

## Dietary fat and physiological determinants of plasma chylomicron remnant homoeostasis in normolipidaemic subjects: insight into atherogenic risk

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### Abstract

TAG depleted remnants of postprandial chylomicrons are a risk factor for atherosclerosis. Recent studies have demonstrated that in the fasted state, the majority of chylomicrons are small enough for transcytosis to arterial subendothelial space and accelerate atherogenesis. However, the size distribution of chylomicrons in the absorptive state is unclear. This study explored in normolipidaemic subjects the postprandial distribution of the chylomicron marker, apoB-48, in a TAG-rich lipoprotein plasma fraction (Svedberg flotation rate (Sf) > 400), in partially hydrolysed remnants (Sf 20–400) and in a TAG-deplete fraction (Sf < 20), following ingestion of isoenergetic meals with either palm oil (PO), rice bran or coconut oil. Results from this study show that the majority of fasting chylomicrons are within the potentially pro-atherogenic Sf < 20 fraction (70–75%). Following the ingestion of test meals, chylomicronaemia was also principally distributed within the Sf < 20 fraction. However, approximately 40% of subjects demonstrated exaggerated postprandial lipaemia specifically in response to the SFA-rich PO meal, with a transient shift to more buoyant chylomicron fractions. The latter demonstrates that heterogeneity in the magnitude and duration of hyper-remnantaemia is dependent on both the nature of the meal fatty acids ingested and possible metabolic determinants that influence chylomicron metabolism. The study findings reiterate that fasting plasma TAG is a poor indicator of atherogenic chylomicron remnant homoeostasis and emphasises the merits of considering specifically, chylomicron remnant abundance and kinetics in the context of atherogenic risk. Few studies address the latter, despite the majority of life being spent in the postprandial and absorptive state.

**Key words:** Dietary fatty acids: Chylomicron remnants: ApoB-48: Postprandial lipaemia

The majority of humans spend most of their 24-h-d in the postprandial state. A typical 24 h TAG profile in individuals consuming three to four meals and snacks per d, showed a 70% increase in TAG concentration that reached baseline for only 3 h in the early morning<sup>(1)</sup>. Therefore, humans are essentially in a constant state of absorptive lipaemia. Following ingestion of dietary fats there is an elevation of TAG-enriched chylomicrons, however this elevation is ordinarily transient. Insulin-stimulated expression of lipoprotein lipase (LPL) bound to glycosylphosphatidylinositol anchored HDL-binding protein 1 mediates the hydrolysis of TAG from chylomicrons, resulting in rapid release of NEFA that can be taken up by endothelial cells via the membrane protein cluster of differentiation 36<sup>(2)</sup>. The post-hydrolysed TAG depleted remnants of chylomicrons and VLDL, are then removed via high-affinity receptor pathways, principally in the liver<sup>(3)</sup>.

Postprandial hypertriacylglycerolaemia is indicative of the net effect of dietary lipid absorption, biogenesis and secretion

of *de novo* chylomicrons and lipolysis by endothelial lipases. Modest intakes of dietary TAG can be mostly accommodated in the constitutively secreted chylomicrons, and may result in only minor changes to particle number<sup>(4,5)</sup>. However, rather than the TAG-rich lipoproteins (TRL) secreted following fat ingestion, accumulation of the post-hydrolysed remnants in plasma poses a greater atherogenic risk<sup>(6)</sup>. Indeed, fasting hypertriacylglycerolaemia, or postprandial dyslipidaemia, is not a requisite surrogate marker of chylomicron remnant accumulation *per se*<sup>(7–9)</sup>, and measurement of chylomicron particle number, rather than TAG concentration provides a more complete marker of pro-atherogenic remnant accumulation<sup>(10)</sup>.

Regulating the plasma abundance of pro-atherogenic chylomicron remnants principally relies on lipoprotein clearance capacity through receptor mediated pathways<sup>(11)</sup>. Chylomicrons share the lipolytic cascade and high affinity clearance pathways with TRL of hepatic origin (VLDL). An accumulation of VLDL

**Abbreviations:** CO, coconut oil; HR, hyper-responder; IAUC, incremental AUC; LPL, lipoprotein lipase; NR, normo-responder; PO, palm oil; RBO, rice bran oil; Sf, Svedberg flotation rate.

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remnants, or indeed LDL as a consequence of receptor insufficiency, often serves as a surrogate marker of clearance deficits of chylomicron remnant lipoproteins, because chylomicron remnants are primarily removed from blood by the apo B/E receptor (LDL-receptor). Although apo B-48, the equivocal marker of chylomicron abundance is not always reported, it is reported to be substantially elevated in subjects with diabetes/ the metabolic syndrome, familial hypertriacylglycerolaemia, familial hypercholesterolaemia and in normolipidaemic subjects with coronary artery disease<sup>(12–15)</sup>. Lipoproteins that are reduced to remnants with a diameter of <70 nm contribute to atherosclerosis through their capacity to deliver cholesterol to the subendothelial space and retention in extracellular matrices<sup>(16–18)</sup>. Indeed, fully hydrolysed chylomicron remnants with diameters of 55 nm have been reported and contain approximately forty times more cholesterol than LDL per particle<sup>(19,20)</sup>.

In healthy Caucasians, the plasma distribution of fasting apo B-48 is principally realised in the small, dense and potentially more atherogenic Svedberg flotation (Sf) <20 lipoprotein fraction<sup>(21)</sup>. However, in this study, we report that this abundance was not normally distributed, suggesting inter-individual variability in basal rates of chylomicron synthesis and/or remnant clearance. Moreover, there is likely to be potential interactive effects with dietary fats and the genetic regulation of chylomicron homeostasis<sup>(22)</sup>. However, the hypothesis of differential synergistic fatty acid/genetically mediated effects on chylomicron remnant abundance *per se* has never been explored directly.

