

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

Yannick Cyr,^{1,*†,§} Hanny Wassef,^{1,*†,§} Simon Bissonnette,^{*,†,§} Valerie Lamantia,^{*,†,§}
Jean Davignon,^{*,†,**,§} and May Faraj^{2,*†,§}

Institut de Recherches Cliniques de Montréal (IRCM),* Montréal, Québec, Canada; Faculty of Medicine,[†] Université de Montréal, Montréal, Québec, Canada; Montreal Diabetes Research Center (MDRC),[§] Montréal, Québec, Canada; and Faculty of Medicine,^{**} McGill University, Montréal, Québec, Canada

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-triolein-labeled NEFA; HOMA-IR, homeostatic model assessment of insulin resistance; ³H-TG, ³H-triolein-labeled TG; ³H-TRL, ³H-triolein-labeled TG-rich lipoproteins; iAUC_{6hrs}, 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.

¹Y. Cyr and H. Wassef contributed equally to this work.

²To whom correspondence should be addressed.

e-mail: may.faraj@umontreal.ca

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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 polyacrylamide 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 (iAUC_{6hrs}). The postprandial clearance rate of chylomicron particles was also calculated as iAUC_{6hrs} 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, 34). Briefly, fasting WAT samples were cleaned, blotted dry, weighed and preincubated in HBSS buffer for ~ 30 min, then transferred into wells containing $500 \mu\text{l}$ ^3H -TRL substrate [95% TG, 1.27 mmol/l TG, and 0.54 mmol/l Tris-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. WAT ^3H -lipids (representing ^3H -TRL hydrolysis and uptake) and medium ^3H -NEFA (representing ^3H -TRL hydrolysis and release) were extracted, counted, and expressed as ^3H -TG substrate hydrolyzed per milligram WAT (12, 28, 29, 34). As a negative control, we measured the background medium ^3H -NEFA content after incubation of the ^3H -TRL substrate for 4 h in the absence of WAT. Medium ^3H -NEFA represented a negligible amount of the total ^3H -dose added ($\sim 0.70 \pm 0.01\%$), indicating that medium ^3H -NEFA and WAT ^3H -lipids were the result of ^3H -TRL hydrolysis. Moreover, we previously reported that the addition of the LPL inhibitor, tetrahydrolipstatin, to the ^3H -TRL substrate markedly reduced intracellular ^3H -lipids in 3T3-L1 adipocytes, indicating that the accumulation of intracellular ^3H -lipids from ^3H -TRL substrate is dependent on LPL activity (34).

To assess NEFA uptake independently of LPL activity, a second set of WAT samples was cleaned, blotted dry, weighed, and preincubated in HBSS buffer for ~ 30 min, then transferred into wells containing $500 \mu\text{l}$ ^3H -oleate-labeled NEFA bound to BSA (1 mmol/l oleate:0.167 mmol/l BSA in DMEM/F12) 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 apoC-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

