White Adipose Tissue Apolipoprotein C-I Secretion in Relation to Delayed Plasma Clearance of Dietary Fat in Humans

Hanny Wassef, Huda Salem, Simon Bissonnette, Alexis Baass, Robert Dufour, Jean Davignon, May Faraj

Objective—White adipose tissue (WAT) dysfunction is characterized by delayed clearance of dietary triglyceride-rich lipoproteins (TRL). We reported that apolipoprotein (apo) C-I, a transferable apolipoprotein that inhibits lipoprotein lipase (LPL) activity, is associated with fasting total and non–high-density lipoprotein apoC-I but not with high-density lipoprotein apoC-I and was the primary predictor of 4-hour postprandial increases in TRL apoC-I. Correction for TRL apoC-I eliminated the association of WAT apoC-I with 6-hour area under the curve of plasma 13C-triglyceride; correction for insulin sensitivity or inflammation did not. Finally, in addition to apoC-I, WAT secreted considerable amount of apoC-II, apoC-III, and apoE over 24 hours; however, only WAT apoC-I secretion was associated with 6-hour area under the curve of plasma 13C-triglyceride.

Conclusion—Increased WAT apoC-I secretion in obese women is associated with delayed postprandial dietary fat clearance mediated by increased TRL apoC-I. Thus, we hypothesize that reducing WAT apoC-I secretion ameliorates WAT dysfunction and associated cardiometabolic risks in humans. (Arterioscler Thromb Vasc Biol. 2012;32:2785-2793.)

Key Words: lipoprotein metabolism ■ postprandial triglyceride clearance ■ stable isotopes ■ subcutaneous white adipose tissue ■ transferable apolipoproteins

Obesity-related metabolic diseases, such as insulin resistance, diabetes mellitus, and atherosclerosis, are attributed to dysfunctional white adipose tissue (WAT). A major characteristic of a dysfunctional WAT is the reduced efficiency of WAT lipoprotein lipase (LPL) to hydrolyze circulating triglyceride-rich lipoproteins (TRL) and to take up and trap LPL-released nonesterified fatty acids (NEFA). This is believed to increase the influx of NEFA and TRL remnants to nonadipose peripheral tissues, including muscle, liver, and pancreas promoting ectopic fat deposition, inflammation, and insulin resistance and increasing the risk for cardiometabolic disease. Factors that promote dysfunctional WAT in some obese subjects are not well understood.

Apolipoprotein (apo) C-I is the smallest of the exchangeable apolipoproteins with a molecular weight of 6.6 kDa. Its mRNA expression is mainly in the liver but also in the skin, lung, spleen, and macrophage. In the fasting state, apoC-I resides mostly on high-density lipoprotein (HDL; ≈80%-90%) but is believed to transfer to TRL, very-low-density lipoprotein (VLDL), and chylomicrons in the postprandial state. ApoC-I has a dual role in lipoprotein metabolism, depending on the lipoprotein with which it is associated. When apoC-I is on HDL, it is protective because it increases HDL cholesterol size and HDL cholesterol concentration. When on TRL, apoC-I is reported to hinder TRL clearance by inhibiting LPL activity, binding NEFA reducing their intracellular availability, and displacing apoE from TRL, thus inhibiting apoE-dependent binding of TRL and TRL remnants (intermediate-density lipoprotein [IDL]) to VLDL receptor, low-density lipoprotein (LDL) receptor, and LDL receptor–related protein. This results in increased plasma triglyceride (TG) that is mainly confined to TRL and IDL. Transgenic mice with hepatic overexpression of human apoC-I have increased TRL apoC-I, impaired TRL hepatic uptake,

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increased VLDL apoB production, and increased VLDL/IDL accumulation. In addition, human apoC-I overexpression in mice have been shown to impair the uptake of fatty acid analogs by WAT. Although studies on the physiological role of apoC-I in humans are limited, similar association of TRL apoC-I with delayed postprandial TRL clearance and the enrichment of TRL with cholesterol were reported in patients with atherosclerosis.

