Delayed clearance of triglyceride-rich lipoproteins in young, healthy obese subjects*

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What is already known about this subject

- Up to 10% of obese subjects can be considered metabolically healthy to date.
- Postprandial triglyceridaemia is well documented in overweight and obese subjects with fasting hypertriglyceridaemia.
- Diverging results exist for chylomicron triglycerides.

What this study adds

- As far as we know, this is the first report that shows that metabolically apparently healthy obese subjects have a delayed postprandial clearance of chylomicron triglycerides.
- This study also points out the possible need of a new and lower reference level for fasting triglyceride levels in obese subjects.

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Summary

Obesity is associated with the metabolic syndrome. The aims were, first, to study the postprandial triglyceride clearance in young, healthy obese subjects and, second, to investigate if fasting triglycerides can predict delayed postprandial triglyceride clearance. Eighteen apparently healthy, obese subjects with no clinical signs of metabolic disturbances participated. Controls were age- and sex-matched, healthy, normal weight subjects. Subclinical markers of metabolic disturbances were assessed by measuring postprandial triglycerides in serum and in chylomicrons by oral fat tolerance test. Postprandial triglyceride clearance for 8 h was assessed indirectly as removal of the lipid from serum during the oral fat tolerance test. Insulin resistance was measured by the homeostasis model assessment of insulin resistance (HOMA-IR). Twelve (66%) of the apparently healthy obese individuals had insulin resistance measured by HOMA-IR. There was a delayed clearance of serum triglycerides and chylomicron triglycerides at 6 h when compared with the control group, while, at 8 h, the differences were only detected for the chylomicron triglyceride clearance. Triglyceride response was significantly greater in the obese subjects. Fasting triglycerides in upper normal level predicted a delayed postprandial triglyceride clearance and insulin resistance. In young, apparently healthy obese subjects early metabolic disturbances including insulin resistance and delayed postprandial triglyceride clearance can be detected. Fasting serum triglyceride in upper normal level predicted delayed postprandial triglyceride clearance and insulin resistance.

Keywords: Chylomicrons, fasting triglycerides, insulin resistance, metabolic healthy obese.

Abbreviations

Apo A-I, apolipoprotein A-I; ApoB-100, apolipoprotein B-100; BMI, body mass index; CM, chylomicrons; CM-TGR, chylomicron triglyceride response; DEXA, dual X-ray absorptiometry; HDL, high-density lipoproteins; HOMA-IR, insulin resistance by the homeostasis model assessment; IR, insulin resistance; LDL, low-density lipoprotein; LPL, lipoprotein lipase; NaCl, natrium chloride; OFTT, oral fat tolerance test; OGTT, oral glucose tolerance test; RM-ANOVA, repeated measure analysis of variance; ROC, receiver-operating characteristic; SE-TG, serum triglyceride; TG, triglycerides; TGR, triglyceride response; TRL, triglyceride-rich lipoproteins; VLDL, very low-density lipoproteins; WBISI, whole body insulin sensitivity index.

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Introduction

Overweight and obesity, the sixth most important risk factor for global death and disease burden (1), are raising global health problems with several metabolic disturbances and comorbidities, such as type 2 diabetes and cardiovascular disease. Environmental challenges such as a sedentary lifestyle and excessive intake of processed food contribute to the increased prevalence of obesity (2,3), with a substantial part of life in the postprandial state. Hypertriglyceridaemia is the typical lipid disturbance in overweight and obesity (4) and contributes to atherosclerosis. A milestone in the understanding of the atherosclerotic process was the proposal of Zilversmith in 1979 (5) of a reduced and prolonged clearance of postprandial accumulation of triglyceride-rich lipoproteins (TRLs) as one of the main pathophysiological events in the atherosclerotic process. This was later supported by studies showing that chylomicron (CM) remnant particles penetrate efficiently and are retained selectively in early atherosclerotic lesions of the vessel wall (6,7), and contribute to coronary atherosclerotic disease by delayed elimination of postprandial TRL (8,9). The enzyme lipoprotein lipase (LPL) plays a pivotal role in the lipoprotein metabolism by hydrolysing TG in CM. Activation of LPL results in hydrolysing of CM and development of TG in small CM remnants (10), hydrolysing TG in very low-density lipoproteins (VLDLs) assembled in the liver, contributing fatty acids to the vascular endothelium and, finally, removing them from the bloodstream (11). LPL activity in the vascular endothelium is regulated by insulin, and the insulin resistance (IR) typically found in overweight and obese individuals (12) may contribute to a delayed removal of postprandial TRL and its highly association to overweight (4) and especially to abdominal obesity (13–16).