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

| | Women (N = 28) | Men (N = 11) | P |
|--|------------------------------------|------------------------------------|------------------|
| Age (years) | 58.5 \pm 4.3 | 58.5 \pm 6.4 | 0.979 |
| Anthropometric parameters | | | |
| Weight (kg) | 77.9 \pm 13.4 | 108.8 \pm 25.8 | <0.001 |
| BMI (kg/m ²) | 31.4 \pm 4.4 | 36.1 \pm 7.0 | 0.017 |
| Waist circumference (cm) | 102.7 \pm 10.7 | 122.3 \pm 14.8 | <0.001 |
| Hip circumference (cm) | 111.6 \pm 9.0 | 116.3 \pm 13.0 | 0.201 |
| Waist/hip ratio | 0.92 \pm 0.06 | 1.05 \pm 0.08 | <0.001 |
| Total fat (kg) | 35.2 \pm 9.4 | 44.2 \pm 16.6 | 0.040 |
| Android fat (kg) | 3.36 \pm 1.05 | 5.37 \pm 2.01 | <0.001 |
| Gynoid fat (kg) | 6.15 \pm 1.61 | 5.85 \pm 2.09 | 0.631 |
| Android/gynoid fat | 0.55 \pm 0.10 | 0.92 \pm 0.10 | <0.001 |
| Adipocyte area (μm^2) | 3,135 \pm 704 | 3,404 \pm 982 | 0.345 |
| Metabolic parameters | | | |
| Systolic blood pressure (mmHg) | 120.4 \pm 17.9 | 136.5 \pm 12.2 | 0.011 |
| Diastolic blood pressure (mmHg) | 76.5 \pm 8.0 | 84.3 \pm 6.8 | 0.009 |
| Plasma glucose (mmol/l) | 5.13 \pm 0.47 | 5.15 \pm 0.25 | 0.860 |
| Plasma insulin ($\mu\text{U}/\text{ml}$) | 14.2 \pm 5.4 | 22.6 \pm 17.9 | 0.031 |
| HOMA-IR [(mmol/l) \times (mU/l)] | 3.27 \pm 1.35 | 5.11 \pm 3.96 | 0.035 |
| Plasma cholesterol (mmol/l) | 5.93 \pm 1.06 | 4.96 \pm 1.20 | 0.015 |
| Plasma VLDL-C (mmol/l) | 1.01 \pm 0.32 | 1.05 \pm 0.36 | 0.703 |
| Plasma IDL-C (mmol/l) | 1.69 \pm 0.41 | 1.34 \pm 0.32 | 0.015 |
| Plasma LDL-C (mmol/l) | 1.95 \pm 0.56 | 1.64 \pm 0.63 | 0.146 |
| Plasma HDL-C (mmol/l) | 1.46 \pm 0.35 | 1.07 \pm 0.26 | 0.002 |
| Plasma TG (mmol/l) | 1.52 \pm 0.84 | 1.76 \pm 0.89 | 0.427 |
| Plasma apoB (g/l) | 1.00 \pm 0.26 | 1.03 \pm 0.27 | 0.829 |
| Plasma apoB48 (mg/l) | 6.62 \pm 4.15 | 7.87 \pm 4.45 | 0.421 |
| LDL diameter (\AA) | 269 \pm 6 | 266 \pm 7 | 0.157 |
| Plasma apoC-I ($\mu\text{mol}/\text{l}$) | 23.6 \pm 5.7 | 17.5 \pm 3.9 | 0.004 |
| WAT apoC-I secretion (pmol/g/4 h) | 86.9 \pm 31.4 | 74.1 \pm 36.6 | 0.279 |
| WAT apoC-I secretion (min–max) | 36.6–155.3 | 23.6–126.9 | — |
| Nutritional parameters ^a | | | |
| Total energy intake (kcal/day) | 1,895 \pm 541 | 2,493 \pm 528 | 0.009 |
| Carbohydrates (%) | 46.3 \pm 5.4 | 47.0 \pm 8.8 | 0.792 |
| Fat (%) | 36.1 \pm 5.1 | 33.2 \pm 6.8 | 0.210 |
| Protein (%) | 16.0 \pm 2.1 | 17.0 \pm 4.0 | 0.412 |
| Alcohol (%) | 1.64 \pm 2.81 | 2.85 \pm 3.09 | 0.298 |
| Saturated fat (%) | 11.1 \pm 2.6 | 10.1 \pm 2.9 | 0.361 |
| Fiber (g/day) | 22.0 \pm 7.2 | 25.6 \pm 8.8 | 0.260 |
| Cholesterol (mg/day) | 253 \pm 117 | 359 \pm 169 | 0.065 |

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

^aData from 16 women and 11 men.

