Relationships between cholesterol homoeostasis and triacylglycerol-rich lipoprotein remnant metabolism in the metabolic syndrome

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ABSTRACT

The dysmetabolic syndrome of insulin resistance and visceral obesity is characterized by elevated plasma concentration of triacylglycerol-rich lipoprotein (TRL) remnants that may be related to increased cardiovascular risk. Perturbed hepato-intestinal cholesterol metabolism may play a contributory role in this abnormality. We therefore investigated the association between plasma markers of cholesterol absorption and synthesis with TRL remnant metabolism in 35 men with the metabolic syndrome (MS). Plasma campesterol:cholesterol and lathosterol:cholesterol ratios were measured as estimates of cholesterol absorption and synthesis respectively. Remnant metabolism was assessed by measuring remnant-like particle-cholesterol (RLP-C), apolipoprotein (apo)B-48 and the fractional catabolic rate (FCR) of a labelled remnant-like emulsion. Compared with controls, subjects with the MS had significantly lower plasma campesterol:cholesterol ratio, but higher lathosterol:cholesterol ratio \((P < 0.05)\). Plasma RLP-C and apoB-48 concentrations were also higher \((P < 0.01)\) and the remnant-like emulsion FCR was lower \((P < 0.05)\). The plasma campesterol:cholesterol ratio was inversely correlated \((P < 0.05)\) with plasma triacylglycerols \((r = -0.346)\), RLP-C \((r = -0.443)\), apoB-48 \((r = -0.427)\) and plasma lathosterol:cholesterol ratio \((r = -0.366)\); the campesterol:cholesterol ratio was also positively correlated with the remnant-like emulsion FCR \((r = 0.398, P < 0.05)\). In multiple regression analysis, the significant correlations between plasma campesterol:cholesterol ratio and plasma triacylglycerols, RLP-C, apoB-48 and FCR of the remnant-like emulsion were independent of age, dietary energy and plasma lathosterol. Our findings suggest that in subjects with the MS alterations in cholesterol absorption and synthesis may be closely linked with the kinetic defects in TRL metabolism.

INTRODUCTION

Subjects with the metabolic syndrome (MS) are characterized by a constellation of risk factors, including abdominal obesity, atherogenic dyslipidaemia [elevated triacylglycerols, low high-density lipoprotein (HDL)-cholesterol, small dense low-density lipoprotein (LDL) particles], hypertension, pro-inflammatory and pro-

Key words: breath test, campesterol, cholesterol absorption, chylomicron remnant metabolism, dyslipidaemia.

Abbreviations: apo, apolipoprotein; FCR, fractional catabolic rate; HDL, high-density lipoprotein; HOMA, homoeostasis model assessment; LDL, low-density lipoprotein; MS, metabolic syndrome; NEFA, non-esterified fatty acid; RLP-C, remnant-like particle-cholesterol; TRL, triacylglycerol-rich lipoprotein; VLDL, very-low-density lipoprotein.

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thrombotic states, as well as insulin resistance [1]. Hypertriglyceridaemia attributable to increased plasma concentrations of hepatic apolipoprotein (apo)B-100 and intestinal apoB-48 is the most consistent lipid disorder in the MS and a risk factor for cardiovascular disease [2]. Although the precise regulation of apoB metabolism has not been fully established, the underlying mechanism may relate to oversecretion and/or impaired clearance of these triacylglycerol-rich lipoprotein (TRL) remnants by high-affinity pathways [3].

The availability of hepatic cholesterol substrate controls the expression of LDL receptors and partly regulates the production of very-low-density lipoprotein (VLDL)-apoB. Fatty acid supply to the liver and hepatic triacylglycerol pools also contribute to the homeostasis of VLDL-apoB metabolism [4]. Intestinally derived chylomicron remnants are known to compete with VLDL remnants for hepatic removal via the same LDL-receptor pathways. Since intestinal cholesterol absorption and hepatic cholesterol synthesis de novo are the two major sources which determine cholesterol content in the liver, these pathways may play a role in regulating TRL remnant metabolism. Quantification of plasma campesterol and lathosterol has been shown to reflect cholesterol absorption efficiency and cholesterol synthesis respectively, in both diabetic and non-diabetic individuals [5,6]. However, the association of cholesterol absorption and synthesis with TRL remnant metabolism has not been fully investigated, particularly in subjects with the MS.

Plasma levels of remnant-like particle-cholesterol (RLP-C) and apoB-48 have been used as static markers for TRL remnant metabolism [7,8]. We have also previously described and validated a ‘breath test’ that provides a functional measurement of chylomicron remnant metabolism [9,10]. The chylomicron remnant breath test may be a useful tool for assessing the kinetics of TRL remnant metabolism in vivo.