We have previously reported that apoC-I is secreted from a human adipocyte cell model, SW872 liposarcoma cells, and that both cellular and medium apoC-I concentrations increased in a differentiation-dependent manner in these cells. To date, the pertinence of apoC-I secretion from WAT and its relation to WAT function and postprandial TRL clearance have never been examined in mice or humans. In view of the documented role of apoC-I on TRL metabolism, the aim of this study was to determine whether in postmenopausal obese women (1) apoC-I is secreted from WAT, (2) increased WAT apoC-I secretion is related to delayed dietary TRL clearance, and (3) WAT secretes other transferable apolipoproteins, apoC-II, apoC-III, and apoE and explores how they relate to perturbations in TRL clearance. Our primary hypothesis was that higher WAT apoC-I secretion is associated with increased TRL apoC-I and delayed dietary fat clearance.

### Materials and Methods

#### Subjects

Twenty postmenopausal women were recruited into this study with the following inclusion criteria: body mass index ≥27 kg/m², age 45–74 years, nonsmoker, sedentary, low-alcohol consumption. The exclusion criteria were as follows: (1) elevated risk of cardiovascular disease (220% of calculated Framingham Risk Score); (2) prior history of cardiovascular events, untreated thyroid disease, diabetes mellitus or fasting glucose >7.0 mmol/L, inflammatory disease, or cancer within the last 3 years; (3) claustrophobia; (4) hemoglobin <120 g/L; (5) blood creatinine >100 µmol/L; (6) hepatic dysfunction (aspartate aminotransferase/alanine aminotransferase >3× the upper limit of normal); (7) problems with blood coagulation; (8) concomitant medications affecting metabolism; (9) known substance abuse; (10) lack of time to participate in the study; (11) have exceeded the annual total allowed radiation dose according to the physician's judgment. All women signed a consent form before initiation of the study, and the study was approved by the ethics board at the Institut de recherches cliniques de Montréal.

#### Anthropometrics and Biochemistry

The study was conducted after 4 weeks of weight stabilization (±2 kg). Body composition was measured by DEXA (General Electric Lunar Corporation, version 6.10.019). Plasma glucose was measured by an automated analyzer (YSI Incorporated), serum insulin by a radioimmunoassay kit (Linco Research), plasma inflammatory markers, lipids, total apoB, and LDL cholesterol by an automated analyzer COBAS INTEGRA 400 (Roche Diagnostics), and plasma apoB48 by an ELISA kit (BioVendor). Fasting insulin sensitivity indices, homeostasis model assessment for insulin resistance and quantitative insulin sensitivity check index, were calculated as published.

#### Lipoprotein Fractionation

Plasma lipoproteins were fractionated based on size and measures the cholesterol concentrations in each fraction. Lipoprotein size and cholesterol concentrations were measured by an automated Food and Drug Administration–approved electrophoresis system (Lipoprint, Quantimetrix), which separates lipoproteins based on size and measures the cholesterol concentrations in each fraction.

