recovery value of the PPC samples was 70-150% and the %CV for the PPC and serum samples was <25%. This level is above that validated by the FDA for LPS quantification. Serum LPS binding protein (LBP) and

sCD14 concentrations were measured using a solid-phase enzyme-linked immunosorbent assays according to the manufacturer's instructions (Hycult Biotechnology, Uden, the Netherlands). Sera were diluted 1/10 with the appropriate buffer and homogenized by vortex before further dilution to 1/200 (sCD14) and 1/1000 (LBP). The detection limits were 4.4 and 1.56 ng/mL and the average intra-assay CVs were 3.9% and 8.5%, and inter-assay CVs were 19.6% and 15.5% for LBP and sCD14, respectively.

Statistical analysis

Associations between normally distributed anthropometric data, metabolic parameters and serum lipoproteins in the whole group, were examined by calculating Pearson's product moment correlation coefficients (r) on raw data. Associations with serum LPS were determined using Spearman's coefficient of rank correlation. Univariate associations between anthropometric, metabolic and dietary variables were examined by a univariate linear regression model, with HDL-cholesterol as the dependent variable. The group was also subdivided into two groups on the basis of serum HDL-cholesterol being above, and below or equal to 1 mmol/L, a clinically recognised cut-off that discriminates CVD risk associated with HDL-cholesterol (21). Between-group differences for normally distributed data were compared using an independent sample *t*-test, with correction for multiple parameter testing. The distribution of serum LPS was not normally distributed and could not be normalised by log transformation, so these data were analysed using a Mann-Whitney U test. A probability level of $\leq 5\%$ was considered significant.

RESULTS

Gut permeability of the large and small intestine combined, showed a greater than 5-fold difference across the group (1.11 to 5.03%). Gut permeability was unrelated to body weight, BMI, serum biomarkers of inflammation and plasma total cholesterol. In contrast, plasma HDL-cholesterol was correlated with intestinal permeability in both the small and large intestine (r= 0.390, P=0.032; r=0.370, P= 0.043; **Figure 1A**). Gut permeability of the small intestine was also significantly correlated with serum TG (r=-0.386, P= 0.035). There was a trend for an association between serum LDL-cholesterol and gut permeability in the small intestine (r= -0.354, P=0.078**, Figure 1B**), and evidence of a much wider distribution of serum TG values at a gut permeability level of less than 1.5% **(Figure 1C)**.

The group expressed a broad range of plasma lipid and lipoprotein concentrations, the mean values of which fell below clinically recognised action limits for these variables **(Table 1)**. There were also subgroups with LDL-cholesterol that would be considered 'borderline high, to high and undesirable' (3.4 - 4.1mmo/l, n=15/30) and 'very high' (>4.9mmol/l, n=3/30). The serum concentration of inflammatory biomarkers were also within their normal reference ranges, however, serum LPS and HDL were highly correlated in the whole group (**Figure 2B**). Subdivision of the group on the basis of a plasma HDL-cholesterol above and below 1mmol/L, a clinically recognised threshold, below which CVD risk is increased significantly **(Table 1)**, also showed serum LPS to be higher in men with raised HDL-cholesterol (P=0.023, **Figure 2A)**. Subdivision of the group also strengthened the significance of the associations between HDL-cholesterol and total, small and large intestinal permeability. All of the directly measured (rather than calculated) variables were examined together by univariate linear regression (**Table 2**). After adjustment for the other variables, the relationship between HDL-cholesterol and the 0-6 h recovery of urinary ^{51}Cr-EDTA and serum LPS remained significant at p=0.004 and p=0.036, respectively.