Dietary fatty acid type has been reported to influence chylomicron metabolism by modulating chylomicron assembly (i.e. microsomal transfer protein activity, lipid droplet formation and stability), LPL mediated hydrolysis and high-affinity clearance pathways<sup>(23,24)</sup>. Among the different types dietary fatty acid there is an abundance of evidence that SFA are pro-atherogenic via pathways that include undefined aberrations in chylomicron metabolism; a broader hypercholesterolaemic induction effect and subsequently heightened vascular inflammation<sup>(25–28)</sup>. Differential effects of fatty acid type on postprandial lipaemia have been widely reported. Exaggerated postprandial lipaemia may be due to overproduction of intestinally derived and hepatically derived TRL, slower clearance by LPL, a rate-regulatory hydrolysis and removal of TAG or accumulation of partially hydrolysed remnant lipoproteins<sup>(1,23,24)</sup>. However, the comparative response to different dietary fats on chylomicron size and importantly remnant abundance is less clear. To address the latter, this study was designed to explore plasma apo B-48 distribution in normolipidaemic but otherwise healthy subjects given different meals rich in either SFA, long-chain *n*-6 PUFA or in medium chain fatty acids.

## Methods

This crossover designed study was conducted at Curtin University, Australia, between August 2014 and December 2015 and approved by the Curtin University Human Research Ethics Committee (HR 151/2013). All participants provided signed informed consent at initial screening. The study was registered with Australian New Zealand Clinical Trials Registry (12614000352606).

## Subjects

Male and female healthy participants aged between 20 and 70 years were recruited by means of standard media and web portals. Before acceptance in the study, potential interested participants were invited to meet the investigators and attended an initial screening. The exclusion criteria were fasting TAG 1.7 mmol/l or more, pregnant, lactating, smoking, excess alcohol intake (>20 g alcohol/d), the use of lipid lowering medication, reported history of cardiovascular, hepatic, renal disease, gastric disturbances or use of drug that affect gastrointestinal motility, diabetes mellitus, unwillingness to avoid consumption of fish oil supplements 3 d before and during the study days, >±5 kg changes in body weight in the previous 6 months, on a dietary advice and allergy or intolerance to any ingredients of the test meals.

Subjects were randomised using the random-number generator function RANDBETWEEN in Microsoft Excel software for the order of the test meals. Sample size required was determined from the sample size of twenty-four in a comparable study in which difference in apo B-48 postprandial response between postprandial hyperlipidaemia and normolipidaemia subjects were detected with a power of 80% and  $\alpha$  of 0.05<sup>(29)</sup>.

## Test meal composition

Each participant consumed an isoenergetic breakfast meal (3100 kJ, 43.4 g fat, 69.4 g carbohydrate and 13.6 g protein) on three occasions in which 40 g of the fat was from one of the following three different cooking oils: palm oil (PO) (Bimoli; PT Indofood Sukses Makmur Tbk), coconut oil (CO) (Eco Food Organics) and rice bran oil (RBO) (Alfa One™ Rice Bran Oil; Hansell Food Group) (Table 1). The test meal was a mixed breakfast meal consisting of pasta (150 g) (Large spiral pasta; San Remo Pasta Ltd), tomato pasta sauce (50 g) (Dolmio Traditional Recipe Classic Tomato Pasta Sauce; Mars Food) added with 40 g test oils, white bread (40 g) (white toaster; Mias Bakery) and 160 ml orange juice (Just Juice, orange juice). Each meal provided 53% of total energy as fat. Fatty acid content of the different oils is provided in Table 1. Fatty acid composition of the cooking oils were analysed in the form of fatty acid

**Table 1.** Fat content of the test oils measured by GC

Fatty acid content (%)	Total SFA	Total MUFA	Total PUFA	12:0 (lauric acid)	14:0 (myristic acid)	16:0 (palmitic acid)	18:0 (stearic acid)	18:1 <i>n</i> -9 (oleic acid)	18:2 <i>n</i> -6 (linoleic acid)
Palm oil	60	31	9	0.1	4.3	42.1	9.8	30.4	8.8
Coconut oil	92	8	0	47.2	27.2	13.3	4.4	7.8	0.0
Rice bran oil	28	40	32	0.1	0.1	24.7	3.2	39.9	32.2

methyl esters by GC (Perkin Elmer AutoSystem XL) equipped with an autosampler, a split-splitless injector, SGE BPX70 GC column and a flame ionisation detector using a method developed in our laboratory.

### Study protocol

Participants attended three postprandial study days separated by at least 4 weeks between each visit. Before each study day, participants were asked to restrain from vigorous physical activity, alcohol and consumption of high-fat meals. They were asked to consume the same amount and food items for breakfast and lunch on the day before each postprandial test day and were provided with a standard commercially available ready meal (SunRice pack meal, 15 g fat; SunRice) for their evening meal before study day. After an overnight fast for at least 12 h, participants were admitted to the research unit. Upon arrival, their body weight, height, waist circumference and blood pressure (Omron) were measured. An initial fasting blood samples were collected before consumption of the test meal. Following consumption of the test meal, participants were given access to plain water with no other food and drinks to be consumed. The test meal was consumed within 15 min and subsequently postprandial blood samples were collected periodically at 4 and 8-h after the test meal for measurement of apo B-48, TAG and cholesterol concentration. Body composition measured by bioelectrical impedance analysis (SECA mBCA 515; SECA GmbH & Co. KG), was determined on the first fat challenge. Fasting and postprandial venous blood samples were collected into BD Vacutainer<sup>R</sup> EDTA tubes (Becton Dickinson) and underwent low-speed centrifugation. Plasma samples were isolated and stored in aliquots at  $-80^{\circ}\text{C}$ .

### Lipoprotein fractionation

Sequential floatation ultracentrifugation was used to collect lipoprotein fractions of  $\text{Sf} > 400$  and  $\text{Sf} 20\text{--}400$  of plasma samples using a modified method that has been used in our laboratory<sup>(30,31)</sup>. In brief, 1 ml plasma was overlaid with a density solution (1.006 g/ml) in 5 ml thin walled tubes (Beckman Coulter). The buoyant chylomicron fraction ( $\text{Sf} > 400$ ) was collected following 30 min of ultracentrifugation at 40 000 rpm,  $20^{\circ}\text{C}$  (AH-650 swing-out rotor; Thermo Scientific) by aspirating approximately 1 ml of the top layer. To collect  $\text{Sf} 20\text{--}400$ , the tube was then topped with density solution (1.006 g/ml) and ultracentrifuged again under the same condition for 20.5 h. Approximately 1 ml of the top layer was aspirated to isolate this fraction. The infranatant remaining in the tube was regarded as the remnant fraction ( $\text{Sf} < 20$ ). To calculate the concentrations of apo B-48, TAG and cholesterol of these fractions, the volume of all supernatants and infranatant collected were taken into account by measuring the collected supernatants or infranatant using a glass bore syringe (Hamilton).