### Table. Baseline Characteristics of Women With Low (n=10) Versus High (n=9) WAT ApoC-I Secretion

<table>
<thead>
<tr>
<th></th>
<th>Low-WAT ApoC-I</th>
<th>High-WAT ApoC-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAT apoC-I, µmol/L per g of fat</td>
<td>0.60±0.04</td>
<td>1.03±0.04*</td>
</tr>
<tr>
<td>Age, y</td>
<td>58.8±1.5</td>
<td>57.3±1.5</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>72.7±1.9</td>
<td>76.1±5.0</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>30.5±1.1</td>
<td>30.6±1.4</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>101.8±2.6</td>
<td>102.6±3.7</td>
</tr>
<tr>
<td>Body fat mass, kg</td>
<td>32.2±1.7</td>
<td>34.1±3.6</td>
</tr>
<tr>
<td>Android fat mass, kg</td>
<td>2.94±0.19</td>
<td>3.33±0.39</td>
</tr>
<tr>
<td>Gynoid fat mass, kg</td>
<td>5.91±0.29</td>
<td>6.3±0.69</td>
</tr>
<tr>
<td>Adipocyte area, µm²</td>
<td>3041±270</td>
<td>3152±201</td>
</tr>
<tr>
<td>Body fat mass, kg</td>
<td>1204±33</td>
<td>1296±51</td>
</tr>
<tr>
<td>Plasma apoE, µmol/L</td>
<td>1.37±0.12</td>
<td>1.57±0.09</td>
</tr>
<tr>
<td>Plasma triglyceride, mmol/L</td>
<td>1.2±0.2</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td>5.5±0.3</td>
<td>6.4±0.5</td>
</tr>
<tr>
<td>Plasma HDL-C, mmol/L</td>
<td>3.5±0.3</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td>Plasma HDL-C, mmol/L</td>
<td>1.8±0.0</td>
<td>2.0±0.0</td>
</tr>
<tr>
<td>Plasma HDL-C, mmol/L</td>
<td>1.5±0.1</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>Plasma triglyceride, mmol/L</td>
<td>1.2±0.2</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>Plasma NEFA, mmol/L</td>
<td>0.56±0.05</td>
<td>0.59±0.05</td>
</tr>
<tr>
<td>Mean LDL size, Å</td>
<td>269.5±1.8</td>
<td>268.2±2.1</td>
</tr>
<tr>
<td>% HDL-C on large HDL</td>
<td>4.3±3.0</td>
<td>29.0±2.1</td>
</tr>
<tr>
<td>% HDL-C on intermediate HDL</td>
<td>51.6±2.3</td>
<td>52.7±1.5</td>
</tr>
<tr>
<td>% HDL-C on small HDL</td>
<td>15.1±1.8</td>
<td>18.3±1.3</td>
</tr>
<tr>
<td>Plasma apoB-48, µg/L</td>
<td>5.4±0.9</td>
<td>7.9±1.7</td>
</tr>
<tr>
<td>Plasma apoE, µmol/L</td>
<td>0.80±0.07</td>
<td>0.97±0.11</td>
</tr>
<tr>
<td>Plasma apoC-I, µmol/L</td>
<td>20.5±1.8</td>
<td>22.7±1.1</td>
</tr>
<tr>
<td>Plasma apoC-II, µmol/L</td>
<td>4.6±0.6</td>
<td>7.1±1.3</td>
</tr>
<tr>
<td>Plasma apoC-III, µmol/L</td>
<td>16.6±1.5</td>
<td>19.1±2.4</td>
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</table>

Data are presented as mean±SEM. Group differences were analyzed by 1-way ANOVA. WAT indicates white adipose tissue; HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; NEFA, nonesterified fatty acids; apo, apolipoprotein.

*P=0.0001.
overall recovery of plasma after FPLC fractionation was assessed as the sum of cholesterol concentrations in the 80 FPLC fractions expressed as percent of total plasma cholesterol, which was 91±2%. The concentration of each parameter measured in the FPLC fractions was determined by multiplying the percent of total parameter in each fraction by the total plasma concentration of that parameter.38,39

Based on the TG and cholesterol contents of the fasting lipoprotein fractions, 3 separate lipoprotein subclasses were identified. First, the large TRL particles eluted in fractions 1 to 16 and contained ≈37% of total TG and ≈35% of total cholesterol (molar ratio of TG:cholesterol, 2.3:1). Second, the intermediate LDL/IDL particles eluted in fractions 17 to 40 and contained ≈49% of total TG and ≈64% of total cholesterol (molar ratio of cholesterol:TG, 6:1). Finally, the HDL particles eluted in fractions 41 to 80 and contained ≈15% of total TG and ≈32% of total cholesterol (cholesterol:TG molar ratio, 10:1). As a control, we correlated the concentration of cholesterol in the fasting TRL, IDL/LDL, and HDL fractions as separated by size on the FPLC and by the Lipoprint. The cholesterol concentrations measured by these 2 techniques were highly correlated (TRL: r=0.82; IDL/LDL: r=0.92; HDL: r=0.84; P<0.0001 for all), which highlights the reliability of the FPLC fractionation of the lipoproteins and cutoff points used.