TABLE 2. Pearson correlation of anthropometric parameters with measured outcomes

| Body Composition | Postprandial Plasma Parameters | | | | | WAT in situ LPL Activity (i.e., ³ H-TRL Substrate) | WAT ³ H-NEFA Uptake (i.e., ³ H-NEFA:BSA Substrate) | |
|--------------------------|---|-------------------------------------|---|--|--|--|--|---------------------------|
| | WAT apoC-I Secretion (pmol/g/4 h) | iAUC _{6hrs} TG (mmol/l) | LOG ₁₀ iAUC _{6hrs} ¹³ C-TG (μmol/l) | iAUC _{6hrs} ¹³ C-NEFA (μmol/l) | iAUC _{6hrs} apoB48 ^b (mg/l) | LOG ₁₀ Medium ³ H-NEFA ^d (nmol ³ H-TG/mg) | WAT ³ H-lipids ^e (nmol ³ H-NEFA/mg) | |
| Weight (kg) | -0.085 | -0.203 | 0.094 | -0.239 | 0.160 | 0.016 | 0.213 | -0.197 |
| BMI (kg/m ²) | -0.025 | -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 | 0.012 | 0.209 | -0.309 |
| Hip (cm) | -0.028 | -0.187 | -0.198 | -0.219 | 0.055 | -0.126 | -0.033 | -0.344 |
| Waist/hip ratio | -0.150 | 0.043 | 0.177 | -0.136 | 0.193 | 0.136 | 0.303 | -0.136 |
| Total fat (kg) | -0.007 | -0.219 | -0.129 | -0.259 | 0.091 | -0.054 | 0.215 | -0.316 |
| Android fat (kg) | -0.098 | -0.210 | 0.001 | -0.282 | 0.131 | 0.023 | 0.002 | -0.341 |
| Gynoid fat (kg) | 0.069 | -0.152 | -0.362^a | -0.204 | -0.064 | 0.005 | 0.130 | -0.419^a |
| Android/gynoid fat | -0.127 | -0.110 | 0.373^a | -0.210 | 0.333^a | 0.057 | 0.213 | -0.043 |

Data in bold represent parameters with significant correlation.

^a $P \leq 0.05$ (two-tailed).

^bN = 38 for missing data.

^cN = 33 for missing data.

^dN = 32 for missing data.

^eN = 28 for missing data.

(Calbiochem, La Jolla, CA), as described above. These experiments model the in vivo interaction of TRL with adipocyte-secreted LPL and apoC-I, as on the luminal surface of endothelial cells. Cells were differentiated for 7 days, as previously described (29, 34), then incubated with the ³H-TRL substrate (0.56 mmol/l TG) in the presence or absence of apoC-I (15 μmol/l) over 5–360 min. To test whether the effect of apoC-I was concentration dependent, adipocytes were incubated with the ³H-TRL substrate in the presence or absence of apoC-I at physiological concentrations (1.5–30 μmol/l) for 4 h. Four hours was selected to simulate the duration of WAT apoC-I secretion. After the 4 h experiment, medium ³H-TG (non-hydrolyzed ³H-TRL substrate), medium ³H-NEFA (hydrolyzed ³H-TRL substrate and released ³H-NEFA), and intracellular ³H-lipids (hydrolyzed ³H-TRL substrate and incorporated ³H-NEFA into adipocyte lipids) were extracted, counted, and expressed per well.

Statistical analysis

Data are presented as mean ± SD. Group differences were assessed by Student's *t*-test. Pearson correlations were used to examine the association between the measured outcomes. Slope analysis indicated no significant sex differences in the direction of association of the independent variables to dependent variables as shown in Table 2, Fig. 2, and supplementary Fig. 2; accordingly, data for both sexes were pooled. Data were log-transformed (base 10) and then entered in the analysis when the test for equal variance of residues failed. Univariate ANOVA analysis was used to adjust the differences between the two groups of low versus high WAT apoC-I secretion for the different parameters. Statistical analysis was performed using SPSS V20 (IBM) and curve analysis using Prism V6.0f (GraphPad Software). Significance was set at $P < 0.05$.

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, and fat mass), particularly central (waist circumference, waist/hip ratio, android fat, and android/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.

Plasma apoC-I is not correlated with clearance of TRL in vivo or by WAT ex vivo

Given the known role of apoC-I in reducing TRL clearance (when on TRL), we first examined whether fasting plasma apoC-I was correlated with indices of delayed postprandial fat clearance in this population. As presented in Fig. 1, there was no association of plasma apoC-I with postprandial plasma clearance of total TG, dietary ¹³C-TG, dietary ¹³C-NEFA, or plasma apoB48 (Fig. 1A–D). Moreover, there was no correlation between plasma apoC-I with in situ LPL activity in WAT ex vivo (Fig. 1E). On the other hand and as previously reported in women, plasma apoC-I correlated with WAT apoC-I secretion and there was no sex-difference in the