### Lipid, insulin and glucose assays

Fasting and postprandial TAG and total cholesterol in plasma and lipoprotein fractions were measured with an enzyme-based colorimetric reagent (Trace; Thermo Fisher Scientific Inc.;  $\text{CV} < 5\%$ ).

HDL was collected using a precipitation method by the addition of phosphotungstic acid in the presence of Mg ions (HDL-cholesterol precipitant Reagent; Randox Laboratories Limited). A modified version of Friedewald formula was used to estimate LDL-cholesterol<sup>(32)</sup>. Fasting insulin and glucose concentrations were analysed by an accredited clinical laboratory (PathWest Laboratory, Fiona Stanley Network) using their automated procedures. The level of insulin resistance was assessed by homoeostasis model of insulin resistance (HOMA-IR)<sup>(33)</sup>. All samples were assayed in duplicate and run within a single batch.

### Apo B-48 determination

Apo B-48 concentration was measured by using a commercial sandwich ELISA method (Shibayagi Human apo B-48 ELISA Kit; Ishihara;  $\text{CV} < 3\%$ ). This method has been validated<sup>(34)</sup>. The determination of apo B-48 concentration in plasma,  $\text{Sf} > 400$  and  $\text{Sf} 20\text{--}400$  fractions was completed based on the manufacturer's instruction with some modification on the dilution factors to ensure that the apo B-48 concentration in those fractions within the standard curve. Fasting and postprandial plasma,  $\text{Sf} > 400$  and  $\text{Sf} 20\text{--}400$  samples were 1 in 300, 1 in 50 and 1 in 100 diluted, respectively. Apo B-48 concentration in  $\text{Sf} < 20$  was calculated from the difference between the concentration of apo B-48 in plasma and in the less dense fractions ( $\text{Sf} > 400$  and  $\text{Sf} 20\text{--}400$ ).

### Statistical analysis

All data were analysed using SPSS version 21 (SPSS Inc.). Data were checked for normality and were natural log transformed where necessary. Postprandial response (incremental AUC (IAUC)) was calculated using the trapezoidal rule<sup>(35)</sup> with some modifications. The IAUC was estimated as the difference between the area under the plasma curve between 0 and 8 h and the area defined below the baseline concentration. However in responses where the 8 h concentration was lower than that at baseline, the baseline AUC represented the area below a line connecting the baseline and 8 h concentration levels. The IAUC represents the increase in area after the response of the fat load above fasting concentrations. The primary outcome of this study was IAUC apo B-48 in plasma,  $\text{Sf} > 400$ ,  $\text{Sf} 20\text{--}400$  and  $\text{Sf} < 20$  fraction. The secondary outcome of this study was IAUC TAG in plasma and lipoprotein fractions and the association between IAUC apo B-48 and a number of metabolic determinants. Postprandial differences between groups following each meal challenge were assessed with Mann-Whitney *U* test. Differences in postprandial responses between meals (PO, CO and RBO) were analysed by linear mixed model with Bonferroni *post hoc* tests to detect significant pairwise differences corrected for multiple comparisons. Spearman correlations were computed to assess the association between parameters. A *P* value  $< 0.05$  was considered as significant.

### Results

Totally, twenty-six participants with normal fasting TAG concentration took part in the single blind, randomised crossover



study, of which twenty-three completed all three meal challenges. In all, three subjects only attended two postprandial visits. None of the participants reported any adverse effects to the test meals. To explore putative interactive effects of the dietary fat challenge with a genetically determined chylomicron distributional response, subjects were considered based on the postprandial TAG response 4 h after consuming the positive lipaemic control SFA-enriched meal (PO) were examined. Subjects were classified as hyper-responders (HR) if plasma TAG exceeded 1.7 mmol/l at the peak absorptive phase of 4 h. Of the twenty-six subjects studied, ten had concentrations  $\geq 1.7$  mmol/l (HR). Totally, sixteen subjects were found to have a 4-h TAG concentration  $< 1.7$  mmol/l following the SFA-enriched meal (normo-responders (NR)).

Table 2 shows that the HR and NR groups had comparable fasting apo B-48. In addition, HR and NR groups were normolipidaemic and comparable for plasma TAG, total cholesterol, LDL-cholesterol and non-HDL-cholesterol. The HR group had a lower concentration of HDL-cholesterol than NR, however this was otherwise within the normal reference range. Both NR and HR groups were normoglycaemic and there was no evidence of insulin resistance based on HOMA or fasting plasma insulin. However, the HR group had significantly greater BMI, waist circumference and fat-free mass than the NR group.

The putative effect of dietary fatty acids on the distribution of chylomicrons in lipoprotein fractions in NR and HR subjects is depicted in Fig. 1. In NR, the plasma IAUC for apo B-48 was comparable for the PO-enriched fat challenge *v.* the RBO diet (23.9 (SE 4.9)  $\mu\text{g} \times 8 \text{ h/ml}$  in PO *v.* 27.2 (SE 3.9)  $\mu\text{g} \times 8 \text{ h/ml}$  in RBO)

(meal effect  $P=0.068$ ). However, there was a modestly lower plasma apo B-48 IAUC response in NR subjects following consumption of the CO diet (18.4 (SE 2.6)  $\mu\text{g} \times 8 \text{ h/ml}$ ). In contrast, HR subjects had approximately 2–3-fold greater IAUC apo B-48 plasma response following ingestion of PO (63.2 (SE 13.4)  $\mu\text{g} \times 8 \text{ h/ml}$ ) relative to the meal challenge with RBO (35.3 (SE 6.2)  $\mu\text{g} \times 8 \text{ h/ml}$ ) or CO (24.4 (SE 4.8)  $\mu\text{g} \times 8 \text{ h/ml}$ ) (meal effect  $P=0.003$ , PO–CO  $P=0.016$ , PO–RBO  $P=0.267$ ).

