WAT apoC-I secretion: role in delayed chylomicron clearance in vivo and ex vivo in WAT in obese subjects

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Abstract Reduced white adipose tissue (WAT) LPL activity delays plasma clearance of TG-rich lipoproteins (TRLs). We reported the secretion of apoC-I, an LPL inhibitor, from WAT ex vivo in women. Therefore we hypothesized that WAT-secreted apoC-I associates with reduced WAT LPL activity and TRL clearance. WAT apoC-I secretion averaged 86.9 ± 31.4 pmol/g/4 h and 74.1 ± 36.6 pmol/g/4 h in 28 women and 11 men with BMI ≥27 kg/m², respectively, with no sex differences. Following the ingestion of a 13C-triolein-labeled high-fat meal, subjects with high WAT apoC-I secretion (above median) had delayed postprandial plasma clearance of dietary TRLs, assessed from plasma 13C-triolein-labeled TGs and apoB48. They also had reduced hydrolysis and storage of synthetic 3H-triolein-labeled (3H)-TRLs in WAT ex vivo (i.e., in situ LPL activity). Adjusting for WAT in situ LPL activity eliminated group differences in chylomicron clearance; while adjusting for plasma apoC-I, 3H-NEFA uptake by WAT, or body composition did not. apoC-I inhibited in situ LPL activity in adipocytes in both a concentration- and time-dependent manner. There was no change in postprandial WAT apoC-I secretion. WAT apoC-I secretion may inhibit WAT LPL activity and promote delayed chylomicron clearance in overweight and obese subjects. We propose that reducing WAT apoC-I secretion ameliorates postprandial TRL clearance in humans.—Cyr, Y., H. Wassef, S. Bissonnette, V. Lamantia, J. Davignon, and M. Faraj. WAT apoC-I secretion: role in delayed chylomicron clearance in vivo and ex vivo in WAT in obese subjects. J. Lipid Res. 2016. 57: 1074–1085.

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The cardiometabolic risk associated with reduced HDL cholesterol (HDL-C) and/or elevated LDL cholesterol (LDL-C) has been well-established, the cardiometabolic risk associated with elevated TG-rich lipoproteins (TRLs) has not. However, elevated concentrations of TRLs have received much interest lately given their association with obesity, insulin resistance, and type 2 diabetes (1, 2). Two recent meta-analyses identified hypertriglyceridemia as a predictor of cardiovascular disease (3, 4). Although controversy exists regarding the independent contribution of fasting hypertriglyceridemia because of its inverse relationship with HDL-C (3, 5), that of postprandial hypertriglyceridemia remains sturdy even after adjustment for HDL-C (5–7). Accordingly, much research has now focused on factors that regulate postprandial TRL clearance.

Clearance of postprandial TRLs is highly dependent on white adipose tissue (WAT). A fundamental function of WAT in the postprandial state is the hydrolysis of the TG core of TRLs through the activity of endothelial LPL and the uptake and storage of released NEFAs (8–10). Dysfunctional WAT has reduced metabolic flexibility and is unable to switch promptly from fasting (catabolic) to postprandial (anabolic) state. This leads to delayed TRL clearance, impaired remodeling of circulating lipoproteins, and increased TRL flux to peripheral tissues; thereby increasing cardiometabolic risks (11, 12). The underlying mechanisms promoting WAT dysfunction and delayed TRL clearance are not fully understood.

Abbreviations: 13C-NEFA, 13C-triolein-labeled NEFA; 13C-TG, 13C-triolein-labeled TG; HDL-C, HDL cholesterol; 3H-NEFA, 3H-tri olein-labeled NEFA; HOMA-IR, homeostatic model assessment of insulin resistance; 3H-TG, 3H-triolein-labeled TG; 3H-TRL, 3H-triolein-labeled TG-rich lipoproteins; iAUC6hrs, incremental area under the curve over 6 hours; IRCM, Institut de recherches cliniques de Montréal; LDL-C, LDL cholesterol; TRL, TG-rich lipoprotein; VLDL-C, VLDL cholesterol; WAT, white adipose tissue.

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Postprandial clearance of TRLs is dependent on multiple transferable apolipoproteins that activate or inhibit LPL activity and TRL clearance, including apoC-I (13–16). apoC-I is a 6.6 kDa apolipoprotein that is primarily secreted from the liver and, in the fasting state, is mainly carried on HDLs (~80%) with a minor fraction carried on TRLs (~7%) (14, 16, 17). ApoC-I enrichment of HDLs is anti-atherogenic, as it promotes increased HDL size and cholesterol content (16–19). On the other hand, apoC-I enrichment of TRLs promotes delayed plasma clearance of TRLs (13, 14, 19). In murine studies, this is reported to be secondary to apoC-I-induced inhibition of LPL activity (20, 21) independent of apoC-III (22) and apoE-dependent clearance of TRLs by VLDL receptor (23), LDL receptor (24), and LDL receptor-related protein (25). The local production of apoC-I from macrophages taken from mice overexpressing human apoC-I has also been reported to bind NEFAs and reduce their esterification (26).