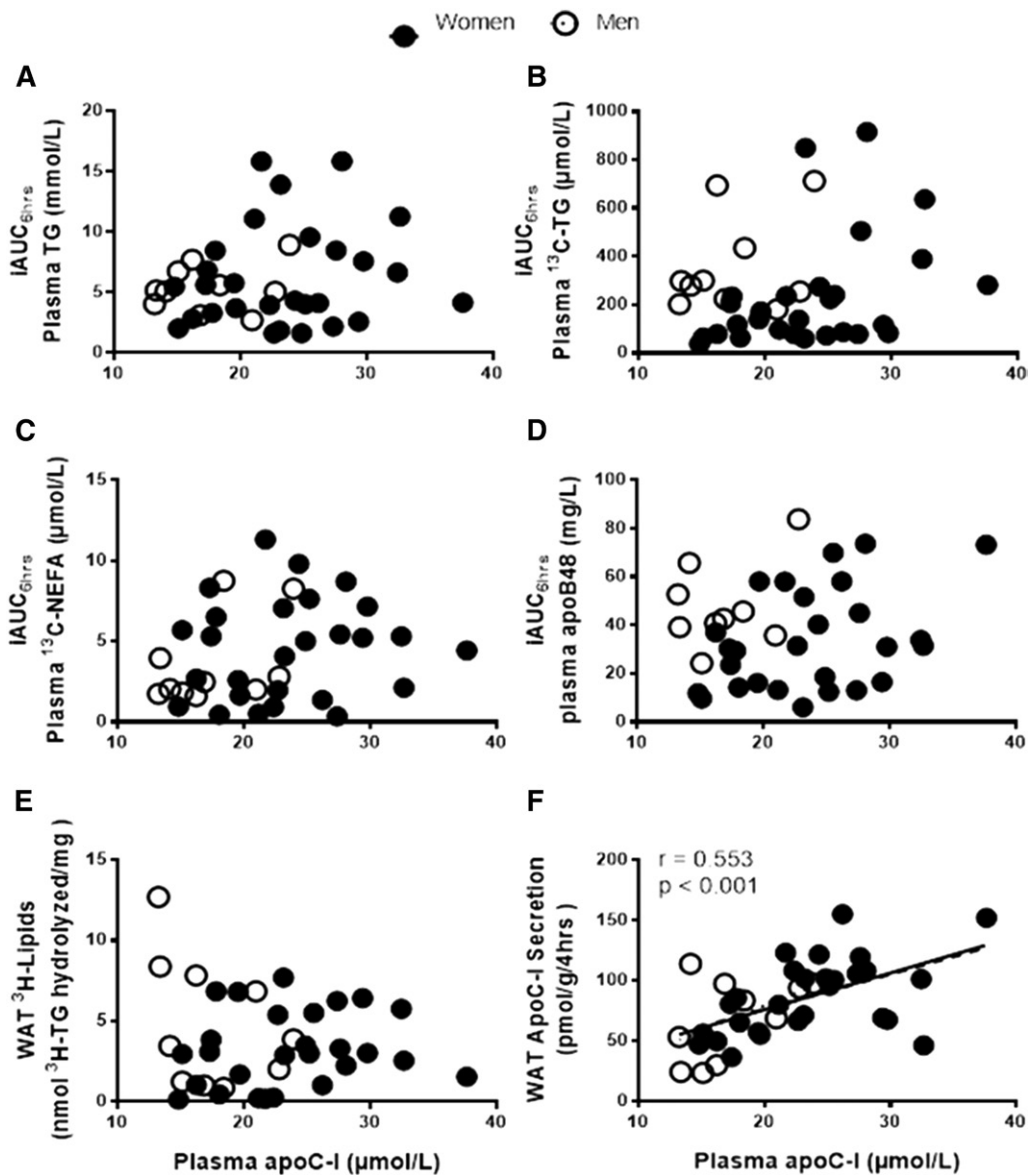


Fig. 1. Association of fasting plasma apoC-I with iAUC_{6hrs} of plasma TG (A), ¹³C-TG (B), ¹³C-NEFA (C), and chylomicrons (apoB48) (D), and with WAT in situ LPL activity measured as WAT ³H-lipids (E) and WAT apoC-I secretion (F) in women (N = 28, closed circles with dashed regression line) and men (N = 11, open circles with dotted regression lines). Solid regression line represents pooled data analysis.

direction of this association ($r = 0.553$ $P < 0.001$; Fig. 1F). Plasma apoC-I correlated with all measures of fasting total plasma cholesterol (supplementary Fig. 2A) and lipoprotein-specific cholesterol (supplementary Fig. 2B–E), but not with plasma TG (supplementary Fig. 2F). It also correlated with fasting concentrations of apoB-lipoproteins, total apoB (supplementary Fig. 2G) and apoB48 (supplementary Fig. 2H), and with fasting plasma NEFA (supplementary Fig. 2I), with no sex differences in the direction of the associations.