Distributional analysis of the heightened apo B-48 IAUC in HR subjects following ingestion of the PO-enriched meal, indicated that approximately half of the exaggerated apo B-48 response was realised in the remnant Sf  $< 20$  fraction (32.1 (SE 6.6)  $\mu\text{g} \times 8 \text{ h/ml}$  in HR *v.* 13.4 (SE 4.3)  $\mu\text{g} \times 8 \text{ h/ml}$  in NR;  $P=0.02$ ). Smaller increases in the apo B-48 IAUC were also observed in HR subjects compared with NR within the Sf 20–400 fraction (14.4 (SE 4.5)  $\mu\text{g} \times 8 \text{ h/ml}$  in HR *v.* 6.6 (SE 1.4)  $\mu\text{g} \times 8 \text{ h/ml}$  in NR;  $P=0.138$ ) and the larger more buoyant lipoprotein isolate Sf  $> 400$  (17.3 (SE 4.8)  $\mu\text{g} \times 8 \text{ h/ml}$  in HR *v.* 4.2 (SE 1.4)  $\mu\text{g} \times 8 \text{ h/ml}$  in NR, respectively;  $P=0.024$ ). Distributional analysis of the apo B-48 IAUC in HR and NR subjects following ingestion of a meal rich in either RBO or CO, did not indicate significant differences between lipoprotein fractions. This finding is consistent with the notion that the disturbed response was specific to the PO fat challenge in HR subjects.

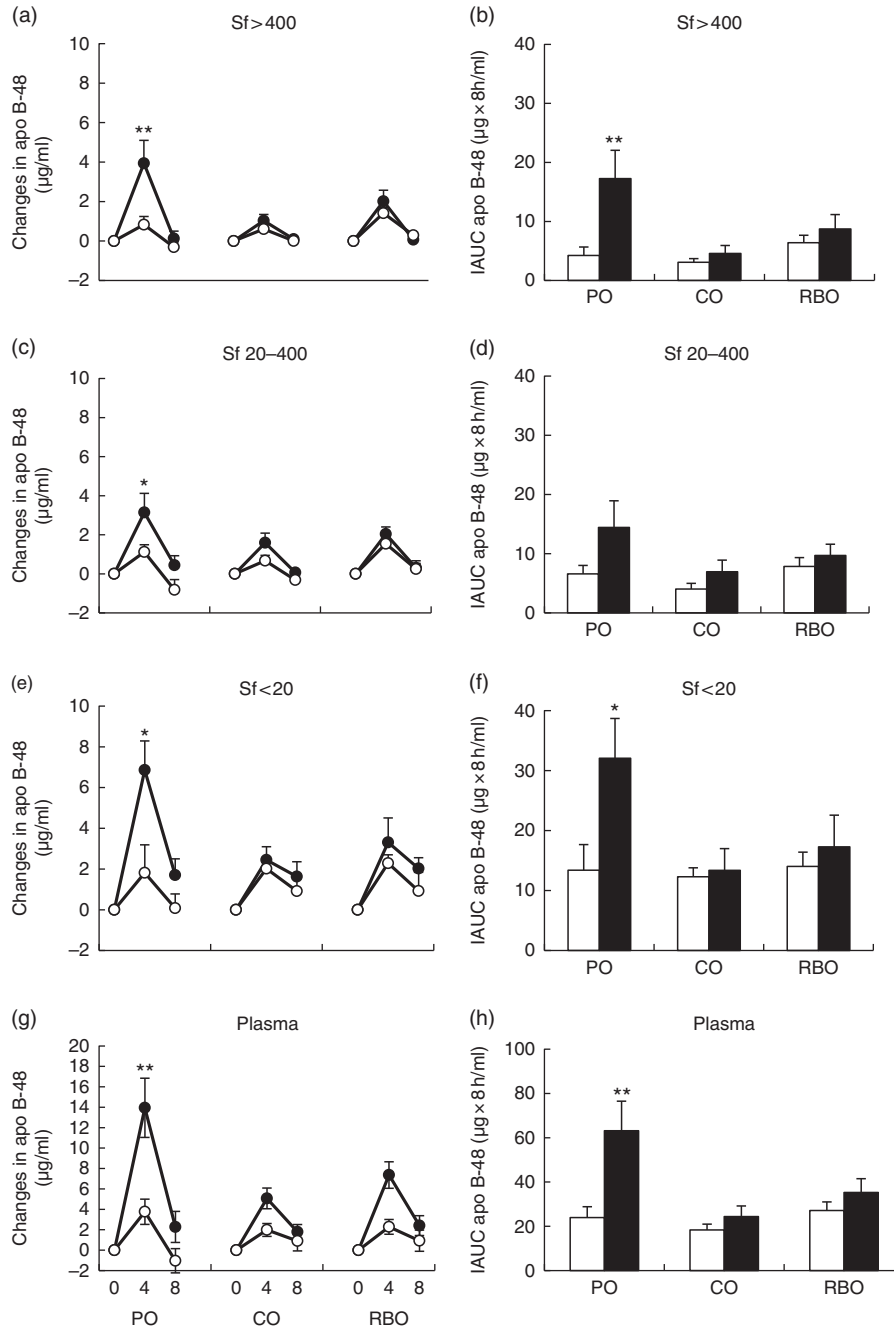
The TAG response in lipoprotein fractions for NR and HR subjects in response to the dietary fat challenges is indicated in Fig. 2. In NR subjects, the plasma IAUC TAG response following the PO meal (1.7 (SE 0.3) mmol  $\times 8 \text{ h/l}$ ) was comparable with the response following ingestion of RBO (2.1 (SE 0.6) mmol  $\times 8 \text{ h/l}$ )