The role of apoC-I in TRL clearance by WAT is, to our knowledge, not examined in humans. We were the first to report the secretion of apoC-I from a human adipocyte model (27) and by human subcutaneous WAT ex vivo (14). Moreover, we demonstrated that WAT secretion of apoC-I, but not of apoC-II, apoC-III, or apoE, was correlated with delayed postprandial plasma clearance of dietary TRL in postmenopausal overweight and obese women (14), while no data existed for men. Given its negative regulation of LPL activity, we hypothesized that WAT apoC-I secretion reduces LPL activity of WAT and, accordingly, TRL clearance by WAT (14). To explore this hypothesis while accounting for sex differences, we examined the association of WAT apoC-I secretion with both TRL clearance ex vivo in WAT, and postprandial plasma clearance of dietary TRLs in vivo in 39 overweight and obese men and postmenopausal women. We further verified the direct effect of purified human apoC-I on the clearance of synthetic TRLs in vitro in adipocytes.

MATERIALS AND METHODS

Study population

Metabolic studies examining TRL clearance in vivo and ex vivo in WAT were conducted between 2010 and 2014 at the Institut de recherches cliniques de Montréal (IRCM). Subjects were recruited by newspaper advertisement and their inclusion and exclusion criteria were previously reported (14, 28–30). In brief, recruited subjects had BMI ≥27 kg/m², were aged 45–74 years, were sedentary nonsmokers with low-alcohol consumption (~2 servings per day), and women were postmenopausal. The exclusion criteria were chronic diseases such as diabetes, cardiovascular, hepatic, or renal diseases, cancer within the past 3 years, problems with blood coagulation, concomitant medications affecting metabolism including lipid lowering and hormone replacement therapy (except thyroid hormone at stable dose), and known substance abuse. All participants signed a consent form before initiating the study, which was approved by the ethics board at IRCM.

Forty-five subjects were recruited, of which six subjects were excluded from the analysis: one woman did not complete the postprandial fat clearance test, two women and two men had yields of WAT biopsies that were too small to be used, and one man had a WAT apoC-I secretion level that was ~4.5-fold above the average rendering him a clear outlier in many correlations. Thus this analysis included 28 women and 11 men.

Anthropometric and metabolic parameters (N = 39)

Subjects underwent a 4 week weight stabilization period (~2 kg) prior to testing to eliminate possible effects of weight fluctuation on the measured metabolic outcomes. Body composition was measured by dual energy X-ray absorptiometry (General Electric Lunar Corp., version 6.10.019), plasma glucose by an automated analyzer (YSI 2300 Stat Plus), serum insulin and C-peptide by radioimmunoassay kits (Millipore Corp.), plasma lipids and apoB by an automated analyzer COBAS Integra 400 (Roche Diagnostics), plasma chylomicron concentration by an ELISA kit against human apoB48 (Biovendor), and plasma apoC-I by an in-house ELISA, as previously reported (14, 31). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to Matthews et al. (32). Lipoprotein cholesterol content and LDL diameter were measured by a polycrylamide gel electrophoresis system (FDA-approved, Lipoprint system; Quantimetrix) (14, 29).

Dietary intake (N = 27)

Dietary intake was assessed using 3 day dietary recall (two weekdays and one weekend day) on a subpopulation of subjects (16 women, 11 men), as previously reported (33). Nutritional data was analyzed using the Food Processor software with a Canadian database version 10 (Esha Research, Salem, OR).

Postprandial fat metabolism (N = 39)

Postprandial dietary fat metabolism was assessed as previously reported (14, 29). In brief, subjects consumed a high-fat meal labeled with 13C-triolein [glycerol tri(oleate-1-13C), 99 atom% 13C; Sigma-Aldrich Canada] standardized according to body surface area (600 kcal/m², 0.017 g 13C-triolein per gram fat, 68% fat, 18% carbohydrate). Plasma concentrations of 13C-triolein-labeled (13C) TG and 13C-NEFA were measured over 0, 1, 2, 4, and 6 h using isotope ratio mass spectrometry (14, 29). The postprandial clearance rates of plasma total TG, 13C-TG, and 13C-NEFA were calculated as the area under the 6 h curve of the incremental increase above baseline levels of plasma lipids (iAUCi6h). The postprandial clearance rate of chylomicron particles was also calculated as iAUCi6h of plasma apoB48.