The mean recorded dietary intakes for the group failed to meet current UK E% dietary recommendations for total carbohydrate (42.4 ± 1.46% vs > 50%), non-milk extrinsic sugars (18.3 ± 1.29% vs <11%), total fat (37.5 ± 1.62% vs <35%), saturated fat (13.6 ± 0.69% vs <11%), and total

dietary fibre (10.4 ± 0.56g vs ≥30g) (22). The protein intake for the group was 14.8 ± 0.48 E%, although it was not possible to differentiate between animal and plant sources. There were significant positive correlations between total protein intake and markers of inflammation (LPS r=0.427, P=0.019; hsCRP r=0.567, P=0.001; and sCD14 r=0.581, P=0.001). Mean alcohol intake for the group was 13.6 ± 1.68g/day (individuals initially reporting intakes of alcohol at recruitment above the then UK guideline recommendation of 21 units (24g alcohol/day) were excluded from the study). Alcohol intake was also correlated with increased markers of inflammation even at the modest levels reported, (hsCRP r=0.445, P=0.015; and sCD14 r=0.442, P=0.016). There were no significant differences in dietary intakes between low and high HDL-cholesterol groups or any bivariate correlations with HDL-cholesterol. However, total carbohydrate, protein and fat were associated with HDL-cholesterol in univariate analysis (**Table 2**). The mean reported energy intake for the group was 9.8 ± 0.36 MJ/day with a mean Reported $Energy_{INTAKE}$: BMR of 1.4 (SEM 0.06) as an index of under-reporting, using predicted BMR (23).

DISCUSSION

Increased gut permeability/impaired gut barrier function, secondary to dysbiosis, is emerging as an early underlying cause of metabolic diseases that may be associated with weight gain and obesity through overfeeding, most notably with dietary fat (24). Abnormally high gut permeability (≥ 2.5%), at levels typically associated with coeliac disease (active > ~4.7%; in remission >~3.0% (25)), inflammatory bowel disease (>~3.47% (26)), non-alcoholic fatty liver disease (NAFLD > ~3.14% (27)) and type 2 diabetes (> ~3.60%) (4), has been linked to the susceptibility of developing these diseases. To the best of our knowledge, the present study provides the first evidence for marked variability in normal gut-barrier function, as measured by differences in gut permeability *in vivo* in men, without current or family history of diabetes or gastrointestinal disease.

The underlying rationale for this study was that gut barrier function may be linked to the concentration of serum lipoproteins, and specifically LDL-cholesterol, by influencing the absorption of dietary and biliary cholesterol. However, there was no evidence from the wide distribution of variables to suggest that increased gut permeability or serum LPS were related to serum LDL-cholesterol. While this finding comes with the caveat that the sample size in this ancillary analysis limited the statistical power, calculated retrospectively as 61% (2-sides, α 0.05), it suggests that barrier function was not compromised, at least to an extent that could produce adverse effects on cholesterol homeostasis in the gut. In contrast, increased gut permeability and serum LPS were unexpectedly associated with elevated plasma HDL-cholesterol, even with the sample size limitation. This finding could be viewed as paradoxical, in that impaired barrier function and local systemic inflammation are commonly found in obesity, metabolic syndrome and type-2 diabetes (1-3), all of which are characterised by a low HDL-cholesterol. Indeed, studies in those with confirmed metabolic diseases such as type 2 diabetes and NAFLD, report an inverse relationship between serum zonulin (modulates intestinal permeability by disassembling intercellular tight junctions between epithelial cells (28)) and HDL-cholesterol (29, 30). However, it should be noted that in our study the ^{51}Cr-EDTA test represents a functional measure of paracellular permeability only and that other biomarkers such as serum zonulin and diamine oxidase (DAO) (31) or faecal levels of alpha-1-

antitrypsin and albumen which have all been shown to correlate with functional intestinal permeability, as reviewed by (32), may have added further information on the mechanism and/or site of the disruption.. The use of inert probes to assess functional permeability has the advantage in that recovery is complete as the label is not metabolised or sequestered, however recovery would be affected by human factors such as delayed gastrointestinal motility or a reduced mucosal blood flow. In healthy individuals this may be less of a confounder than in gastrointestinal disease patients. In the future DAO would need to be used as more specific marker of small intestinal integrity, as in the gut, the enzyme is localised to the tip of the enterocyte villi.