**Table 2.** Subject characteristics (Mean values with their standard errors; medians, 25th and 75th interquartile (IQR) ranges)

Parameters	Total (n 26)		NR (n 16)		HR (n 10)	
	Mean	SE	Mean	SE	Mean	SE
Men/women	11/15		4/12		7/3	
Age (years)	41	3	39	4	43	6
Apo B-48 ( $\mu\text{g/ml}$ )						
Median	6.3		7.1		5.1	
25th and 75th IQR	4.8–9.4		5.2–11.3		4.2–7.8	
TAG (mmol/l)	1	0.1	0.9	0.1	1.1	0.1
Total cholesterol (mmol/l)	5.5	0.2	5.4	0.3	5.4	0.4
LDL-cholesterol (mmol/l)	3.3	0.2	3.2	0.3	3.6	0.4
HDL-cholesterol (mmol/l)	1.7	0.1	1.8	0.1	1.4	0.1*
Non-HDL-cholesterol (mmol/l)	3.8	0.2	3.6	0.3	4.1	0.4
Glucose (mmol/l)	5.5	0.1	5.5	0.1	5.5	0.1
Insulin (mIU/l)						
Median	6.8		6.8		7	
25th and 75th IQR	4.2–11.1		4.1–9.8		5.1–12.2	
HOMA-IR						
Median	1.7		1.7		1.7	
25th and 75th IQR	1–3		1–2.3		1.3–3.2	
Waist circumference (cm)	83.7	2.7	77.9	3.2	93.1	2.8**
BMI ( $\text{kg/m}^2$ )	25.8	0.8	24.1	1	28.6	0.9**
Fat mass index ( $\text{kg/m}^2$ )	7.7	0.7	7.2	1	8.5	1.1
Fat-free mass index ( $\text{kg/m}^2$ )	18	0.5	16.8	0.5	19.9	0.7**
Systolic blood pressure (mmHg)	122	3	119	4	127	5
Diastolic blood pressure (mmHg)						
Median	72		71		73	
25th and 75th IQR	67–78		67–77		68–81	

NR, normo-responder; HR, hyper-responder; HOMA-IR, homeostasis model of insulin resistance.

\*  $P < 0.05$ , \*\*  $P < 0.01$ ; HR compared with NR group. *P* values were based on independent *t* test and Mann–Whitney test for mean and medians.



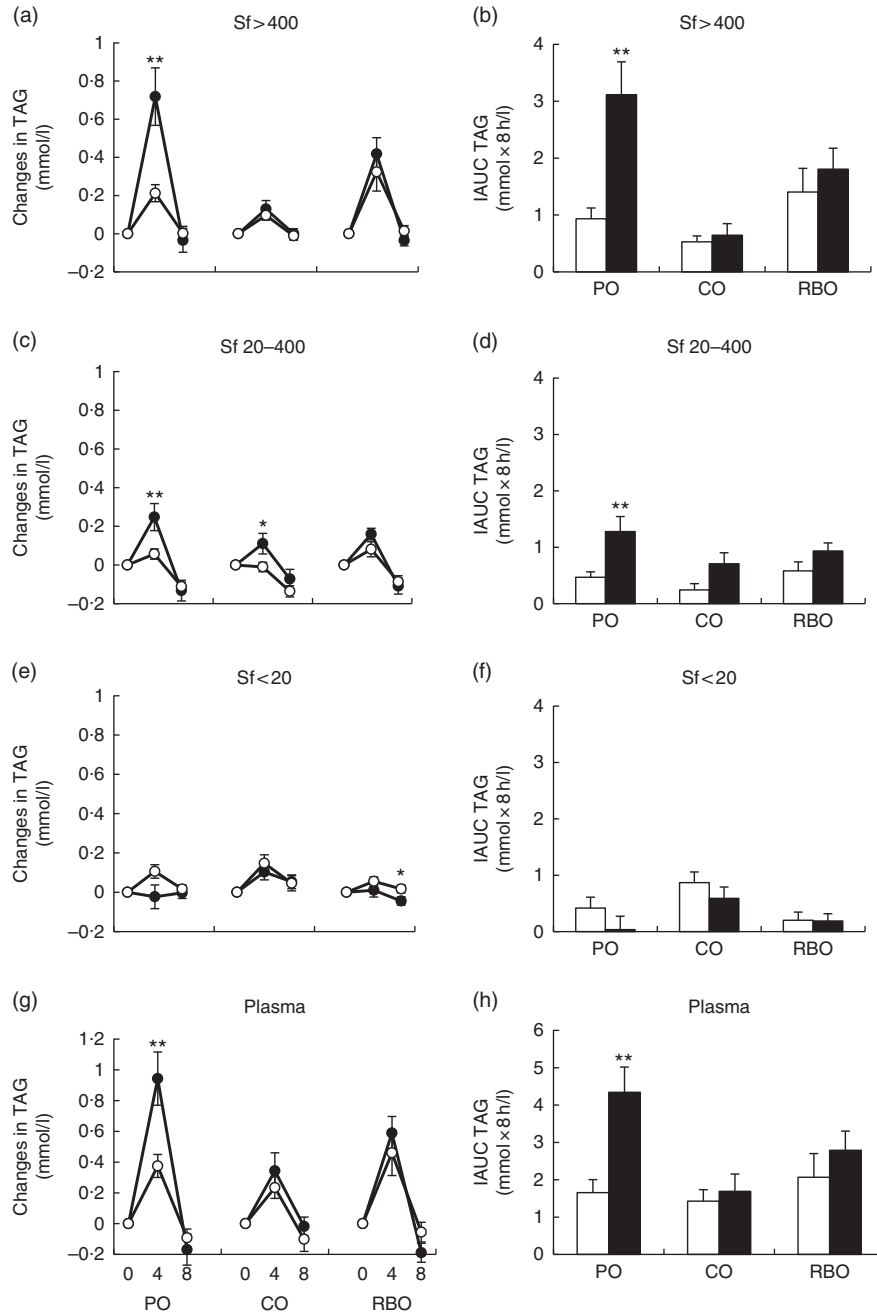
**Fig. 1.** Changes in apo B-48 concentration (a), (c), (e), (g) and incremental AUC (IAUC) of apo B-48 (b), (d), (f), (h)) in Svedberg flotation rate (Sf) > 400, Sf 20–400, Sf < 20 fraction and plasma following mixed meal containing palm oil (PO), coconut oil (CO) and rice bran oil (RBO). ● and ■ represent hyper-responder (HR) group; ○ and □ represent normo-responder (NR) group. \* *P* value < 0.05, \*\* *P* value < 0.01, against NR group.