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

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 | 112.0 ± 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 ± 1.43 | 0.653 |
| Gynoid fat (kg) | 6.22 ± 1.44 | 5.92 ± 2.01 | 0.602 |
| Android/gynoid fat | 0.64 ± 0.20 | 0.66 ± 0.19 | 0.752 |
| Adipocyte area (μm ²) | 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 | 0.236 |
| 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 ^a (mg/l) | 6.29 ± 4.10 | 7.61 ± 4.31 | 0.337 |
| LDL diameter (Å) | 269 ± 5 | 267 ± 6 | 0.351 |
| Plasma apoC-I (μmol/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 ^b | | | |
| 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.

^aN = 5 men in high WAT apoC-I secretion group.

^bN = 13 (five men) and N = 14 (six men) in low versus high WAT apoC-I secretion groups, respectively.

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 ¹³C-NEFA (Fig. 2C) (Note that removing the outlier point in plasma ¹³C-NEFA in the high secretion group, which is ~8-fold higher than the average, 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 ¹³C-TG and chylomicrons (Fig. 2, P ≤ 0.05 for all). Adjusting for android/gynoid fat ratio rendered group differences in iAUC_{6hrs} of ¹³C-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 iAUC_{6hrs} 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 ¹³C-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 ³H-TRL and uptake and incorporation of LPL-generated ³H-NEFA as WAT ³H-lipids. We also measured the accumulation of ³H-NEFA in the medium over 4 h. As presented in **Fig. 3**, subjects with high WAT apoC-I had reduced WAT ³H-lipids compared with subjects with low WAT apoC-I (high = 3.15 ± 1.97 nmol vs. low = 5.09 ± 3.21 nmol ³H-TG hydrolyzed per milligram WAT,