Since our observation was in men with a wide range of serum cholesterol levels, the associations with elevated HDL might represent a distinct, subclinical phenomenon, that is expressed at the upper end of the range of gut permeability. A positive association between HDL-cholesterol and intestinal permeability has been reported recently in pregnant women (33). While this finding was linked specifically to maternal lipid metabolism, it provides further evidence in support of this association being an *in vivo* phenomenon in otherwise healthy individuals.

Up to 30% of the steady-state serum HDL-cholesterol pool may be derived from the small intestine, but whether the contribution of this gut-derived HDL-cholesterol confers any benefit to cardiovascular health is unknown, since this will depend on the functional properties of HDL in preventing cardiovascular atherosclerosis, and not on the serum concentration of HDL-cholesterol.

Serum HDL-cholesterol was first established as a discriminating biomarker of CVD risk in large prospective cohort studies in the 1970s, with a concentration of HDL-cholesterol below 1mmol/L being used subsequently as a metric to forecast the 10 year risk of suffering a cardiovascular event (21). Conversely, there is much less evidence that raising serum HDL-cholesterol reduces CVD risk, primarily because not all HDL is equivalent in its functional capacity to confer protection against CVD (8). Intestinal permeability regulates molecular trafficking between the intestinal lumen and the

submucosa, and high levels of cytokines further contribute to intestinal barrier dysfunction by altering the structure and localisation of tight junctions (34). The gut lumen is a prominent source of persistent immune-stimulation which can result in sub-clinical mucosal inflammation through the transfer of LPS and other bacterial products (3). The significant associations between increased gut permeability, gut-derived LPS and plasma HDL-cholesterol in the present study, suggest that the elevated HDL may be an acute phase reactant, produced in response to local inflammation in the gut, since its principal apoprotein, apo A-I, is synthesised in the gut and recognised as an acute phase protein (15). Low doses of LPS (3ng/kg) given to humans have been shown to increase acute phase proteins e.g. serum amyloid A (SAA), CRP, apo A-I. In mammals, SAA is the major acute phase reactant that is transported in serum HDL. Increased SAA in HDL under inflammatory conditions has been implicated in reducing the functional capacity of HDL, which includes its ability to inhibit inflammation and promote cholesterol efflux (35-37). However, since the chronic low-grade inflammation found in metabolic disease and systemic infection are usually associated with low serum HDL-cholesterol, this suggests that inflammation that is localised in the gut, may have a unique effect in stimulating the synthesis of apo A-I and HDL.

The evidence for an inverse association between intestinal permeability and serum TG (Figure 2C) was created largely by the data of participants with serum TG close to or above 1.5mmol/l. This is recognised as a critical threshold for serum TG, above which serum TG becomes associated with dyslipidaemia, dysglycaemia and insulin resistance (38). Because serum TG is regulated by many factors, it is possible that its concentration in serum, especially above 1.5mmol/l, may not be related directly to intestinal permeability, but acting as a marker for an as yet unidentified factor. An inverse association between intestinal permeability and serum TG also seems counterintuitive, especially in light of the evidence for increased intestinal permeability in type 2 diabetes (4).

High-fat diets have consistently been linked with metabolic endotoxaemia and metabolic disease, partly due to the translocation of LPS across the enterocyte during chylomicron formation after fat-feeding (39). Dietary saturated fat is also associated with increased serum HDL-cholesterol, possibly by stimulating the synthesis of apo A-I in the small intestine (40). There was no evidence in the

present study of associations between the intake of saturated fat and gut permeability or inflammatory markers, but protein intake showed significant positive associations with LPS and sCD14, and a trend for an association with LBP. CD14 and LBP make-up integral parts of the LPS cellular receptor, increasing the likelihood of an LPS-induced pro-inflammatory cascade. Protein intake was also significantly associated with hsCRP, a clinical marker of immune activation. The products of the colonic proteolysis of dietary protein (ammonia and phenol) have been shown to impact on the expression of tight junction proteins, reducing gut barrier integrity *in vitro* (41). In humans, high phenol levels have been linked to disruption of the gut barrier (42), which provides evidence to implicate dietary protein as a possible cause of the local inflammation.