or CO (1.4 (SE 0.3) mmol × 8 h/l) (meal effect *P* = 0.414). In contrast, in HR subjects the IAUC response for TAG following the PO-enriched meal was substantially greater than either the RBO (4.3 (SE 0.7) *v.* 2.8 (SE 0.5) mmol × 8 h/l, respectively) or the CO-enriched meal (1.7 (SE 0.5) mmol × 8 h/l) (meal effect *P* = 0.001). Distributional analysis of the heightened TAG IAUC response indicated in HR subjects was principally realised with a 3-fold increase in the largest and most buoyant lipoprotein fraction Sf > 400 compared with NR (3.1 (SE 0.6) *v.* 0.9 (SE 0.2) mmol × 8 h/l, respectively; *P* < 0.0005). Some increase in

TAG IAUC was also observed within the Sf 20–400 fraction of HR subjects compared with NR (1.3 (SE 0.3) *v.* 0.5 (SE 0.1) mmol × 8 h/l, respectively; *P* = 0.003). Although relatively modest in the context of total TAG response, within the Sf < 20 fraction there was less of a TAG response in HR subjects compared with NR subjects (0.1 (SE 0.2) *v.* 0.4 (SE 0.2) mmol × 8 h/l, respectively; *P* = 0.272).

Suggested metabolic determinants of chylomicron homeostasis in HR and NR subjects is depicted in Table 3. Fat-free mass determined by bioelectrical impedance analysis was





**Fig. 2.** Changes in TAG concentration ((a), (c), (e), (g)) and incremental AUC (IAUC) of TAG ((b), (d), (f), (h)) in Svedberg flotation rate (Sf) > 400, Sf 20–400, Sf < 20 fraction and plasma following mixed meal containing palm oil (PO), coconut oil (CO) and rice bran oil (RBO). ● represents hyper-responder (HR) group; ○ represents normo-responder (NR) group. \* *P* value < 0.05, \*\* *P* value < 0.01, against NR group.

generally strongly associated with the apo B-48 IAUC for the Sf > 400 and the Sf 20–400 fractions in HR subjects across all dietary treatment arms. However, measures of fat-free tissue, or conversely adiposity, were not associated with the Sf < 20 remnant fraction in HR subjects. In the HR subjects, insulin sensitivity indicated by HOMA-IR score and fasting plasma insulin concentration were strongly associated with the apo B48 IAUC in the Sf > 400 and to a lesser extent the Sf 20–400 fraction in subjects given RBO or CO. However, the association was not evident in HR subjects when challenged with the PO-enriched

meal. Fasting TAG, often considered a surrogate marker of chylomicronaemia, was not associated with chylomicron abundance in any of the lipoprotein fractions or dietary treatment arms considered in HR subjects. Instead, strong associations between fasting apo B-48 concentration and apo B-48 IAUC were observed across all dietary treatment arms in the HR group.

In NR subjects, fat-free mass did not correlate with apo B-48 IAUC for any of the lipoprotein fractions against any of the three meal challenges provided, contrasting with the findings in HR subjects. HOMA and fasting plasma insulin showed association

**Table 3.** Correlations between apo B-48 incremental AUC (IAUC) (0–8 h) in different fractions following palm oil (PO), coconut oil (CO) and rice bran oil (RBO) meal and baseline metabolic parameters in normo-responder (NR) (*n* 16) and hyper-responder (HR) (*n* 10) groups