Our knowledge of lipid disturbances in young, healthy overweight and obese subjects is poor, especially for postprandial triglyceride metabolism focusing on CM. Both delayed postprandial plasma TG response, in obese men and women (17), and serum TG (SE-TG) (18) in overweight men have been detected, but as far as we know no differences have been found in the CM compartment.

Therefore, in this report postprandial TG metabolism in serum and in CM has been studied in young, apparently healthy, obese subjects without signs of metabolic disturbances, including normolipidaemia.

Methods

Participants

Volunteers were recruited from the Centre of Obesity, Department of Gastroenterology and Nutrition, University Hospital of North Norway. Posters were also used to recruit obese individuals and healthy controls to participate in the study. The inclusion criteria for the obese subjects were body mass index (BMI) >30 kg m^{-2}, age 18–40 years old, normotensive, normoglycaemic, normolipaemic, no history of diabetes and not pregnant. Exclusion criteria were smoking, serious mental and somatic diseases, and patients on anti-obesity drugs. The inclusion and exclusion criteria for the age- and sex-matched healthy controls were the same, except having normal weight (BMI < 25).

Participants were informed and signed a written consent. Of the 40 obese subjects who were screened for participation, 13 did not answer after receiving more info, and nine were excluded mainly because of high blood pressure and high blood lipids. The remaining 18 subjects were eligible to participate, and were defined as 'healthy obese'. A 'healthy obese' individual is, in this study, defined as an obese individual whom would be classified as apparently healthy by standard clinical evaluation and biochemical measurements by a general practitioner.

Height, body weight, BMI and waist circumference were measured and blood tests were drawn. These included fasting glucose (FG), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and TG.

Dual X-ray absorptiometry (DEXA; Lunar Prodigy Advance, GE Healthcare, USA) was collected at baseline for all subjects. The DEXA measured total fat percentage, abdominal fat percentage, total fat mass (kg) and total muscle mass (kg).

Oral glucose tolerance test (OGTT)

OGTT was conducted using an oral intake of 75 g of glucose in solution, after a 12 h night-fast. Blood tests were drawn from the antecubital vein before ingestion (0 min) and at 30, 60, 90 and 120 min after intake. Glucose and insulin were measured at all time points.

Oral fat tolerance test

On the test day, the participants did an 8 h oral fat tolerance test (OFTT) to indirectly measure TG clearance. OFTT has been proven to be a good, indirect and qualitative measure of triglyceride clearance (19). Three days prior to testing, the subjects had a normal food intake, no alcohol intake and had abstained from heavy physical activity. The participants fasted for 12 h before the start of the test. They were at rest and not allowed to smoke, chew a gum or drink anything other than water during the test day. The OFTT was conducted using a test meal prepared from standard sour cream porridge and double cream, together containing 70% calories of fat, of which 66% was saturated fat, 32% was monounsaturated fat and 2% was polyunsaturated fat (20). A freshly prepared test meal

was served with two teaspoons of white sugar (10 g of carbohydrates), cinnamon and one glass (100 mL) of calorie-free lemonade. The participants were served a weight-adjusted meal (1 g of fat per kg body weight) at 08:00 h (baseline) and the meal was consumed within a 15-min period. The participants were offered a 500-mL calorie-free beverage and one fruit (pear or apple) at 12:00 h (4 h). Blood samples for serum and Ethylenedia-minetetraacetic acid (EDTA) plasma for isolation of CM were collected before the test meal (baseline) and every second hour over the next 8 h. The TG clearances at 6 and 8 h were calculated by the following formula: Clearance 6 h = 100 × (1 − ({[TG (6 h) − TG (0 h)]/[TG (max) − TG (0 h)]}). Triglyceride response (TGR) in CM and in SE-TG was calculated as the mean of the two highest postprandial values minus the baseline value of SE-TG (21).