CONCLUSION

This study provides new evidence for significant variation in gut permeability in men without metabolic or gastrointestinal disease, but no evidence to support a link between this phenomenon and variation in serum LDL cholesterol. The positive associations between increased gut permeability, serum HDL-cholesterol and LPS are consistent with HDL being an acute phase reactant to local gastrointestinal inflammation. These findings may have negative implications for the role of HDL as a biomarker of CVD risk, but need to be confirmed in future studies that measure acute phase reactants in HDL such as SAA, and HDL function. The potential for dietary macronutrients to be an underlying cause of this pro-inflammatory cascade is in accord with epidemiological evidence to link diet with metabolic disease through a putative, gut-dependent mechanism.

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Table 1. Fasting metabolic markers showing bivariate correlation with serum HDL-cholesterol.

	Cohort mean (range)	Low HDL (≤1.0mmol/l) n=9	High HDL (>1.0mmol/l) n=21	Between Group P-value	Correlation with HDL (r)	P Value
HDL Cholesterol (mmol/l)	1.26 (0.54-2.09)	0.87 (0.05)	1.44 (0.06)	<0.001		
BMI (kg/m^2)	25.6 (19.5 – 36.2)	26.7 (1.21)	25.2 (0.96)	ns	-0.403	0.027
LDL Cholesterol (mmol/l)	3.41 (1.98-4.96)	3.20 (0.25)	3.50 (0.16)	ns	-0.53	ns
Total Cholesterol (mmol/l)	5.12 (3.0-6.85)	4.58 (0.29)	5.35 (0.17)	0.026	0.430	0.018
Fasting Triacylglycerol (mmol/l)	0.98 (0.53-2.11)	1.08 (0.18)	0.90 (0.04)	ns	-0.251	ns
Intestinal Permeability (% 0-6h ^{51}Cr-EDTA recovery)	1.40 (0.34 – 2.58)	1.08 (0.18)	1.54 (0.14)	0.056	0.392	0.032
Intestinal Permeability (% 6-24h ^{51}Cr-EDTA recovery)	2.85 (1.11-5.03)	2.27 (0.26)	3.11 (0.20)	0.023	0.432	0.016
Serum lipopolysaccharide (EU/mL)	0.78 (0.25-1.8)	0.45 (0.15)	0.85 (0.07)	0.028*	0.410*	0.023
Lipolysaccharide binding protein (µg/mL)	11.2 (4.24-26.6)	10.4 (1.52)	11.4 (1.06)	ns	-0.082	ns
sCD14 (µg/mL)	1.06 (0.56-2.23)	0.99 (0.10)	1.09 (0.08)	ns	0.231	ns
Interleukin-6 (pg/mL)	19.6 (0.3-91.55)	21.8 (7.32)	19.5 (4.55)	ns	0.238	ns
Tumour necrosis factor alpha (pg/mL)	11.8 (5.0-72.21)	11.7 (2.88)	12.2(3.59)	ns	0.099	ns
hsCRP (mg/L)	2.26 (0.16-5.37)	1.21 (0.36)	2.75 (1.25)	ns	0.004	ns
Fasting plasma glucose (mmol/l)		4.5 (0.12)	4.7 (0.14)	ns	0.287	ns

*Data for LPS could not be normalised by log-transformation and so non-parametric analysis was used.