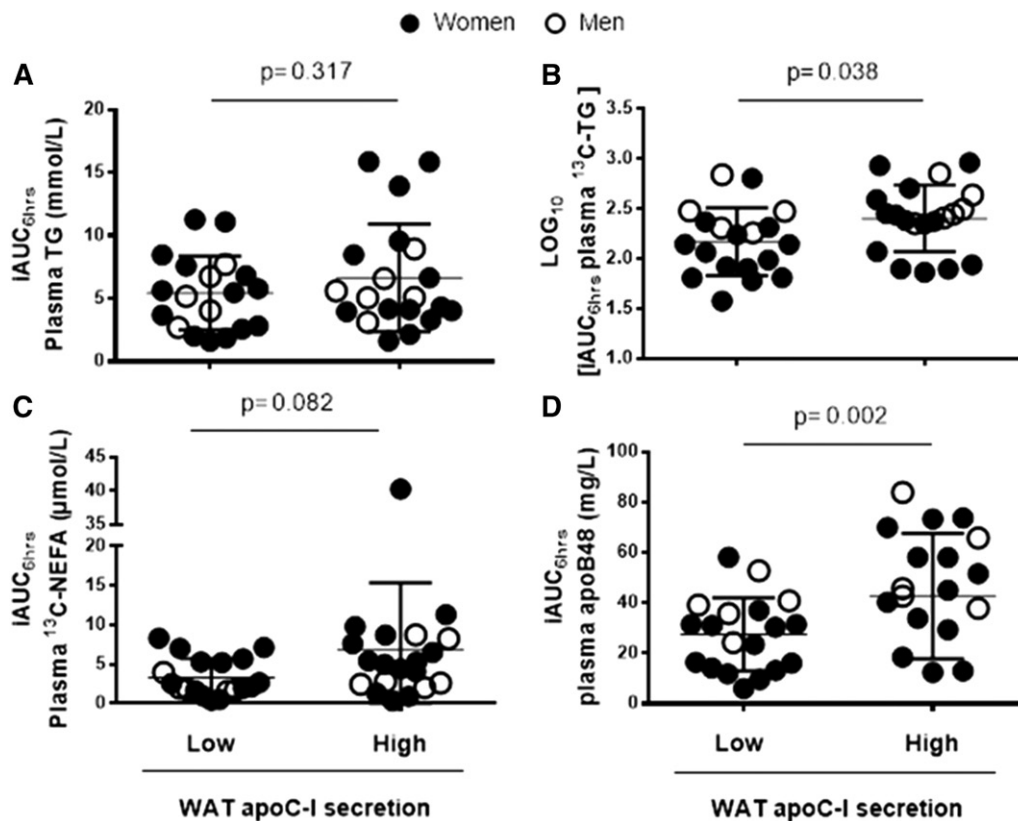


Fig. 2. Group differences in postprandial plasma clearance of TG (A), ^{13}C -TG (B), ^{13}C -NEFA (C), and chylomicrons (apoB48) (D) in subjects with low (N = 19) versus high (N = 20) WAT apoC-I secretion (except for (D) where N = 5 men in the high group for missing data). Women are represented as solid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

$P = 0.043$; Fig. 3A). However, there were no group differences in medium ^3H -NEFA (Fig. 3B, equivalent to high = 1.00 ± 0.70 nmol vs. low = 2.19 ± 2.68 nmol ^3H -TG hydrolyzed per milligram WAT).

To verify whether the group difference in WAT ^3H -lipids was dependent on LPL activity, we incubated a separate set of WAT samples with ^3H -NEFA:BSA for 4 h. In contrast to when ^3H -TRL was used, there were no group-differences in WAT ^3H -lipids when ^3H -NEFA:BSA was used (high = 3.62 ± 2.46 nmol vs. low = 3.71 ± 1.56 nmol ^3H -NEFA per milligram WAT, $P = 0.911$, Fig. 3C). Adjusting for BMI; total, android, or gynoid fat; or android/gynoid ratio did not affect group differences in Fig. 3A–C. This suggests that the effect of WAT apoC-I on TRL clearance ex vivo in WAT is LPL dependent.

To verify whether the group differences in postprandial plasma clearance of dietary TRL were dependent on WAT in situ LPL activity, we adjusted for in situ LPL activity. Adjustment for WAT ^3H -lipids (i.e., Fig. 3A) eliminated group differences in $\text{iAUC}_{6\text{hrs}}$ plasma ^{13}C -TG and apoB48, while adjustment for LPL-released ^3H -NEFA in the medium (i.e., Fig. 3B) only eliminated group differences in plasma clearance of ^{13}C -TG, but not apoB48 ($P = 0.038$). Adjustment for WAT ^3H -lipids from ^3H -NEFA:BSA sources (i.e., Fig. 3C) also had no effect. Taken together, these data suggest that delayed postprandial plasma clearance of dietary TRL in subjects with high WAT apoC-I secretion

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.

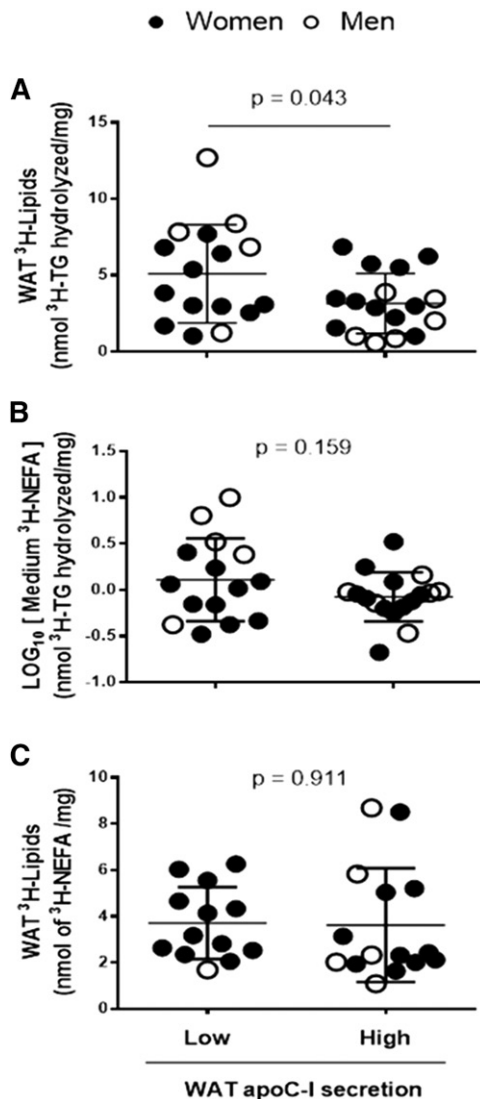


Fig. 3. Group differences in in situ LPL activity measured as WAT ³H-lipids (A) and as medium ³H-NEFA (B) using ³H-TRL substrate (1.27 mmol/l TG) and ³H-NEFA uptake and incorporation into WAT ³H-lipids using ³H-NEFA:BSA substrate (C) in subjects with low (N = 16) versus high (N = 17) WAT apoC-I secretion for (A, B), and low (N = 13) versus high (N = 15) WAT apoC-I secretion for (C). Women are represented as solid circles and men as open circles.