ApoC-I secretion from subjects’ WAT samples ex vivo (N = 39)

Subjects’ fasting WAT samples were collected by needle biopsy from the right hip (gynoid) area under local anesthesia (Xylocaine, 20 mg/ml; Astrazeneca) as previously described (14, 29). WAT samples were cleaned and preincubated in 1 ml HBSS for 30 min to wash out products of cellular damage induced by the biopsy. WAT samples were blotted dry, weighed, and then incubated in DMEM/F12 containing 5% fetal bovine serum. Fasting WAT apoC-I secretion was assessed as the 4 h accumulation of apoC-I in the incubation medium using an in-house ELISA, as previously reported (14, 31). Postprandial WAT samples were also collected from the left hip 4 h after the ingestion of the high-fat meal in a subpopulation of 19 subjects (11 men, 8 women), and postprandial WAT apoC-I secretion was measured. WAT apoC-I secretion in the fasting and postprandial states represents average WAT apoC-I accumulation in three to four wells, with two to four WAT pieces per well, for a total of 5–10 mg WAT per well.
WAT in situ LPL activity and NEFA uptake ex vivo (N = 33)

The hydrolysis of synthetic 3H-triolein-labeled (3H-)TRL and the uptake and incorporation of the released 3H-NEFA is a reflection of in situ LPL activity. It was assessed in subjects’ WAT samples, as previously published (12, 29). Briefly, fasting WAT samples were cleaned, blotted dry, weighed and preincubated in HBSS buffer for ~30 min, then transferred into wells containing 500 μl 3H-TRL substrate [95% TG, 1.27 mmol/l TG, and 0.54 mmol/l Tri-HCL (pH 7.2) in DMEM/F12, 5.1% BSA, and 7.5% fasting human serum, emulsified by sonication] and incubated for an additional 4 h at 37°C on a shaking plate at 300 rpm (12, 29). WAT 3H-lipids were extracted, counted, and expressed as 3H-NEFA uptake per WAT (12, 29). Due to insufficient WAT yields in all included subjects (N = 39), 33 subjects had data for WAT experiments with 3H-TRL and 28 had data for WAT experiments with 3H-NEFA. WAT experiments with 3H-TRL and 3H-NEFA represent the average of WAT samples in three to six wells, with two to four WAT pieces per well, for a total of 5–10 mg WAT per well.

Adipocyte area (N = 39)

The areas of adipocytes of the fasting hip WAT samples were measured by digital imaging as previously reported (14, 28, 29). The average surface area of 986 ± 399 adipocytes, in six fields of view in three WAT slides, is reported per subject.

Direct effect of apolipoprotein C-I on in situ LPL activity in 3T3-L1 adipocytes

The in situ LPL activity of 3T3-L1 adipocytes was determined in the presence or absence of human VLDL-extracted apoC-I

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**TABLE 1.** Fasting baseline characteristics of the study population (N = 39)

|                     | Women (N = 28) | Men (N = 11) | P     |
|---------------------|---------------|-------------|-------|
| **Age (years)**     | 58.5 ± 4.3    | 58.5 ± 6.4  | 0.979 |
| **Anthropometric parameters** |               |             |       |
| Weight (kg)         | 77.9 ± 13.4   | 108.8 ± 25.8| <0.001|
| BMI (kg/m²)         | 31.4 ± 4.4    | 36.1 ± 7.0  | 0.017 |
| Waist circumference (cm) | 107.7 ± 10.7 | 122.3 ± 14.8| <0.001|
| Hip circumference (cm) | 111.6 ± 9.0 | 116.3 ± 13.0| 0.201 |
| Waist/hip ratio     | 0.92 ± 0.06   | 1.05 ± 0.08 | <0.001|
| Total fat (kg)      | 35.2 ± 9.4    | 44.2 ± 16.6 | 0.040 |
| Android fat (kg)    | 3.36 ± 1.05   | 5.37 ± 2.01 | <0.001|
| Gynoid fat (kg)     | 6.15 ± 1.61   | 5.85 ± 2.09 | 0.651 |
| Android/gynoid fat  | 0.55 ± 0.10   | 0.92 ± 0.10 | <0.001|
| **Adipocyte area (μm²)** | 3,135 ± 704   | 3,404 ± 982 | 0.345 |
| **Metabolic parameters** |             |             |       |
| Systolic blood pressure (mmHg) | 120.4 ± 17.9 | 136.5 ± 12.2 | 0.011 |
| Diastolic blood pressure (mmHg) | 76.5 ± 8.0   | 84.3 ± 6.8  | 0.009 |
| Plasma glucose (mmol/l) | 5.15 ± 0.47  | 5.15 ± 0.25 | 0.860 |
| Plasma insulin (μU/ml) | 14.2 ± 5.4   | 22.6 ± 17.9 | 0.031 |
| HOMA-IR [(mmol/l) × (mU/l)] | 3.27 ± 1.35  | 5.11 ± 3.96 | 0.035 |
| Plasma cholesterol (mmol/l) | 5.93 ± 1.06  | 4.96 ± 1.20 | 0.015 |
| Plasma VLDL-C (mmol/l) | 1.01 ± 0.32  | 1.05 ± 0.36 | 0.703 |
| Plasma IDL-C (mmol/l) | 1.69 ± 0.41  | 1.34 ± 0.32 | 0.015 |
| Plasma LDL-C (mmol/l) | 1.95 ± 0.56  | 1.64 ± 0.63 | 0.146 |
| Plasma HDL-C (mmol/l) | 1.46 ± 0.35  | 1.07 ± 0.26 | 0.002 |
| Plasma TG (mmol/l) | 1.92 ± 0.84  | 1.76 ± 0.89 | 0.427 |
| Plasma apoB (g/l) | 1.00 ± 0.26  | 1.03 ± 0.27 | 0.829 |
| Plasma apoB48 (mg/l) | 6.02 ± 4.15  | 7.87 ± 4.45 | 0.421 |
| LDL diameter (Å) | 290 ± 6      | 266 ± 7     | 0.157 |
| Plasma apoC-I (μmol/l) | 23.6 ± 5.7   | 17.5 ± 3.9  | 0.004 |
| WAT apoC-I secretion (pmol/g/4 h) | 86.9 ± 31.4  | 74.1 ± 36.6 | 0.279 |
| WAT apoC-I secretion (min-max) | 36.0–155.5   | 25.6–126.9  | —     |
| **Nutritional parameters** |             |             |       |
| Total energy intake (kcal/day) | 1,895 ± 541  | 2,493 ± 528 | 0.009 |
| Carbohydrates (%) | 46.3 ± 5.4   | 47.0 ± 8.8  | 0.792 |
| Fat (%)           | 36.1 ± 5.1   | 35.2 ± 6.8  | 0.210 |
| Protein (%)       | 16.0 ± 2.1   | 17.0 ± 4.0  | 0.412 |
| Alcohol (%)       | 1.64 ± 2.81  | 2.85 ± 3.09 | 0.298 |
| Saturated fat (%) | 11.1 ± 2.6   | 10.1 ± 2.9  | 0.361 |
| Fiber (g/day)     | 22.0 ± 7.2   | 25.6 ± 8.8  | 0.260 |
| Cholesterol (mg/day) | 253 ± 117   | 359 ± 169   | 0.065 |