Table 2 Univariate linear regression of anthropometric, dietary and metabolic variables with HDL-Cholesterol as dependent variable, adjusted $r^2 = 0.875$, p=0.10

Co-variate	B	SE	Beta	P Value
Age	-0.002	0.008	-0.41	0.797
Weight	0.008	0.010	.295	0.446
Waist Circumference	-0.018	0.18	-0.565	0.366
Systolic blood pressure	-0.025	0.16	-0.789	0.176
Diastolic blood pressure	0.005	0.10	0.116	0.602
Glucose	-0.251	0.147	-0.365	0.148
NEFA	-0.091	0.286	-0.052	0.764
Total cholesterol	0.330	0.083	0.757	0.010
Triglyceride	-0.368	0.189	-0.343	0.110
Lipopolysaccharide	0.231	0.081	0.431	0.036
hsCRP	-0.030	0.029	-0.378	0.346
LBP	-0.011	0.16	-0.129	0.532
CD14	0.473	0.188	0.435	0.053
IL-6	-0.007	0.002	-0.367	0.037
TNF-α	0.019	0.009	0.700	0.087
Insulin	0.038	0.012	0.797	0.027
Dietary total carbohydrate	-0.095	0.017	-1.557	0.003
Dietary protein	-0.122	0.042	-0.566	0.035
Dietary fat	-0.079	0.120	-1.219	0.001
Dietary fibre	-0.10	0.004	-0.242	0.068
Alcohol intake	0.000	0.007	-0.010	0.954
Small intestinal permeability	0.368	0.073	0.593	0.004
Large intestinal permeability	-0.266	0.182	-0.312	0.204