Isolation of CMs

CM were isolated by overlayering 8 mL of EDTA plasma with 5 mL of natrium chloride (NaCl) solution (a density of 1.006 kg L^{-1} NaCl solution with 0.02% sodium azide and 0.01% EDTA) in a cellulose nitrate tube (Beckman Instruments Inc., CA, USA) and centrifuged in a Beckman SW40 Ti swinging bucket rotor (Beckman Coulter Inc., Brea, California, USA) at 20 000 rpm for 1 h at 20° C (22). The CM, with Svedberg flotation rates >400 × 10^{-13} s, was carefully removed by aspiration from the top of the tubes by (23) dividing into three aliquots in cryovials, flushed with nitrogen and frozen at −70° C until further analysis.

Serum lipid and apolipoprotein measurements

Serum lipids were analysed on a Hitachi 737 automatic analyser (Boehringer Mannheim GmbH, Mannheim, Germany) according to manufacturer's recommendations. Total cholesterol (our laboratory reference value was 18–29 years: 2.9–6.1 mmol L^{-1}, 30–49 years: 3.3–6.9 mmol L^{-1}, ≥50 years: 3.9–7.8 mmol L^{-1}) was measured with an enzymatic colorimetric method (CHOD-PAP) and HDL cholesterol (our laboratory reference value was for women: 1.0–2.7 mmol L^{-1} and men: 0.8–2.1 mmol L^{-1}) was assayed by the same procedure after precipitation LDL with heparin and manganese chloride as described by Burstein *et al*. (24). TG concentration in serum (our laboratory reference value was 0.5–2.6 mmol L^{-1}) and in CM was determined with an enzymatic colorimetric test (GPO-PAP). LDL cholesterol was calculated by the formula of Friedewald *et al*. (25): LDL cholesterol = Total cholesterol − HDL cholesterol − 0.47 × serum triglycerides. Apolipoproteins A-I and B-100 (ApoB-100) were measured immunochemically by rate nephelometry using the Array Protein System from Beckman Instruments Inc. (Brea, CA, USA).

Measurements for insulin sensitivity

Serum insulin was analysed directly through a commercial enzyme-linked immunosorbent assay (ELISA) kit (DRG insulin ELISA kit, DRG Instruments GmbH, Marburg, Germany). IR determination by the homeostasis model assessment of IR (HOMA-IR) and supplementary by the whole body insulin sensitivity index (WBISI) were calculated. IR was calculated as follows: HOMA-IR = Fasting insulin (FI) (mU L^{-1}) × FG (mmol L^{-1})/22.5 (26), WBISI = 10 000/[FI (mU L^{-1}) × FG (mg dL^{-1}) × mean insulin (mU L^{-1}) × mean glucose (mg dL^{-1})]1/2 (27). The cut-off value of 95% confidence interval (CI) of HOMA-IR in the normal weight subjects was considered as the limits of normality (28–30).

Statistics

Statistics were calculated on SPSS 19 IBM for Windows (SPSS Inc., Chicago, IL, USA). Microsoft Excel (Microsoft corp., Redmond, Washington, USA) was used for calculating HOMA-IR, WBISI, TG clearance and TGR. Normal distribution was detected by determination of skewness and histograms. Parametric statistics were performed when either raw or transformed data resembled normal distribution; otherwise, non-parametric tests were used. Tests for independent or paired samples were used as appropriate. A repeated measure analysis of variance (RM-ANOVA) was used to analyse data from the OFTT. Corrections for deviation from the assumption of sphericity were used as appropriate. An RM-ANOVA was performed to modulate postprandial TG profile as predicted by weight group and fasting TG group (all subjects were ranked according to fasting TG and split in two equal groups with cut-off of 1.02 mmol L^{-1}). Two-sided *P*-values < 0.05 were considered statistically significant. The receiver-operating characteristic curves of HOMA-IR for fasting TG, total fat percentage, abdominal fat percentage and BMI were depicted, and the optimal cut-offs were determined by 95% CI for the normal weight controls.

The study was approved by the Regional Committee of Medical Ethics of North Norway and the Norwegian Social Science Data Services.

Results

Subject characteristics

The anthropometric, clinical and metabolic characteristics for the normal weight subjects and the healthy obese subjects are shown in Table 1. As expected, the anthropometric data showed several differences. The healthy obese subjects had several metabolic parameters that were significantly increased compared with controls, although still within the normal range (Table 1).