Data are presented as average ± SD. min, minimum; max, maximum. Data in bold represent parameters with significant group differences.

*Data from 16 women and 11 men.
Baseline characteristics of the study population are presented in Table 1. Both groups were obese, but men had higher indices of insulin resistance (fasting plasma insulin and HOMA-IR), blood pressure, and adiposity (BMI, weight, waist/hip ratio, android fat, gynoid fat), particularly central (waist circumference, android fat), blood pressure, and adiposity (BMI, weight, android fat, gynoid fat ratio). Women had higher total IDL and HDL-C. Men had a higher total daily energy intake; however there were no sex-differences in the percent of energy from carbohydrate, fat, protein, alcohol, or saturated fat, nor in relation to total daily intake of fiber and cholesterol. Notably, although women had higher total plasma apoC-I, there were no sex differences in fasting WAT apoC-I secretion, suggesting that other apoC-I sources, such as the liver, may be different between the two sexes.

Given the phenotype of this population, we examined whether adiposity or fat distribution correlated with the measured outcomes (Table 2, supplementary Fig. 1). There were no associations between several markers of adiposity with total apoC-I, WAT apoC-I, postprandial fat clearance (total TG, dietary 13C-TG, dietary 13C-NEFA, or apoB48). WAT in situ LPL activity (WAT 3H-lipids and medium 3H-NEFA generated from the hydrolysis of 3H-TRL substrate), or 3H-NEFA uptake (from 3H-NEFA:BSA substrate). Gynoid fat mass, which is metabolically protective, was associated with faster postprandial plasma clearance of 13C-TG and lower WAT 3H-NEFA:BSA uptake. On the other hand, higher abdominal relative to gynoid adiposity was associated with delayed postprandial plasma clearance of 13C-TG and apoB48.

### RESULTS

Baseline characteristics of the study population are presented in Table 1. Both groups were obese, but men had higher indices of insulin resistance (fasting plasma insulin and HOMA-IR), blood pressure, and adiposity (BMI, weight, android fat). Women had a higher total daily energy intake; however there were no sex-differences in the percent of energy from carbohydrate, fat, protein, alcohol, or saturated fat, nor in relation to total daily intake of fiber and cholesterol. Notably, although women had higher total plasma apoC-I, there were no sex differences in fasting WAT apoC-I secretion, suggesting that other apoC-I sources, such as the liver, may be different between the two sexes.