In the present study, we aimed to investigate the association between cholesterol metabolism, measured indirectly with plasma concentrations of campesterol and lathosterol, and TRL remnant metabolism, based on the breath test with measurements of fasting RLP-C and apoB-48.

MATERIALS AND METHODS

Subjects

Thirty-five men with the MS [1], while consuming an ad libitum weight-maintenance diet, were recruited from the community. The MS was defined by National Cholesterol Education Program (NCEP) criteria according to the presence of at least three of the following risk factors [1]: waist circumference ≥ 102 cm, blood pressure ≥ 130/ ≥ 85 mmHg, triglycerides > 1.7 mmol/l, HDL-cholesterol < 1.05 mmol/l and fasting glucose > 6.1 mmol/l. None of the subjects had diabetes mellitus (excluded by oral glucose tolerance test), apo E2/E2 or E4/E4 genotypes, macroproteinuria, raised creatinine (> 120 μmol/l), hypothyroidism or abnormal liver enzymes, or consumed more than 30 g alcohol/day. None reported a history of cardiovascular disease, or was taking medication or other agents known to affect lipid metabolism. Nine age- and sex-matched, normolipidaemic (plasma triglycerides < 1.7 mmol/l, total cholesterol < 5.2 mmol/l) non-obese men were also recruited for comparison purposes by newspaper advertisement from the community. They were chosen to be free of all factors comprising the MS. All subjects provided informed consent and the study was approved by the Ethics Committee of the Royal Perth Hospital.

Clinical protocols

All subjects were admitted to the metabolic ward in the morning after a 14-h fast. They were studied in a semirecumbent position and allowed to drink only water. Venous blood was collected for measurements of biochemical analytes. Arterial blood pressure was recorded after 3 min in the supine position using a Dinamap1846 SX/P monitor (Critikon Inc., Tampa, FL, U.S.A.). Dietary intake was assessed for energy and major nutrients, using at least two 24 h dietary diaries, with the DIET 4 Nutrient Calculation Software (Xyris Software, Highgate Hill, Queensland, Australia).

The sterile isotopically labelled chylomicron remnant-like emulsion (14 ml), containing triolein (135 mg), phosphatidylethanolamine (75 mg), cholesterol (24 mg) and cholesteryl [14C]oleate, was injected intravenously into an antecubital vein via a 21-gauge butterfly needle [10]. End-expiratory breath samples were collected into a Vacutainer Tube at baseline and post-intervention every 10 min for the first hour, every 20 min for the second hour, every 30 min for the next 5 h, and hourly for another 3 h. Participants were then given a snack and allowed to go home. Two additional breath samples were collected on the following days (24 h). During the first 10 h of collection of breath samples, participants sat quietly in a chair and were allowed to drink only water.

Biochemical analyses

Fasting triacylglycerols and cholesterol were assayed using standard enzymic methods for triacylglycerols and cholesterol and HDL-cholesterol (Roche Molecular Biochemicals, Mannheim, Germany). LDL-cholesterol was calculated using the Friedewald equation. Non-HDL-cholesterol was derived as total cholesterol minus HDL-cholesterol. Plasma apoA-I and apoB-100 were determined by immunonephelometry. Plasma RLP-C was determined with a JIMRO-II assay kit (Japan Immunoresearch Laboratories, Takasaki, Japan) using an immununoseparation method as described by Nakajima.
Cholesterol absorption and triacylglycerol-rich lipoprotein remnant metabolism

et al. [11]. apoB-48 was measured by SDS/PAGE and enhanced chemiluminescence, as described previously [12]. Plasma non-esterified fatty acids (NEFAs) were measured with a kit (Randox, Crumlin, Country Antrim, Northern Ireland, U.K.). Plasma insulin was measured by radioimmunoassay (DiaSorini srl, Saluggia, Italy). Plasma glucose concentration was measured by a hexokinase method. Insulin resistance was estimated using the homoeostasis model assessment (HOMA) score [13]. ApoE genotype was determined by the method of Hixson and Vernier [14]. Plasma liver and muscle enzymes were also analysed using routine methods. Plasma lathosterol and campesterol concentrations were measured by gas chromatography as described previously [15,16]. Briefly, 100 µl plasma was saponified with 6 M KOH solution and then extracted with hexane. The non-saponifiable plasma lipids were silylated with N,O-bis(trimethylsilyl)trifluoracetamide (‘BSTFA’) (Sigma, St. Louis, MO, U.S.A.) and then evaporated under nitrogen. The samples were reconstituted in 100 µl decane in preparation for gas chromatography analysis (Varian CP-3800 gas chromatograph; Varian Analytical Instruments, Walton-on-Thames, Surrey, U.K.). Plasma concentrations of campesterol and lathosterol were expressed in mmol × 10⁻² per mol of cholesterol.