Figure 1

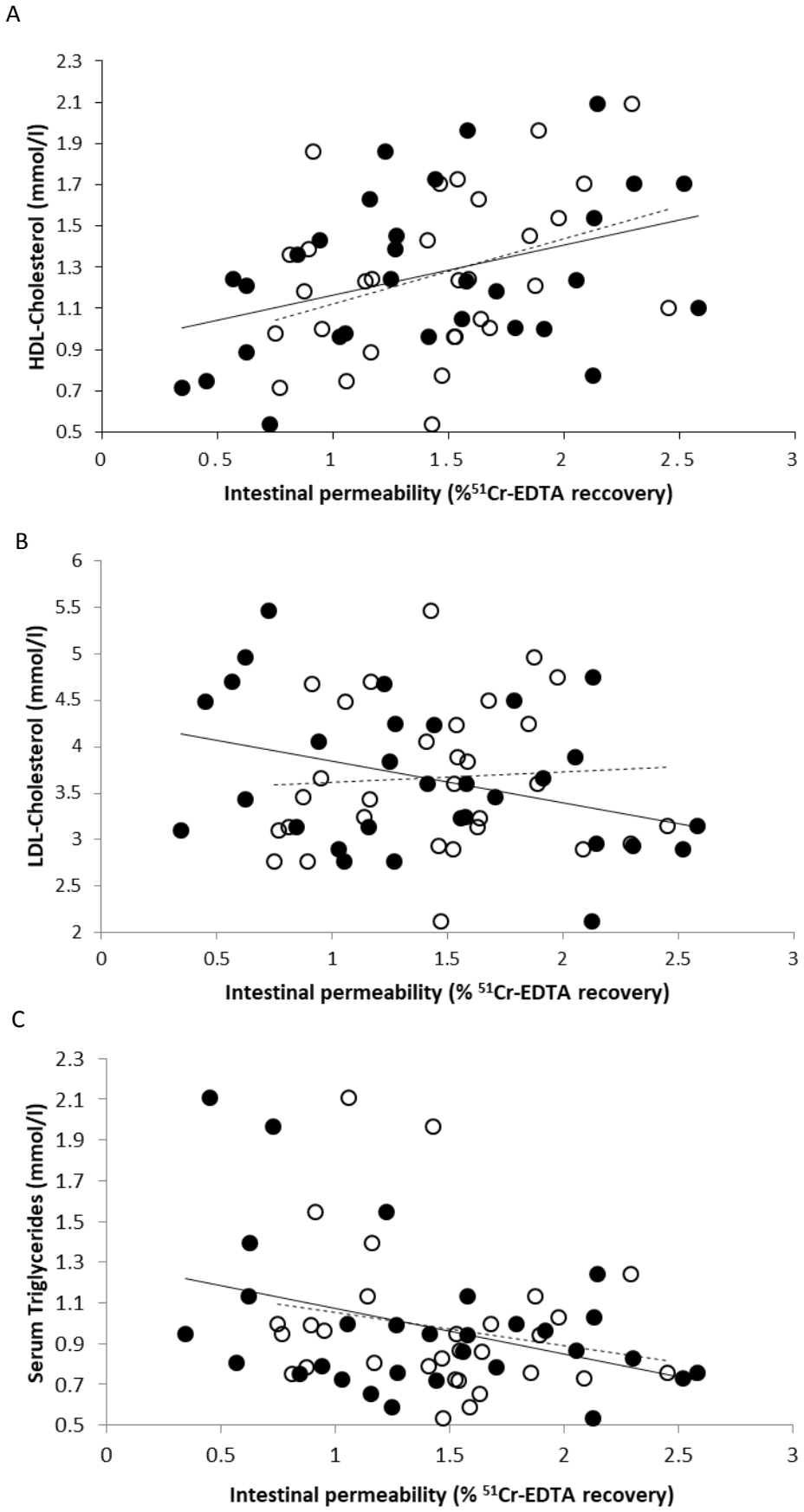
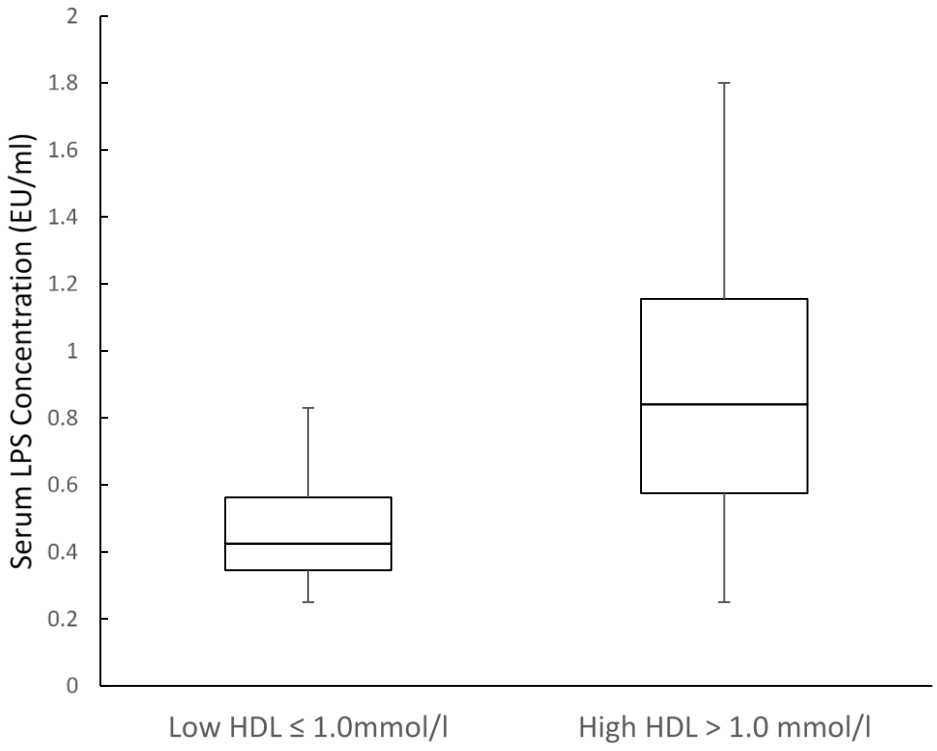

A
HDL-Cholesterol (mmol/l)
Intestinal permeability (%51Cr-EDTA reccovery)
B
LDL-Cholesterol (mmol/l)
Intestinal permeability (% 51Cr-EDTA recovery)
C
Serum Triglycerides (mmol/l)
Intestinal permeability (% 51Cr-EDTA recovery)

Figure 2

A


Serum LPS Concentration (EU/ml)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Low HDL ≤ 1.0mmol/l
High HDL > 1.0 mmol/l