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### Adipose tissue apoC-I and TRL clearance

| Body Composition | Postprandial Plasma Parameters | WAT in situ LPL Activity (i.e., 3H-TRL Substrate) | WAT 3H-NEFA Uptake (i.e., 3H-NEFA:BSA Substrate) |
|------------------|--------------------------------|-----------------------------------------------|--------------------------------------------------|
| Weight (kg) | -0.085 | -0.203 | 0.094 | -0.239 | 0.160 | 0.016 | 0.213 | -0.197 |
| BMI (kg/m²) | -0.095 | -0.168 | -0.118 | -0.292 | 0.111 | -0.091 | 0.168 | -0.341 |
| Waist (cm) | -0.135 | -0.100 | -0.011 | -0.244 | 0.156 | -0.126 | 0.209 | -0.309 |
| Hip (cm) | -0.028 | -0.187 | -0.198 | -0.219 | 0.055 | -0.136 | 0.303 | -0.136 |
| Waist/hip ratio | -0.150 | 0.043 | 0.177 | -0.136 | 0.195 | -0.054 | 0.215 | -0.316 |
| Total fat (kg) | -0.007 | -0.219 | -0.129 | -0.259 | 0.094 | 0.023 | 0.002 | -0.341 |
| Android fat (kg) | -0.098 | -0.210 | 0.001 | -0.282 | 0.131 | -0.005 | 0.130 | -0.419 |
| Gynoid fat (kg) | 0.069 | -0.152 | -0.362 | -0.204 | -0.064 | -0.057 | 0.213 | -0.043 |
| Android/gynoid fat | -0.127 | -0.110 | 0.373 | -0.210 | 0.333 | Data in bold represent parameters with significant correlation. |
We first examined whether the same conclusion could be drawn for both sexes. Subjects were divided into two groups based on median WAT apoC-I secretion per sex (women = 83.2 pmol/g/4 h and men = 83.8 pmol/g/4 h) to assure an equal number of men and women in each group and eliminate possible sex-differences in the measured outcomes. As presented in Table 3, there were no group differences in any of the measured parameters related to adiposity, fat distribution, or adipocyte size. There were no group differences in fasting plasma metabolic parameters, lipids, apoB-lipoprotein particle numbers (total apoB or apoB48), total daily energy intake, and nutritional parameters. Subjects with high WAT apoC-I secretion ex vivo have delayed postprandial plasma clearance of dietary TRL in vivo.

We previously reported that postmenopausal obese women with high WAT apoC-I secretion had delayed postprandial plasma clearance of dietary TG when compared with women with low WAT apoC-I secretion (14). We first examined whether the same conclusion could be drawn for both sexes. Subjects were divided into two groups based on median WAT apoC-I secretion per sex (women = 83.2 pmol/g/4 h and men = 83.8 pmol/g/4 h) to assure an equal number of men and women in each group and eliminate possible sex-differences in the measured outcomes. As presented in Table 3, there were no group differences in any of the measured parameters related to adiposity, fat distribution, or adipocyte size. There were no group differences in fasting plasma metabolic parameters, lipids, apoB-lipoprotein particle numbers (total apoB or apoB48), total daily energy intake, and nutritional parameters. Subjects with high WAT apoC-I secretion had higher fasting plasma apoC-I and VLDL cholesterol (VLDL-C). This is in line with the positive correlation of WAT apoC-I
Adipose tissue apoC-I and TRL clearance further increased the significance of the group differences in iAUC6hrs of apoB48. Adjusting for plasma apoC-I had no effect on the group differences in clearance of apoB48 ($P = 0.005$), but eliminated group differences in dietary $13\text{C}$-TG. Thus the association of WAT apoC-I secretion with postprandial plasma clearance of chylomicrons was independent of adiposity, fat distribution, and fasting plasma apoC-I.

Subjects with high WAT apoC-I secretion have reduced WAT in situ LPL activity ex vivo To test the hypothesis that local WAT apoC-I secretion associates with reduced WAT in situ LPL activity, we measured the hydrolysis of $3\text{H}$-TRL and uptake and incorporation of LPL-generated $3\text{H}$-NEFA as WAT $3\text{H}$-lipids. We also measured the accumulation of $3\text{H}$-NEFA in the medium over 4 h. As presented in Fig. 3, subjects with high WAT apoC-I had reduced WAT $3\text{H}$-lipids compared with subjects with low WAT apoC-I (high = $3.15 \pm 1.97$ nmol vs. low = $5.09 \pm 3.21$ nmol $3\text{H}$-TG hydrolyzed per milligram WAT, with delayed TRL clearance shown previously (14). Thus overall, the two groups had similar anthropometric, metabolic, and nutritional characteristics in the fasting state (Table 3).

Subjects with high WAT secretion had delayed postprandial plasma clearance of dietary TG (Fig. 2B) and apoB48 (Fig. 2D), with no group differences in that of total TG (Fig. 2A) or dietary $13\text{C}$-NEFA (Fig. 2C) (Note that removing the outlier point in plasma $13\text{C}$-NEFA in the high group, has no effect on the group differences).