<i>R</i> between apo B-48 IAUC and parameters	PO				CO				RBO			
	Plasma	Sf > 400	Sf 20–400	Sf < 20	Plasma	Sf > 400	Sf 20–400	Sf < 20	Plasma	Sf > 400	Sf 20–400	Sf < 20
<b>NR</b>												
Apo B-48	-0.211	-0.629	-0.132	-0.193	-0.246	-0.411	-0.343	-0.046	0.053	-0.071	-0.115	0.191
TAG	0.2	-0.211	0.154	-0.029	-0.075	0.139	0.171	-0.2	0.715**	0.456	0.429	0.635**
Total cholesterol	0.35	-0.046	0.221	0.086	-0.082	0.036	0.086	-0.161	0.471	0.3	0.476	0.321
LDL-cholesterol	0.611*	0.054	0.468	0.389	0.179	0.304	0.354	0.057	0.5*	0.279	0.479	0.406
HDL-cholesterol	-0.47	0.032	-0.484	-0.509	-0.377	-0.495	-0.399	-0.273	-0.205	-0.115	-0.13	-0.219
Non-HDL-cholesterol	0.525*	-0.054	0.386	0.329	0.122	0.227	0.273	0.013	0.527*	0.277	0.446	0.455
Glucose	-0.009	-0.307	-0.036	0.172	-0.057	0.084	0.025	0.001	0.001	0.087	0.041	-0.015
Insulin	-0.107	0.121	0.089	-0.439	-0.182	0.136	0.096	-0.425	0.432	0.553*	0.600*	0.059
HOMA-IR	-0.104	0.125	0.096	-0.429	-0.171	0.154	0.114	-0.429	0.421	0.559*	0.606*	0.026
BMI	0.3	-0.132	0.404	0.279	0.189	0.143	0.279	0.118	0.385	-0.091	0.079	0.612*
Waist circumference	0.418	0.068	0.575*	0.157	0.232	0.382	0.479	0.036	0.626**	0.226	0.432	0.662**
Fat-free mass index	-0.097	-0.311	0.184	0.154	0.157	0.197	0.175	0.055	-0.087	-0.093	-0.138	-0.105
Systolic	-0.011	-0.095	0.018	0.004	0.009	0.263	0.309	-0.075	0.187	0.175	0.108	0.196
Diastolic	0.141	0.032	0.404	-0.123	0.079	0.481	0.436	-0.166	0.394	0.202	0.252	0.284
<b>HR</b>												
Apo B-48	0.810*	0.643	0.952**	0.667	0.806**	0.588	0.721*	0.345	0.588	0.43	0.758*	0.261
TAG	0.357	0.452	0.5	-0.024	0.358	0.539	0.564	-0.224	0.37	0.418	0.479	-0.079
Total cholesterol	0.263	0.001	0.419	0.168	-0.085	-0.146	0.043	-0.122	-0.079	0.067	0.267	-0.328
LDL-cholesterol	0.214	-0.167	0.357	0.238	0.006	-0.152	0.042	0.079	-0.03	-0.018	0.248	-0.2
HDL-cholesterol	0.263	-0.228	0.12	0.311	-0.354	-0.591	-0.433	-0.03	-0.439	-0.396	-0.165	-0.299
Non-HDL-cholesterol	0.204	0.001	0.395	0.072	0.073	0.061	0.213	-0.073	0.122	0.182	0.359	-0.195
Glucose	0.216	0.335	0.539	-0.084	0.31	0.529	0.547	-0.146	0.322	0.675*	0.632*	-0.255
Insulin	-0.048	0.476	0.024	-0.357	0.321	0.770**	0.6	-0.2	0.176	0.721*	0.358	-0.261
HOMA-IR	-0.073	0.512	0.049	-0.439	0.264	0.742*	0.62	-0.313	0.117	0.706*	0.325	-0.325
BMI	-0.405	-0.619	-0.595	-0.333	-0.042	0.018	-0.164	0.333	0.079	-0.176	-0.273	0.188
Waist circumference	0.071	-0.405	-0.143	0.19	0.2	0.03	-0.115	0.612	0.285	-0.152	0.018	0.333
Fat-free mass index	0.635	0.731*	0.659	0.407	0.894**	0.833**	0.796**	0.353	0.766**	0.48	0.632*	0.498
Systolic	-0.095	-0.19	-0.095	-0.262	0.152	0.333	0.188	0.164	0.115	0.309	0.248	-0.224
Diastolic	-0.429	-0.452	-0.571	-0.357	-0.164	-0.055	-0.115	0.188	-0.382	-0.079	-0.212	-0.358

\* *P* < 0.05, \*\* *P* < 0.01 (Spearman's correlation).

in the Sf > 400 and the Sf 20–400 for the RBO challenge, but this was not indicated for either the PO or CO meal challenges. In NR group, apo B-48 IAUC in Sf < 20 was also strongly associated with waist circumference and BMI following RBO meal.

For HR and NR subjects, there was no significant association between total cholesterol, LDL-cholesterol, HDL-cholesterol, fasting glucose or blood pressure and IAUC apo B-48 responses with the lipoprotein fractions indicated.

### Discussion

This study explored the postprandial plasma distribution of apo B-48 in normolipidaemic subjects following ingestion of iso-energetic meals enriched in either PO, RBO or CO. Approximately 40% of subjects studied demonstrated substantially exaggerated postprandial lipaemia in response to the SFA-rich PO diet (HR group), despite that these subjects did not exhibit an increased fasting concentration of chylomicron particles compared with the NR group. Moreover, significant heterogeneity was found within the responses of the otherwise healthy subjects with normal fasting plasma lipid concentrations, implying variation is due to the individual determinants of chylomicron homeostasis.

The rationale to consider HR *v.* NR was driven based on the findings from Sato *et al.*<sup>(29)</sup>. Their results suggest that normolipidaemic subjects with TAG concentration postprandial  $\geq 1.7$  mmol/l may have a delayed clearance of TRL. The finding of the present study demonstrates that consideration of

heterogeneity based on classical metabolic parameters such as the metabolic syndrome did not effectively segregate those with the exaggerated response to the SFA-rich meal. Hence analogous to classical dietary challenge assessment, we instead considered the postprandial response and set a cut-off value of 1.7 mmol/l TAG at peak postprandial phase based on recommendation below this concentration for fasting<sup>(36)</sup>.

The study confirms that contrary to historic considerations, the majority of fasting chylomicrons are within the potentially pro-atherogenic Sf < 20 fraction (70–75%)<sup>(37)</sup>. In both HR and NR subjects the most substantive apo B-48 IAUC response was realised in the Sf < 20 fraction, at least twice that compared with the Sf 20–400 or the Sf > 400 fractions. The findings indicate that following the ingestion of dietary fats, chylomicronaemia is principally realised in pro-atherogenic particles, which can be delivered through transcytotic processes directly to the sub-endothelial space. This study demonstrates that within normolipidaemic subjects significant heterogeneity in the magnitude and duration of hyper-remnantaemia is dependent both on the nature of the fatty acids ingested and the likely individuals' metabolic response. A significant subset of individuals studied had substantially elevated apo B-48 IAUC, specifically indicated when consuming a fat challenge rich in SFA. Paradoxically, the same HR subjects had comparable apo B-48 response and distribution between lipoprotein fractions to NR subjects when challenged with meals containing either RBO or CO. The metabolic aberration in these normolipidaemic HR subjects in response to a PO-enriched fat challenge was not detected in correlation analysis by indices of the metabolic syndrome.

The study findings reiterate that fasting plasma TAG is a poor indicator of chylomicronaemia and potential postprandial atherogenicity<sup>(38)</sup>. It emphasises the merits of considering specifically, chylomicron remnant homeostasis and response in the context of atherogenic risk. Few studies address the latter, despite the majority of life being spent in the postprandial and absorptive state.