B

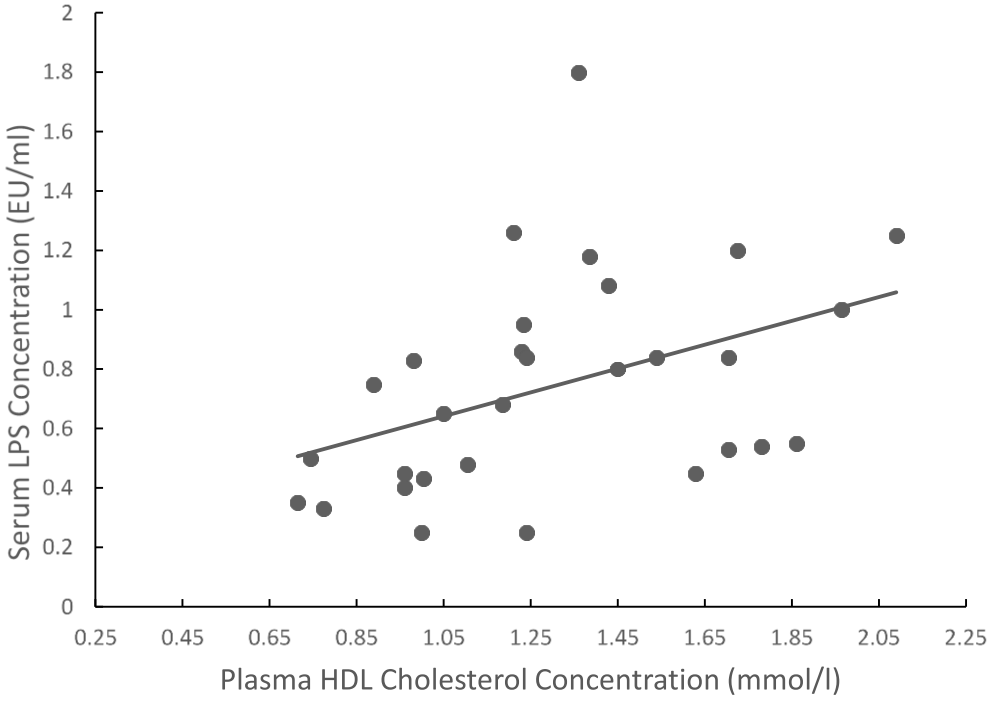

Serum LPS Concentration (EU/ml)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0.25
0.45
0.65
0.85
1.05
1.25
1.45
1.65
1.85
2.05
2.25
Plasma HDL Cholesterol Concentration (mmol/l)

Figure Legends

Figure 1A . Bivariate correlation between intestinal permeability and fasting HDL cholesterol, n=30. Small intestine, 0-6h recovery (closed circle, solid line) r=0.389, p=0.032; large intestine, 6-24h recovery (open circle, dashed line) r=0.372, p=0.043.

Figure 1B. Bivariate correlation between intestinal permeability and fasting LDL-cholesterol, n=30. Small intestine, 0-6h recovery (closed circle, solid line) r=-0.354, p=0.078; large intestine, 6-24h recovery (open circle, dashed line) r=0.065, p=0.622

Figure 1C. Bivariate correlation between intestinal permeability and fasting plasma triglyceride, n=30. Small intestine, 0-6h recovery (closed circle, solid line) r=-0.386, p=0.035; large intestine, 6-24h recovery (open circle, dashed line) r=-0.205, p=0.278

Figure 2A. Box plot of plasma lipopolysaccharide concentrations in non-diabetic men with low (≤ 1mmol/l, n=9) or high (> 1mmol/l, n=21) HDL-cholesterol levels. Boxes represent the 25^{th} and 75^{th} percentiles, bands inside the box is the median, and the whiskers display the full ranges (from minimum to maximum), p=0.028.

Figure 2B. Spearman's coefficient of rank correlation between plasma HDL-cholesterol and plasma lipopolysaccharide concentration, n=30, p=0.023.