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Table 1 Anthropometric, metabolic and clinical characteristics at baseline between normal weight and obese subjects

Variables	Baseline	
	Normal weight subjects ($n = 17$)	Obese subjects ($n = 18$)
Sex (M/F)	2/15	3/16
BMI (kg m^{-2})	22 (21, 23)	37 (34, 38)***
Total fat percent (%)	27 (24, 30)	49 (46, 51)***
Abdominal fat percent (%)	28 (24, 31)	56 (54, 58)***
Systolic BP (mmHg)	108 (102, 114)	124 (118, 130)***
Diastolic BP (mmHg)	65 (62, 68)	73 (69, 76)***
Glucose (mmol L^{-1})	4.5 (4.3, 4.7)	4.8 (4.5, 5.0)
Insulin (μmol L^{-1})	5.1 (4.0, 6.2)	12.1 (9.8, 14.5)***†
HOMA-IR	1 (0.8, 1.2)	2.6 (2.0, 3.2)***†
WBISI	157 (130, 191)‡	62 (52, 74)***‡
Total cholesterol (mmol L^{-1})	4.5 (4.1, 4.8)	4.2 (3.9, 4.5)
LDL cholesterol (mmol L^{-1})	2.7 (2.3, 3.0)	2.7 (2.4, 3.1)
HDL cholesterol (mmol L^{-1})	1.6 (1.4, 1.8)	1.2 (1.0–1.3)***
HDL/LDL ratio	0.7 (0.5, 0.8)	0.5 (0.4, 0.6)*†
TG (mmol L^{-1})	0.9 (0.7, 1.0)	1.3 (1.1, 1.6)**

Values are mean (95% CI).
†Mann–Whitney non-parametric test.
‡Geometric mean.
*$P < 0.05$.
**$P < 0.005$.
***$P < 0.0001$.
BMI, body mass index; BP, blood pressure; CI, confidence interval; HDL, high-density lipoprotein; HOMA-IR, insulin resistance by the homeostasis model assessment; LDL, low-density lipoprotein; TG, triglyceride; WBISI, whole body insulin sensitivity index.

Insulin sensitivity

There was a significant difference in FI and a close to significant difference in fasting plasma glucose at baseline (Table 1). The cut-off value of the HOMA-IR defined by 95% CI on the normal weight subjects was defined as IR and was calculated to be >1.83. Twelve of the 18 healthy obese subjects (66%) had IR measured by HOMA-IR. Furthermore, insulin sensitivity was significantly lower (higher HOMA-IR, lower WBISI) in the healthy obese subjects (Table 1).

Postprandial triglyceride profiles

Results from the OFTT were analysed by RM-ANOVA, and the estimated marginal means are shown in Fig. 1.

Serum triglyceride

Grand (all time points) mean of the total SE-TG levels was 0.54 mmol L^{-1} (0.20–0.94) higher in healthy obese subjects when compared with the normal weight subjects ($P = 0.01$). A significant interaction between time and subject group was detected ($P = 0.004$; Greenhouse–
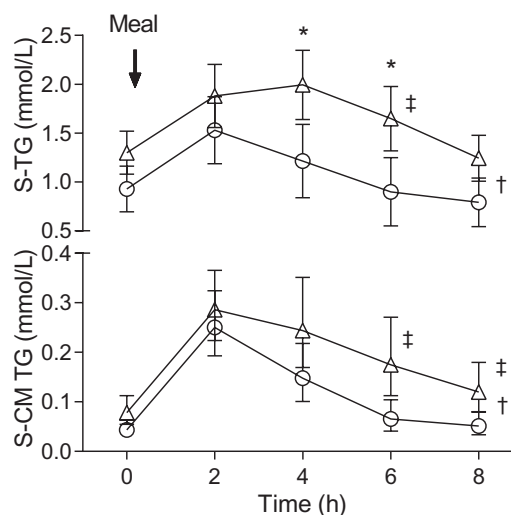
Figure 1 Oral fat tolerance test in apparently healthy, obese and normal weight subjects at study baseline. Upper part shows total serum triglycerides (SE-TG) and lower part shows chylomicron triglycerides (CM-TG). At 0 h, the subject ingested a standard meal. Asterisks denote significant difference (repeated measure analysis of variance [RM-ANOVA]: interaction time × group; post hoc comparison, Bonferroni correction). Dagger denotes a significant difference in grand mean (RM-ANOVA, between subjects). Double dagger denotes significant difference in triglyceride clearance at 6 or 8 h (Wilcoxon). Triangles represent obese subjects; circles represent normal weight subjects. Values are estimated marginal means (95% confidence interval).

Geisser). The contrast showed significantly higher SE-TG levels for the obese subjects at time points 4 ($P = 0.015$) and 6 h ($P = 0.009$) compared with the normal weight subjects (Fig. 1).