Given that WAT is a source of apoC-I, we examined whether adjusting the group differences (high vs. low WAT apoC-I secretors) for adiposity affected the outcomes. Adjusting for BMI, total fat, android fat, gynoid fat, or android/gynoid ratio retained group differences in plasma $13\text{C}$-TG and chylomicrons (Fig. 2, $P \leq 0.05$ for all). Adjusting for android/gynoid fat ratio rendered group differences in iAUC6hrs of $13\text{C}$-NEFA significant ($P = 0.038$, with exclusion of the outlier point in the high group), and further increased the significance of the group differences in iAUC6hrs of apoB48. Adjusting for plasma apoC-I had no effect on the group differences in clearance of apoB48 ($P = 0.005$), but eliminated group differences in dietary $13\text{C}$-TG. Thus the association of WAT apoC-I secretion with postprandial plasma clearance of chylomicrons was independent of adiposity, fat distribution, and fasting plasma apoC-I.

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### Table 3. Fasting baseline characteristics of the two groups with low versus high WAT apoC-I secretion separated based on median WAT apoC-I secretion per sex

|                  | Low WAT apoC-I | High WAT apoC-I | $P$  |
|------------------|----------------|-----------------|------|
| Women:men        | 14:5           | 14:6            |      |
| WAT apoC-I secretion (pmol/g/4 h) | **55.3 ± 17.3** | **109.8 ± 19.1** | <0.001 |
| WAT apoC-I secretion (min–max)       | 23.6–80.72     | 83.8–155.3      |      |
| Age (years)      | 56.8 ± 4.9     | 60.0 ± 4.6      | 0.051 |
| Anthropometric parameters            |                |                 |      |
| Weight (kg)      | 87.8 ± 25.3    | 85.6 ± 19.7     | 0.759 |
| BMI (kg/m$^2$)   | 33.4 ± 6.2     | 32.1 ± 5.1      | 0.482 |
| Waist circumference (cm)             | 109.7 ± 15.7   | 106.8 ± 14.1    | 0.551 |
| Hip circumference (cm)               | 113.9 ± 9.8    | 112.9 ± 11.1    | 0.574 |
| Waist/hip ratio | 0.96 ± 0.09    | 0.95 ± 0.10     | 0.830 |
| Total fat (kg)   | 38.6 ± 12.7    | 37.0 ± 12.3     | 0.674 |
| Android fat (kg) | 4.05 ± 1.87    | 3.81 ± 1.45     | 0.653 |
| Gynoid fat (kg)  | 6.22 ± 1.44    | 5.92 ± 2.01     | 0.692 |
| Android/gynoid fat | 0.64 ± 0.20 | 0.66 ± 0.19     | 0.752 |
| Adipocyte area ($\mu$m$^3$)          | 3,172 ± 876    | 3,254 ± 715     | 0.751 |
| Metabolic parameters               |                |                 |      |
| Systolic blood pressure (mmHg)       | 120.5 ± 15.4   | 129.2 ± 19.4    | 0.133 |
| Diastolic blood pressure (mmHg)      | 77.1 ± 9.1     | 80.3 ± 7.5      | 0.256 |
| Plasma glucose (mmol/l)              | 5.03 ± 0.37    | 5.23 ± 0.45     | 0.144 |
| Plasma insulin (µU/ml)               | 19.4 ± 14.7    | 13.9 ± 4.6      | 0.124 |
| HOMA-IR [([mmol/l] × (mU/l))          | 4.33 ± 3.29    | 3.28 ± 1.20     | 0.186 |
| Plasma cholesterol (mmol/l)          | 5.32 ± 0.97    | 5.99 ± 1.27     | 0.073 |
| Plasma VLDL-C (mmol/l)               | **0.92 ± 0.27** | **1.12 ± 0.35** | 0.048 |
| Plasma IDL-C (mmol/l)                | 1.52 ± 0.41    | 1.67 ± 0.42     | 0.287 |
| Plasma LDL-C (mmol/l)                | 1.75 ± 0.47    | 1.98 ± 0.68     | 0.216 |
| Plasma HDL-C (mmol/l)                | 1.27 ± 0.28    | 1.43 ± 0.43     | 0.186 |
| Plasma TG (mmol/l)                   | 1.42 ± 0.71    | 1.75 ± 0.95     | 0.229 |
| Plasma apoB (g/l)                    | 0.93 ± 0.24    | 1.07 ± 0.28     | 0.123 |
| Plasma apoB48 ($\text{mg/l}$)        | 6.29 ± 4.10    | 7.61 ± 4.31     | 0.357 |
| LDL diameter (Å)                     | 269 ± 5        | 267 ± 6         | 0.351 |
| Plasma apoC-I ($\mu$m/l)             | **19.8 ± 5.6** | **24.0 ± 5.4**  | **0.024** |
| Plasma apoC-I (min–max)              | 13.2–32.7      | 14.1–37.6       | —     |
| Nutritional parameters$^a$           |                |                 |      |
| Total energy intake (kcal/day)       | 2,067 ± 659    | 2,205 ± 565     | 0.563 |
| Carbohydrates (%)                    | 46.8 ± 6.7     | 46.5 ± 7.7      | 0.917 |
| Fat (%)                              | 36.2 ± 4.1     | 33.7 ± 7.1      | 0.291 |
| Protein (%)                          | 15.3 ± 2.4     | 17.4 ± 3.2      | 0.072 |
| Alcohol (%)                          | 1.79 ± 2.00    | 2.45 ± 3.65     | 0.565 |
| Saturated fat (%)                    | 11.2 ± 2.7     | 10.2 ± 2.8      | 0.342 |
| Fiber (g/day)                        | 23.7 ± 9.7     | 23.2 ± 6.1      | 0.878 |
| Cholesterol (mg/day)                 | 271 ± 123      | 320 ± 168       | 0.403 |