Apo B-48, an equivocal marker of chylomicron homeostasis and distribution, was principally indicated within the smallest and most dense plasma lipoprotein fraction measured (Sf < 20). These particles typically have a diameter 30–55 nm, sufficient to be captured within the non-specific transcytotic vesicles that deliver small constant amounts of plasma lipoprotein to the subendothelial space (<70 nm)<sup>(16,20)</sup>. It is a reasonable proposition therefore that a greater understanding of chylomicron atherogenicity would require better appreciation of distributional considerations in the absorptive state; a pathophysiological period that occupies the majority of our 24-h-d. In NR subjects, the plasma response and the distribution of apo B-48 was comparable for dietary challenges enriched in either PO, RBO or CO suggesting that vascular exposure was otherwise equivalent. In this context, atherogenic risk might be similar. However, other critical factors in the atherogenic cascade including entrapment, uptake by macrophages, mitochondrial respiratory activity or stimulation of inflammatory pathways may still render chylomicron remnants derived from PO a greater risk than remnants derived from RBO or CO in NR subjects.

In normotriglyceridaemic HR individuals, atherogenic risk may be exacerbated beyond the effects of the fatty acid *per se*, but as a consequence of heightened vascular exposure to chylomicrons. Despite the metabolic aberration in these individuals principally being indicated in the lipolytic cascade, nonetheless the substantive accumulation and response was for the large part realised within the dense Sf < 20 fraction. On the basis that chylomicronaemia was observed in HR subjects following the PO meal and not when challenged with either RBO or CO, we suggest that the metabolic defect is indicated specifically in the metabolism of the particles enriched in PO. Given that the response to PO was not exaggerated in all subjects (only in the HR group), one interpretation is that the defect may not be due to the fatty acid composition in *de novo* chylomicrons. Instead, the defects may be due to the complement of regulating apo that are associated with chylomicrons, which differs between individuals (between NR and HR groups). Differential expression of key apo involved in lipolysis (e.g. apo CII/CIII), or remnant clearance (apo E isoforms) may be a genetic point of synergistic regulation of PO-enriched chylomicrons<sup>(39,40)</sup>. Meal rich in SFA (PO-based meal) has been reported to modulate the amount of apoC-II, apoC-III and apo E than meal rich in MUFA and PUFA<sup>(40)</sup>. Since the exaggerated apo B-48 response was observed in the HR group following PO meal but not CO or RBO meal or in the NR group, this raises the possibility of interactions between type of dietary fatty acid (PO-based meal) and individual metabolic response. Alternatively, abundance or isoforms of lipolytic enzymes or receptor clearance proteins may explain the interactive effects with dietary lipids on chylomicron abundance. The latter seems

less likely given that there was no difference between NR and HR in response to RBO- or CO-enriched meal challenges. Correlation analysis indicated for both NR and HR subjects, a consistently strong association between fat-free mass and apo B-48 homeostasis, particularly within the Sf > 400 and the Sf 20–400 fractions. In the absence of a frank lipolytic defect in HR subjects (normal fasting TAG, normal response to RBO and CO), one explanation for the heightened apo B-48 response in HR subjects principally within Sf > 400 and Sf 20–400 (but not in Sf < 20) might be in a modest hydrolytic defect in muscle tissue-associated LPL, given the consistent association between fat-free mass (inverse association with fat mass) and apo B-48 abundance within larger more lipoprotein fractions. The latter is an interesting consideration given that HR subjects had greater fat-free mass and that muscle and adipose tissue LPL are differentially regulated<sup>(41)</sup>. Insulin stimulates adipose tissue LPL but inhibits expression of muscle tissue LPL. Meals high in SFA content have been reported to decrease insulin sensitivity<sup>(42,43)</sup> via accumulation of TAG and ceramide in muscle, activation of protein kinase C, NF- $\kappa$ B and the subsequent inflammatory genes<sup>(44)</sup>. By extension, muscle-mediated LPL activity may have been attenuated in HR subjects<sup>(45)</sup>. Nonetheless, the data from this study suggest that any such putative tissue-bed lipolytic defect in HR subjects would be a differential interaction between enzyme and substrate (chylomicron), because RBO and CO challenges were comparable between HR and NR subjects<sup>(11)</sup>.

Measures of obesity suggested the HR subjects exhibited greater central obesity and were more overweight than the NR subjects, however there was no evidence of insulin resistance, or the metabolic syndrome based on current combined criteria *per se*. Insulin sensitivity is indicated in the context of glucose metabolism and plasma insulin homeostasis, surrogate markers that may be inappropriate for identifying key regulatory determinants of chylomicron homeostasis and metabolism.

In these otherwise healthy HR, the findings indicate that classical markers of plasma lipid homeostasis provide no valuable insight into chylomicron metabolism, remnant homeostasis and by extension atherogenic risk. Plasma TAG, cholesterol within the LDL, HDL and non-HDL-cholesterol were otherwise normal and not associated with apo B-48 IAUC measures.

The limitations of the present study are the relatively small number of participants and the unbalanced sex distribution between groups, nonetheless the study was sufficiently powered to demonstrate treatment effects. In the present study, the limited number of postprandial time points for blood collection in each postprandial course may miss an early or late apo B-48 or TAG peak resulting in underestimation or overestimation of apo B-48 postprandial response. However three time points blood collection for oral fat tolerance test have been shown to have high correlation with hourly blood collections following an oral fat tolerance test<sup>(46)</sup>. The current guidelines adopted by several countries have used non-fasting TAG concentration of 1.89 or 2 mmol/l or more as the cut-off value for increased risk of CVD events<sup>(47)</sup>. Further examination with a higher cut-off value (i.e. 2 mmol/l) is warranted. Analysis of apo C, apo E and LPL are necessary due to their role in lipolysis and remnant





uptake. In the present study, we did not examine LPL activity and those apo (apo C-II, apo C-III, apo E) therefore we could not determine which key apo or enzymes interacts synergistically with dietary fatty acid in the HR group.

In conclusion, we have demonstrated that some healthy normotriglyceridaemic individuals are susceptible to develop exaggerated chylomicron remnant aemia in response to meals enriched in SFA derived from PO. HR subjects were susceptible to a greater exposure of atherogenic small-sized remnants in response to PO-enriched meals with modest or no effects indicated for RBO and CO. Silent hyper-remnant aemia in response to PO-enriched meals in some normolipidemic subjects may contribute to atherogenic risk, but not in others.

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