CM triglyceride

The healthy obese subjects had overall 1.8 (1.3–2.7) times higher CM-TG (grand mean, transformed raw data, $P = 0.002$) (Fig. 1). No difference in time course between subject groups was detected.

Triglyceride clearance

The results of total SE-TG and CM-TG clearance calculations are presented in Table 2. At 6 h, a significant difference between subject groups in both SE-TG ($P < 0.001$) and CM-TG ($P = 0.011$) clearance is noted, whereas at 8 h the differences between subject groups can only be detected in CM-TG clearance ($P = 0.007$).

TGR

The results of the serum TGR (SE-TGR) and CM-TGR are shown in Table 2. There was a significant difference between subject groups in both SE-TGR ($P = 0.013$) and CM-TGR ($P = 0.006$). Significant correlations were also found between SE-TGR and each of the variables BMI,

Table 2 Postprandial triglyceride (TG) clearance, chylomicron triglyceride (CM-TG) clearance, triglyceride response (TGR) and chylomicron triglyceride response (CM-TGR) after oral fat tolerance test at baseline

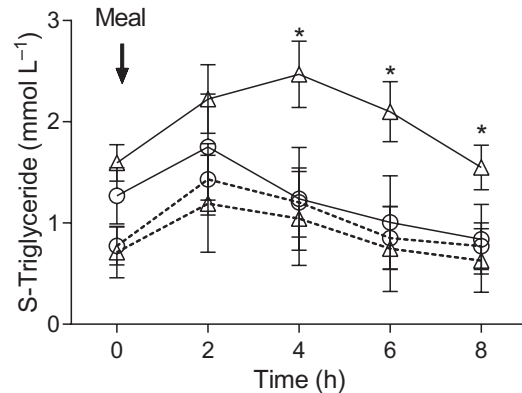|	Normal weight	Obese	*P*
6 h SE-TG clearance (%)*	115 (163)	61 (96)	0.000
8 h SE-TG clearance (%)*	125 (257)	103 (100)	0.123
6 h CM-TG clearance (%)*	88 (110)	60 (104)	0.011
8 h CM-TG clearance (%)*	98 (97)	78 (114)	0.007
TGR*	0.34 (1.8)	0.63 (1.5)	0.013
CM-TGR*	0.15 (0.36)	0.22 (0.5)	0.006

Values are median (range).
*Mann–Whitney *U*-test.

Figure 2 Total serum triglycerides (SE-TG) in oral fat tolerance test in normal weight and apparently healthy, obese subjects. Estimated marginal means (standard error of the mean, SEM) of the observed interaction between weight group, fasting triglyceride group and time is plotted. Triangles represent obese subjects; circles represent normal weight subjects. Solid lines indicate fasting triglyceride level >1.02 mmol L^{-1}; dashed lines indicate fasting triglyceride level ≤1.02 mmol L^{-1}. Asterisks indicate significant contrast. Values are estimated marginal means (95% confidence interval).

total bodyweight (kg), total fat mass (kg) and abdominal fat percentage (data not shown). The cut-off value of the grand SE-TGR and CM-TGR defined by 95% CI on the normal weight subjects is defined abnormal (pathological), and was calculated to be SE-TGR > 0.64. Nine (50%) of the healthy obese subjects had an abnormal high SE-TGR (>0.64). The cut-off value of the grand CM-TGR was calculated to be CM-TGR > 0.28. Seven (39%) of the healthy obese subjects had an abnormally high CM-TGR (>0.28). Significant correlations were also found between CM-TGR and each of the variables BMI, total bodyweight (kg), total fat mass (kg) and abdominal fat percent (data not shown).

Fasting triglyceride as predictor of postprandial triglyceride clearance

We then studied if fasting TG could reflect the postprandial TG profile. A significant interaction between fasting TG vs. SE-TG at various time points was detected in each group (*P* = 0.008; Greenhouse–Geisser). The contrasts showed significantly higher SE-TG levels for the obese subjects at time points 4 (*P* = 0.023) and 6 h (*P* = 0.013) compared with the normal weight subjects, when adjusted for fasting TG level. The results of SE-TG clearance calculations are presented in Table 2.