Data in bold represent parameters with significant group differences. min, minimum; max, maximum.

$^a$N = 5 men in high WAT apoC-I secretion group.

$^b$N = 13 (five men) and N = 14 (six men) in low versus high WAT apoC-I secretion groups, respectively.
may be dependent on reduced WAT LPL activity and not NEFA uptake (whether NEFA was LPL-released or BSA-bound).

WAT apoC-I secretion is not subject to postprandial fluctuation

We examined whether WAT apoC-I secretion was changed postprandially in a subpopulation of 19 subjects. There were no significant postprandial changes in WAT-apoC-I secretion in the entire group (fasting: 86.2 ± 40.5 pmol/g/4 h vs. postprandial: 80.4 ± 60.5 pmol/g/4 h, N = 19) nor per group of low WAT apoC-I secretion (fasting: 46.6 ± 18.2 pmol/g/4 h vs. postprandial: 58.4 ± 44.4 pmol/g/4 h, N = 8) or high WAT apoC-I secretion (fasting: 115.0 ± 23.2 pmol/g/4 h vs. postprandial: 96.4 ± 67.3 pmol/g/4 h, N = 11). Moreover, as in the fasting state, there were no sex-differences in postprandial WAT apoC-I secretion (11 men: 72.4 ± 72.8 pmol/g/4 h vs. 8 women: 91.4 ± 39.8 pmol/g/4 h). There were small associations between postprandial WAT apoC-I secretion with the measured outcomes related to TRL clearance in plasma and in WAT, likely due to the smaller sample size. However, postprandial WAT apoC-I secretion was negatively associated with 3H-TRL hydrolysis by WAT over 4 h ($r = -0.832, P < 0.001$). The postprandial changes in WAT apoC-I secretion, whether expressed as absolute or percent changes, were not associated with any of the measured outcomes.

Fig. 2. Group differences in postprandial plasma clearance of TG (A), $^{13}$C-TG (B), $^{13}$C-NEFA (C), and chylomicrons (apoB48) (D) in subjects with low (N = 19) versus high (N = 20) WAT apoC-I secretion (except for (D) where N = 5 men in the high group for missing data). Women are represented as solid circles and men as open circles.
Adipose tissue apoC-I and TRL clearance

also concentration dependent, reaching a maximum inhibition of $^3$H-TRL hydrolysis and incorporation as intracellular $^3$H-lipids at 15 μM (≈78%, Fig. 4D). Notably, the lack of accumulation of LPL-released $^3$H-NEFA in the medium of the apoC-I-incubated adipocytes (Fig. 4B) suggests that apoC-I does not hinder the uptake and esterification of NEFA.

DISCUSSION

In this study of 39 overweight and obese men and postmenopausal women, we present novel data demonstrating that when compared with subjects with low WAT apoC-I, subjects with high WAT apoC-I secretion have: 1) delayed postprandial plasma clearance of dietary, but not total, TG and TRL in vivo; 2) reduced in situ LPL activity ex vivo in WAT; and 3) no differences in NEFA uptake and storage in vivo or ex vivo in WAT. Adjustment for plasma apoC-I, adiposity, fat distribution, or NEFA uptake ex vivo by WAT did not eliminate group differences in apoB48 clearance; however, adjustment for WAT in situ LPL activity did. There were no sex-differences in any measured outcomes related to WAT apoC-I secretion or TRL clearance in vivo or ex vivo in WAT. Finally, human apoC-I directly inhibited in situ LPL activity resulting in the media-accumulation of TRL in both a concentration- and time-dependent manner. Taken together, while the clinical data cannot establish causality, the combination of in vivo, ex vivo, and in vitro studies suggests that local WAT apoC-I secretion inhibits plasma clearance of dietary TRL by reducing LPL activity at the WAT.