Kinetic analysis
A compartmental model describing the appearance of labelled CO₂ in breath was developed using the SAAM II program (SAAM Institute, Seattle, WA, U.S.A.) as described previously [17]. The compartmental model was fitted to the observed ¹³C CO₂ breath test, and estimates of the fractional catabolic rate (FCR) were then determined.

Statistical analysis
Data were expressed as the means ± S.D. or S.E.M. Group characteristics were compared by Student’s t tests, after logarithmic transformation of skewed variables where appropriate. Associations were examined by simple and multiple linear regression methods. Statistical significance was defined at the 5% level using a two-tailed test.

RESULTS
Table 1 shows the clinical and biochemical characteristics in the two groups. Compared with the non-obese controls, the subjects with the MS were centrally obese and hypertensive (P < 0.05). Although plasma glucose and NEFAs were not significantly different between the groups, the MS subjects had significantly elevated fasting insulin concentrations and HOMA scores (P < 0.01). Twenty-five of the MS men were E3/E3 homozygotes for the apo gene, two were E2/E3 heterozygotes and eight were E3/E4 heterozygotes. Five of the lean men were E3/E3 homozygotes, two were E2/E3 heterozygotes and one was an E3/E4. One subject in the non-obese group did not consent to give blood for DNA analysis. There were no statistically significant differences in the frequency distribution of E alleles between the groups. Mean daily energy intake was significantly higher in the MS than control subjects (9985 ± 226 kJ versus 7001 ± 677 kJ, P < 0.001). The proportion of energy intake from carbohydrates, protein, fat and alcohol did not differ between the two groups.

Table 2 shows the plasma lipids, lipoproteins, apolipoproteins, non-cholesterol sterols and the FCR of the remnant-like emulsion in the subjects studied. Compared with control subjects, the MS group had significantly
higher plasma cholesterol, triacylglycerol, non-HDL-cholesterol, LDL-cholesterol and apoB-100 \((P < 0.01)\), but lower HDL-cholesterol \((P < 0.05)\). The MS subjects had significantly \((P < 0.05)\) lower plasma campesterol:cholesterol but higher lathosterol:cholesterol ratios compared with controls. Lathosterol:campesterol ratio, another marker of cholesterol synthesis \([5]\), was also higher in the MS than in the non-obese group \((P < 0.05)\).

There was also a significant increase in plasma concentrations of RLP-C and apoB-48 compared with controls \((P < 0.001)\). The FCR of the remnant-like emulsion was significantly lower in the MS subjects compared with the non-obese group.

Figure 1 shows the correlations of plasma campesterol:cholesterol ratio with triacylglycerols \((r = -0.346, P < 0.05)\), RLP-C \((r = -0.443, P < 0.05)\), apoB-48 \((r = -0.427, P < 0.05)\) and the FCR of the remnant-like emulsion \((r = 0.398, P < 0.05)\) in the subjects with the MS. In multiple regression analysis, the significant correlations between plasma campesterol:cholesterol ratio and plasma triacylglycerols, RLP-C, apoB-48 and the FCR of the remnant-like emulsion were independent of age, dietary energy and plasma lathosterol. Plasma campesterol:cholesterol ratio was also associated with plasma lathosterol:cholesterol ratio \((r = -0.366, P < 0.05)\). However, plasma campesterol:cholesterol ratio was significantly associated only with plasma RLP-C \((r = -0.419, P < 0.05)\) and the FCR of the remnant-like emulsion \((r = 0.4232, P < 0.05)\) after adjusting for plasma NEFA and waist-to-hip ratio.

Plasma lathosterol:campesterol ratio was also significantly correlated positively with plasma RLP-C \((r = 0.408, P < 0.05)\) and negatively with the FCR of the remnant-like emulsion \((r = -0.397, P < 0.05)\).