A significant interaction between time, subject group and fasting TG category (low normal or high normal; cut-off 1.02 mmol L^{-1}) was found (*P* = 0.007; Greenhouse–Geisser). The contrast showed significantly higher postprandial SE-TG levels for the obese subjects with a higher fasting SE-TG (>1.02 mmol L^{-1}) at time points 4 (*P* = 0.006), 6 (*P* = 0.006) and 8 h (*P* = 0.028). The subjects with high-normal fasting SE-TG had also significantly higher SE-TG (*P* = 0.029) and CM-TG clearance (*P* = 0.027) at 6 h, while the difference at 8 h was only detected in CM-TG clearance (*P* = 0.044). Thus, the 12 obese subjects with high-normal fasting TG calculated as

TG >1.02 mmol L^{-1} had a significant delay in TG clearance (Fig. 2).

Finally, there was a significant non-parametric correlation between insulin sensitivity and postprandial SE-TG clearance at 6 h (HOMA-IR; ρ = −0.636, *P* = 0.000, WBISI; ρ = 0.632, *P* = 0.000; see Fig. 3) and CM-TG clearance at 6 h (HOMA-IR; ρ = −0.493, *P* = 0.005, WBISI: ρ = 0.555, *P* = 0.001), while at 8 h the significant correlation was only found between insulin sensitivity and CM-TG clearance (HOMA-IR; ρ = −0.527, *P* = 0.002, WBISI; ρ = 0.528, *P* = 0.002). In addition, fasting HDL cholesterol significantly correlated to the postprandial CM-TGR (ρ = −0.520, *P* = 0.004), SE-TGR (ρ = −0.429, *P* = 0.013), SE-TG clearance (ρ = 0.757, *P* = 0.000) and CM-TG clearance at 6 h (ρ = 0.540, *P* = 0.002) and CM-TG at 8 h (ρ = 0.608, *P* = 0.000).

Correlation between insulin sensitivity and postprandial TGR

There was significant non-parametric correlations between HOMA-IR and WBISI and both SE-TGR (HOMA-IR; ρ = 0.395, *P* = 0.021, WBISI; ρ = −0.384, *P* = 0.025) and CM-TGR (HOMA-IR; ρ = 0.393, *P* = 0.021, WBISI ρ = −0.375, *P* = 0.049), respectively. Furthermore, 77% of the obese subjects who had a pathological SE-TGR (>0.64), and 86% who had a pathological CM-TGR (>0.28), also had IR measured by HOMA-IR. Of the 12 subjects having IR measured by HOMA-IR, 58% of these subjects also had a pathological SE-TGR, and 50% of the 12 had an abnormally high CM-TGR.

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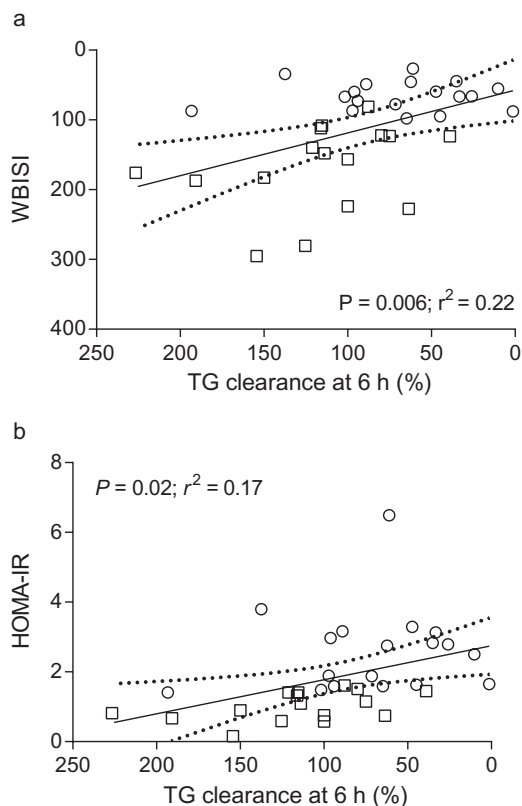
Figure 3 Indirect total serum triglycerides (SE-TG) clearance at 6 h vs. insulin sensitivity (whole body insulin sensitivity index, WBISI; a) and insulin resistance (insulin resistance by the homeostasis model assessment, HOMA-IR; b). Normal weight subjects are shown as squares, obese subjects as circles. Linear regression line is based on all subjects. Dashed lines indicate 95% confidence interval for prediction.