Direct effect of human apoC-I on in situ LPL activity in 3T3-L1 adipocytes

To explore the direct effect of apoC-I on ³H-TRL hydrolysis by adipocytes' secreted LPL and their storage by adipocytes, we measured in situ LPL activity in 3T3-L1 adipocytes in the presence or absence of human VLDL-extracted apoC-I. Synthetic ³H-TRLs (0.56 mmol/l TG) were incubated with differentiated 3T3-L1 adipocytes with or without physiological concentrations of apoC-I (15 μmol/l) for 6 h. ApoC-I induced a time-dependent reduction in the hydrolysis and incorporation of ³H-TRL substrate as intracellular ³H-lipids by ~4-fold (Fig. 4A), and reduced the accumulation of LPL-generated ³H-NEFA in the medium by ~2-fold (Fig. 4B). Subsequently, there was an accumulation of ³H-TRL in the medium of apoC-I-incubated adipocytes (Fig. 4C). The inhibitory effect of apoC-I at 4 h was

also concentration dependent, reaching a maximum inhibition of ³H-TRL hydrolysis and incorporation as intracellular ³H-lipids at 15 μM (~78%, Fig. 4D). Notably, the lack of accumulation of LPL-released ³H-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 (HpaI) (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

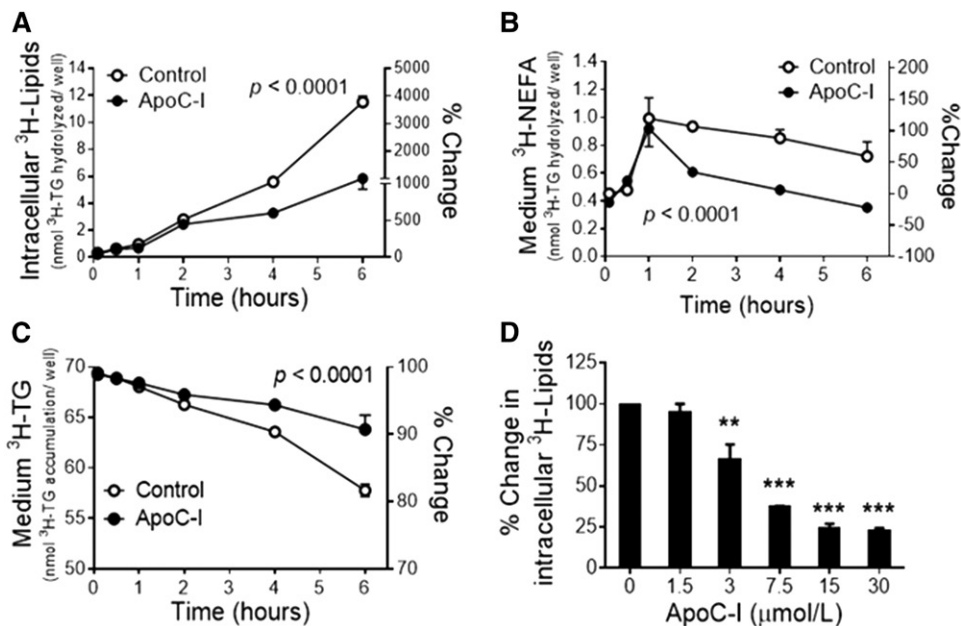


Fig. 4. Time-dependent effects of human VLDL-extracted apoC-I (15 μ M) on 3 H-TRL hydrolysis and incorporation as intracellular 3 H-lipids (A), 3 H-TRL hydrolysis and 3 H-NEFA release into the medium (B), 3 H-TRL accumulation in the medium (C), and concentration-dependent effect of human apoC-I on 3 H-TRL hydrolysis and incorporation as intracellular 3 H-lipids over 4-h in 3T3-L1 adipocytes (D).