**DISCUSSION**

The major findings were that in men with the MS plasma campesterol:cholesterol ratio (an index of cholesterol absorption) was inversely associated with plasma triacylglycerol, RLP-C and apoB-48 concentrations, and was also positively correlated with the FCR of a chylomicron remnant-like emulsion. These associations were independent of age, dietary intake, HOMA score and plasma lathosterol concentration. This suggests that alterations in the homoeostasis of cholesterol absorption are closely linked with defects in TRL remnant metabolism. We also confirm that compared with non-obese controls, men with the MS have lower cholesterol absorption efficiency, higher cholesterol synthesis and defective TRL remnant metabolism.

Consistent with previous reports in Type II diabetes and obesity \([6,18,19]\), our present data showed that subjects with the MS had low cholesterol absorption and high cholesterol synthesis. Low cholesterol absorption efficiency may be due to increased cholesterol synthesis. This notion is supported by the inverse association between plasma ratios of campesterol:cholesterol and lathosterol:cholesterol in the present study. Elevated
cholesterol synthesis de novo increases biliary excretion of cholesterol. The increased biliary cholesterol pool competes with dietary cholesterol and non-cholesterol sterols for intestinal absorption, resulting in reduction of cholesterol absorption efficiency [18,19]. An inverse relationship between dietary cholesterol intake and cholesterol absorption efficiency has been clearly demonstrated [20], and the same presumably holds for the mass of biliary cholesterol entering the small intestine. This is supported by demonstrations that weight reduction or statin therapy decreases cholesterol synthesis and biliary cholesterol, resulting in an increase in cholesterol absorption efficiency [19,21]. A recent study [22] also showed that biliary cholesterol secretion is inversely associated with plasma plant sterol levels. In the present study, we used the campesterol:cholesterol and lathosterol:cholesterol ratios to estimate cholesterol absorption and synthesis respectively. However, these markers are strictly not definitive measures of cholesterol homoeostasis. Although we concluded that our obese subjects had low cholesterol absorption efficiency, we note that this may chiefly apply to the fractional absorption of cholesterol. Hence, in the presence of a large biliary flux of cholesterol in obesity the absolute mass transport of cholesterol to the liver may be normal, which is consistent with other reports [18,19]. Therefore it might have been preferable to measure cholesterol absorption and synthesis using dual labelling and sterol balance techniques. Nevertheless, the quantification of these markers, in particular the campesterol:cholesterol ratio, is strongly associated with direct measure of cholesterol absorption [5].

Our present data extend previous studies of TRL metabolism in diabetes and obesity [23–26] by employing a stable isotope chylomicron remnant-like emulsion and measurements of fasting plasma RLP-C and apoB-48 in subjects with MS defined using recognized criteria [1]. Insulin resistance in the MS is associated with dyslipidaemia [27], and has two potential effects on chylomicron remnant metabolism. First, it downregulates LDL-receptor expression [28], and secondly, it increases hepatic cholesterol synthesis and VLDL secretion [29]. These effects could increase competition between chylomicron and VLDL remnants for hepatic receptors, thereby impairing the uptake of chylomicron remnants by this pathway [30]. Accordingly, we found that our MS subjects had elevated RLP-C and apoB-48 concentrations and decreased FCR of the remnant-like emulsion.

Although we suggest that subjects with the MS have low cholesterol-absorption efficiency and delayed chylomicron remnant clearance, we found that an increase in plasma campesterol:cholesterol ratio was significantly negatively associated with elevated TRL remnants. This statistical association could reflect the positive effect of increased cholesterol synthesis (which was inversely correlated with cholesterol absorption) on hepatic VLDL-apoB secretion [31], and subsequent competition of VLDL and other TRLs for common removal pathways [30]. We also speculate that a relative increase in intestinal cholesterol absorption would increase the cholesterol content in the liver, thereby reducing cholesterol synthesis de novo with two potential consequences: first, a decrease in the secretion of VLDL-apoB and, secondly, upregulation of LDL-receptor expression [4,32]. These effects would accordingly enhance the removal of both chylomicron and VLDL remnants. The role of genetic factors in controlling these aforementioned mechanisms requires further investigation [33].

In conclusion, we suggest that in subjects with the MS the disturbances in TRL metabolism may be related to the combination of increased cholesterol synthesis and decreased cholesterol absorption, which results in a net decrease in catabolism and increase in plasma concentrations of TRL remnants. These abnormalities may eventually be causally related to an increased risk of cardiovascular disease and require further investigation with respect to the effects of pharmacotherapies (e.g. statin, insulin sensitizers, ezetimibe or dietary phytosterol supplements) that influence cholesterol synthesis and absorption.

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REFERENCES


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33 Berge, K. E., von Bergmann, K., Lutjohann, D. et al. (2002) Heterogeneity of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. J. Lipid Res. 43, 486–494