Fasting triglycerides as predictor of insulin sensitivity

Fasting TG as a predictor for IR defined as HOMA-IR > 1.83 (see above) had the sensitivity of 83% and the specificity of 86% with a cut-off value of TG > 1.13 mmol L^{-1}. BMI as a predictor of IR measured by HOMA-IR had the sensitivity of 92% and the specificity of 81%, with a BMI cut-off >29.95. In this study, total body fat percentage as even a better documented predictor of IR [31] had the sensitivity of 92% and the specificity of 72%, with a cut-off total body fat percentage of >41%. Abdominal fat percentage as a predictor of IR had in this study the best sensitivity of 100% and the specificity of 82%, with a cut-off >51%. With a model combining BMI and fasting TG (BMI × fasting TG) as predictors of IR measured by HOMA-IR, the sensitivity was 92% and the specificity was found to be 86% with a cut-off value of >31.3.

Discussion

In this study, we have shown that young, apparently healthy obese subjects have a postprandial delayed metabolism of SE-TG and CM-TG when measured indirectly by OFTT. When adjusted for fasting TG at baseline, the obese subjects still had higher postprandial SE-TG levels compared with the normal weight controls. The obese subjects with a fasting TG > 1.02 mmol L^{-1} and TG > 1.13 mmol L^{-1} had a significantly delayed SE-TG and CM-TG clearance, and pathological insulin sensitivity, respectively. Our data indicate that young, apparently healthy obese subjects have early metabolic disturbances.

Insulin sensitivity

In agreement with other studies (28–30), 66% of the apparently healthy obese subjects had IR defined by HOMA-IR (>1.83) based on 95% CI of the normal weight subjects. This limit is in well agreement with other studies performing comparisons of HOMA-IR to the gold standard method glucose clamp (29). In our study with a median BMI around 35, two-thirds had IR, and this is similar to that observed in other reports with the same BMI (28–30). In the postprandial state, IR is associated with increased intestinal production of CM (32). The RM-ANOVA model of postprandial TG clearance did not include HOMA-IR in addition to fasting TG, in part, because of the low statistical power and, in part, because the statistical model becomes unstable when entering closely correlated variables, such as HOMA-IR and fasting TG. To explore this further, we tested replacing fasting TG with HOMA-IR; however, this model was inferior to the model presented here and explained less of the variance in the data set. In our study, as expected, the postprandial TG clearance, SE-TGR and CM-TGR was significantly correlated to the insulin sensitivity, reflecting the LPL activity in the endothelium (33). There are some factors related to IR that we did not measure in this study. Among them are hepatic lipid content, gastric motility, leptin, adiponectin and modified VLDL export. It would be of interest to investigate the combined relationship among these factors and postprandial triglyceride clearance in a future study.

Characteristics of postprandial triglyceride metabolism

Using an OFTT test, there was a delayed peak and clearance of postprandial SE-TG and CM-TG in the obese subjects compared with the normal weight controls. Moreover, CM-TG was the most sensitive test as significant differences were observed both after 6 and 8 h postprandial. A postprandial delayed SE-TG clearance is well documented in overweight and obese subjects with fasting

hypertriglyceridaemia (for review, see (33)), whereas diverging results exist for CM-TG (15). Moreover, similar studies in healthy, obese subjects with normal fasting TG few reports exist, and especially for CM-TG. In two studies of obese subjects with normal fasting TG levels, a delayed postprandial metabolism of TG was observed, but the study groups were small (17,18). As far as we know, no reports exist for postprandial CM-TG. The postprandial TG in serum and in CM has been reported to be a key marker, and a more sensitive risk factor for atherosclerosis than the corresponding fasting levels (34) (for review see (35)). The greater the magnitude and duration of the postprandial TG response, the arterial wall will be more exposed to postprandial TRL. The longer duration of the postprandial TRL in the bloodstream will give more time to replace cholesterol ester in LDL and HDL, favouring the transformation of LDL to be a smaller and more pro-atherogenic particle, and making HDL more dysfunctional. Moreover, the mechanism of TRL's influence on lowering the HDL level is believed to be due to the enrichment of TG to the HDL particle, which leads to increased catabolism of Apo-A-I HDL (for review see (36)).