Identifying the physiological role of apoC-I in humans has been challenging because, to date, there are no primary dyslipidemias attributed to an apoC-I mutation or polymorphism. In fact, only a single case-report of complete apoC-I deficiency was found in which the patient had combined deficiency of apoC-I and apoC-II and suffered from hypertriglyceridemia, hypercholesterolemia, and type 2 diabetes (35). ApoC-I polymorphisms are equally rare, as only two have been reported: a polymorphism in the promoter region (Hpa1) (36, 37) and an apoC-I structural variant (T45S) (38). However, their effects on lipid metabolism in vivo remain ambiguous and depend on the population examined. For example, reduced plasma apoC-I secondary to the T45S polymorphism was reported to be associated with reduced percent body fat and waist circumference and unchanged BMI, plasma TG, and cholesterol in aboriginal Canadians (39). However, this variant was associated with elevated BMI and diabetes in subjects of American Indian and Mexican ancestry (38). While apoC-I polymorphism was not assessed here, plasma apoC-I was not associated with adiposity or plasma clearance of TRLs (TG or apoB48) in this study. It should be recalled, however, that in humans the major fraction of fasting apoC-I is carried on HDL (≈80%) where it is considered protective and unrelated to TRL clearance (14, 16, 17). Thus, the uneven distribution of apoC-I on plasma lipoproteins may play a major role in the
was not, however, associated with NEFA uptake, as measured by the plasma clearance of $^{13}$C-NEFAs and the WAT uptake of $^3$H-NEFAs in subjects with high, compared with low, WAT apoC-I secretion. Moreover, physiological concentrations of human apoC-I did not induce the accumulation of $^3$H-NEFA in the adipocyte culture medium. Because NEFA accumulation inhibits LPL activity (34, 44–46), the lack of NEFA accumulation with apoC-I addition supports that the inhibition of LPL was due to apoC-I. This also suggests that the principal mechanism by which apoC-I inhibits TRL clearance in WAT is via the inhibition of LPL activity, not NEFA uptake. While this is in line with reduced VLDL-like particle clearance by WAT in mice overexpressing apoC-I (21), it opposes the proposed role of overexpression of apoC-I in hindering NEFA uptake in mice (26, 42).

The in situ experiments with WAT and adipocytes shown here model the in vivo interaction of adipocyte-secreted apoC-I with LPL and chylomicrons. LPL is anchored to the endothelial surface by glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1), where it exerts its lipolytic activity on circulating TRLs (47). The anchoring of LPL on the endothelial surface is vital for its activity because in vivo human studies show that, although plasma LPL mass increases in the postprandial state, it is mostly inactive (>95%) (48). Adipocyte-secreted apoC-I also has to cross the endothelium to influence LPL-mediated chylomicron lipolysis. This has yet to be examined.

Increased WAT apoC-I secretion was associated with delayed postprandial plasma clearance of dietary TRL particles ($^{13}$C-TG and apoB48), but not of total plasma TG or inconsistent relation of plasma apoC-I with lipid metabolism in humans.

Here, we demonstrate for the first time how apoC-I secreted from human WAT impedes in situ LPL activity and TRL clearance in WAT. WAT plays an important role in the clearance of dietary lipids in response to postprandial signals in humans (8, 12, 40). Tracer in vivo human studies reported that the trapping of LPL-generated dietary NEFAs by subcutaneous WAT is almost absent at fasting, increases to ~100% 1 h after eating, and returns to 10–30% 6 h after eating a meal (10). On the other hand, the role of apoC-I on TRLs has solely been examined in vitro and in murine models. apoC-I was found to inhibit the binding of LPL to lipid emulsions, decreasing TG hydrolysis and rendering unbound-LPL more prone to inactivation (41). ApoE knockout mice overexpressing human apoC-I have reduced uptake of $^3$H-TG-derived NEFAs from intravenously administered $^3$H-VLDL-like particles only in WAT (21). Moreover, apoC-I overexpression in wild-type mice was shown to impair the uptake of intravenously injected fatty acid analog by WAT, but not by other tissues (42). Endogenous apoC-I produced locally in vitro from macrophages of mice overexpressing human apoC-I was also reported to bind and reduce NEFA esterification (26). While overexpression of apoC-I was proposed to protect against obesity in mice (26), it consistently led to postprandial hyperlipidemia believed to be secondary to the inhibition of LPL activity, TRL clearance, and NEFA uptake (20, 21, 26, 42, 43), and to the overproduction of hepatic VLDL-TG and apoB without a change of TG absorption (21).

In our hands, WAT apoC-I secretion, but not total plasma apoC-I, was associated with reduced TRL clearance in vivo and ex vivo in human WAT. WAT apoC-I secretion
apoC-I secretion. Future studies need to evaluate the regulation of WAT apoC-I secretion by apoC-I polymorphism or variables not examined in this study, such as WAT inflammation, which can modulate both WAT in situ LPL activity and apoC-I secretion.

In conclusion, WAT apoC-I secretion is associated with reduced WAT in situ LPL activity and delayed postprandial plasma clearance of chylomicrons in obese men and postmenopausal women independent of adiposity, body fat distribution, WAT NEFA uptake, or plasma apoC-I. We hypothesize that targeting the reduction in WAT apoC-I secretion ameliorates plasma clearance of TRLs and its associated cardiometabolic risk factors.

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