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 \sim 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

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 LPL needs to cross the endothelium and reach the luminal surface in order to exert its lipolytic activity on TRLs (49). Consequently, one would assume that 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

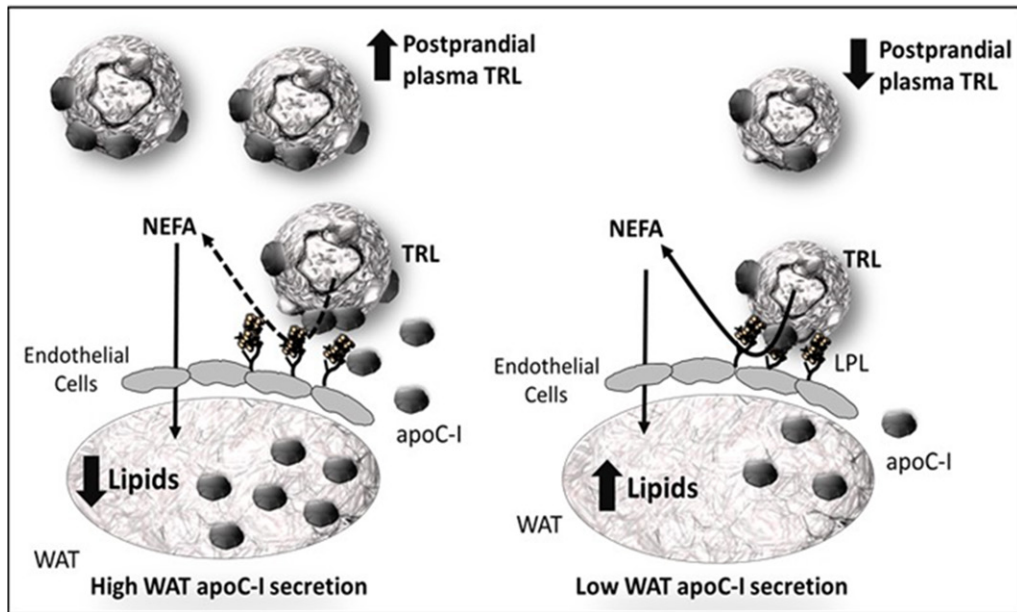


Fig. 5. Proposed model for the interaction of WAT-secreted apoC-I with TRL and LPL on the endothelial surface of WAT. Increased apoC-I secretion from WAT enriches TRL with apoC-I, which inhibits the activity of LPL on the endothelium of WAT and the hydrolysis of postprandial TRL without affecting the uptake of NEFA into WAT. This results in decreased clearance and incorporation of TRL into WAT lipids and increased postprandial plasma concentrations of TRLs (dashed arrow for inhibited and solid arrow for unaffected process).

apoB. This may be due to the minor contribution of dietary particles to total plasma concentrations (<2%) (29). Alternatively, WAT apoC-I secretion may have a greater effect on chylomicrons than on VLDL clearance because hepatic endoplasmic reticulum-derived VLDL transport vesicles, but not prechylomicrons, were reported to contain apoC-I in rats (50). This suggests that the enrichment of chylomicrons with apoC-I takes place in the plasma. Because the WAT represents a physiological site for the interaction with chylomicrons, it is logical to propose that WAT apoC-I secretion enriches chylomicrons, thereby delaying their clearance by endothelial LPL activity (Fig. 5). This is supported by: 1) our previous findings that WAT apoC-I secretion associates with TRL apoC-I content and that adjustment for TRL apoC-I eliminates the association of WAT apoC-I with delayed ^{13}C -TG clearance in women (14); and 2) by the present study in which adjustment for WAT in situ LPL activity eliminates the association of WAT apoC-I with delayed clearance of dietary TRLs in men and women.

Finally, we observed a 7-fold variation in the secretion of apoC-I from WAT among our participants, whereas the plasma apoC-I variation was only 3-fold. Given that WAT apoC-I secretion associates with the fasting and postprandial enrichment of nonHDL lipoproteins with apoC-I (14), increased plasma retention time of TRLs in subjects with high WAT apoC-I secretion may explain higher plasma apoC-I concentrations. Factors affecting the variability of WAT apoC-I secretion, whether genetic or environmental, were not determined in this study. However, none of the examined parameters related to nutritional intake, body composition, and metabolic profile predicted

WAT 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. **Fig.**

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