In our study, the fasting TG levels were significantly higher, and the fasting HDL cholesterol was significantly lower in the healthy obese subjects than in the normal weight controls, although within the normal range. As expected the fasting HDL cholesterol significantly correlated to the postprandial CM-TGR, SE-TGR, SE-TG clearance and CM-TG clearance at 6 h, and CM-TG at 8 h. This implies that an OFTT can be performed to unmask early changes in the TG metabolism in overweight and obese subjects that may have strategic therapeutical implications. The OFTT may be a way to identify overweight and obese subjects at high risk of developing the metabolic disturbances.

There are several lifestyle factors contributing to delayed clearance of postprandial triglycerides. Among them are low level of physical activity, low intake of omega 3 fatty acids and high alcohol intake. Some studies have shown that low fat and high carbohydrate diets may increase the postprandial triglycerides. The study subjects did a 12-h fast before the OFTT, in agreement with other studies (20,21,37). However, if the subjects would have a longer fasting period, one might hypothesize an even lower postprandial triglyceride level in both groups. However, to explore real-life reflection of postprandial triglycerides in the study subjects, 12 h fast is considered enough to reach a fasting state. This was also reflected on the TG profile during the OFTT of duration of 8 h. In addition, the study subjects had a 'normal' food intake, without extensive amount of fat and no alcohol 3 days prior to the OFTT, to reflect the reference population. However, they did not fill out a 3-day food or activity diary and the amount of omega-3 fatty acids in the diet was not investigated; this

may be a weakness of the study. On the other hand, the participants did not follow a specific diet at inclusion, as we wanted to explore the reference population in a non-dieting group.

Fasting triglyceride as predictor of postprandial lipid profile

All of the apparently healthy obese subjects included in the study had fasting TG in the normal range. In addition, the obese subjects with high-normal fasting TG (>1.02 mmol L^{-1}) had delayed postprandial TG clearance compared with both the obese subjects with low normal fasting TG and normal weight subjects. This indicates that in healthy, obese subjects fasting TG in the upper normal range predicts a delayed postprandial TG clearance both in serum and in CM. It is well known that fasting TG levels are strongly associated to the postprandial TG metabolism (38). Another strong influence on the postprandial TG profile is abdominal obesity (13–16). All of the apparently healthy, obese subjects had abdominal obesity according to the WHO and International Diabetes Federation (waist circumference >88 cm [80] for women and >102 cm [94] for men). In our study, abdominal fat percentage was strongly correlated to postprandial TG profile as expected (13–16).

Fasting triglycerides as a predictor for insulin sensitivity

IR is closely related to postprandial lipid metabolism because of insulin's influence on LPL activity in the vascular endothelium (12). In our study, 77% of the obese subjects who had a pathological SE-TGR, and 86% who had a pathological CM-TGR, also had IR measured by HOMA-IR. Moreover, we also found that fasting TG, in the normal range, predicted IR with a sensitivity of 83% and the specificity of 86% with a cut-off value of TG > 1.13 mmol L^{-1}. Our findings that fasting TG in the normal range in obese subjects can predict IR is of great utility in clinical practice. The calibrated cut-off of fasting TG of 1.13 mmol L^{-1} should therefore be followed up with a validation study.

The advantage of this study is that it focuses on TRLs, with a specific focus on CMs on apparently healthy, non-dieting, obese subjects.

However, in our study there are also some areas with limitations. First, the sex distribution is unbalanced among our participants. This indicates a need to confirm our results in a larger study population to explore sex differences. Second, a quantitative and direct estimate of the triglyceride clearance like isotopic labelling is the gold standard; in our study, triglyceride clearance was estimated indirectly. However, OFTT has been proven to have a

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strong correlation to triglyceride clearance when compared with other methods (19). Third, LPL activity (11) was not measured that would also be of importance in a future study.

Conclusion

In apparently healthy obese subjects, early metabolic disturbances with delayed metabolism of postprandial SE-TG and CM can be observed. When adjusted for fasting TG at baseline, the obese subjects still had higher postprandial SE-TG levels compared with normal weight controls. A sub-analysis revealed that fasting TG level in the higher normal range predicted delayed postprandial TG clearance and IR, but only in obese subjects. Further studies should focus on a new and possibly lower normal range for fasting TG in obese subjects, as this may have therapeutic implications.

Conflict of Interest Statement

No conflict of interest was declared.

Author contributions

MAL designed the study, conceived and carried out the experiments and data collection, analysed the data and wrote the manuscript. RG analysed the data, supervised the manuscript and generated figures. SL contributed to the design of the study. OSM carried out some of the tests. JF designed the study and supervised the manuscript. All the authors had final approval of the article before submission.

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