Secretion of apoC-I, apoC-II, apoC-III, and apoE from Women’s Fasting VAT Samples

Fasting subcutaneous VAT samples were collected from the woman’s right hip by needle biopsy under local anesthesia (Xylocaine 20 mg/mL, AstraZeneca) at T=−1 hour, as described,40 and kept in warm Hank’s buffered salt solution with 0.035% NaHCO₃, penicillin/streptomycin/fungizone mix (100 U/mL:100 μg/mL:0.25 μg/mL), 50 μg/mL gentamicin, and 5 mmol/L HEPES. Clean fresh VAT samples (5–10 mg) were incubated in DMEM/F12 (600 μL mixture) containing 5% fetal bovine serum, and VAT apoC-I secretion over 4 hours was assessed as apoC-I accumulation in the medium as measured by in-house ELISA.15 Women were separated based on median VAT apoC-I secretion over 4 hours, which corresponds to the same energy expenditure, by indirect calorimetry, and collection of breath fat, 68% fat, 18% carbohydrate). Serial postprandial measurements of HDL:

Dietary Fat Clearance

Dietary fat clearance was assessed in vivo as previously published.18 Briefly, fasting metabolic rate, breath, and blood samples were collected 1 hour after the fasting VAT biopsies, followed by the consumption of a 13C-labeled high-fat meal (600 kcal/m², 0.017 g 13C-triolein/g fat, 68% fat, 18% carbohydrate). Serial postprandial measurements of energy expenditure, by indirect calorimetry, and collection of breath and blood samples were then conducted. The concentrations of 13C-TG and 13C-NEFA and 13C recovered in breath CO₂ were calculated as described in the online-only Data Supplement.

Statistical Analysis

Data are presented as means±SEM. VAT biopsy could not be obtained from 1 woman, thus data analysis was based on 19 women. Pearson correlation was used to examine the association between the variables; presented significant correlations by Pearson were also significant by Spearman. Stepwise forward regression analysis was used to predict the 6-hour area under the curve (AUC₆₉₆) of 13C-TG and 13C-NEFA. Group differences in the time curves were analyzed by repeated measures (RM) 2-way or 3-way ANOVA with interaction; the correction of Greenhouse-Geisser was also applied to be more robust for sphericity. When interaction was significant, intrasubject differences was analyzed by paired t test, whereas intersubject differences were analyzed by unpaired t test. Statistical analysis was performed using SPSS V13 (SPSS Inc, Chicago, IL), and significance was set at P≤0.05.

Results

WAT biopsies extracted from obese postmenopausal women secreted variable amounts of apoC-I over 4 hours into the VAT medium (0.366–1.232 μmol/L apoC-I per g/hours). Accordingly, we separated the women based on their median VAT apoC-I secretion, which equaled 0.804 μmol/L per g VAT/4 hours, into 2 groups termed low- and high-WAT apoC-I (Table). There were no group differences in any of the measured parameters related to body composition, adipocyte size, or plasma metabolic profile, including inflammatory markers (high-sensitivity C-reactive protein, orosomucoid, and haptoglobin) and fasting insulin sensitivity indices (homeostasis model assessment for insulin resistance and quantitative insulin sensitivity check index). There were also no group differences in total plasma lipids, LDL and HDL size, and transferable apolipoproteins measured; apoC-I, apoC-II, apoC-III, and apoE. Thus, the 2 groups had no apparent metabolic discrepancies in the fasting state.

After the ingestion of the 13C-triolein–labeled high-fat meal, there were no significant group differences in total postprandial plasma TG or glucose (Figure 1A and 1B), whether examined by curve analysis (RM ANOVA) or as increment increase in AUC₆₉₆ of plasma TG and glucose. On the other hand, although total plasma NEFA decreased between 1 and 2 hours in both groups, NEFA suppression was lower in women with high-WAT apoC-I (Figure 1C; P=0.025), suggesting lower insulin-mediated inhibition of WAT lipolysis in this group. Women with high-WAT apoC-I had a greater AUC₆₉₆ of insulin (high=250±22 μU/mL versus low=178±24 μU/mL; P=0.040, Figure 1D) and a smaller ratio of AUC₆₉₆ of glucose to AUC₆₉₆ of insulin (high=0.13±0.01 mol/U versus low=0.21±0.03 mol/U; P=0.039). Because both groups had similar postprandial glyceremia, this suggests lower systemic postprandial insulin sensitivity in women with high-WAT apoC-I.

Women with high-WAT apoC-I had delayed plasma clearance of dietary 13C-TG (P=0.035, RM ANOVA; Figure 2A) and higher AUC₆₉₆ (high=189.5±62.1 μmol/L versus low=47.7±13.1 μmol/L; P=0.031). In addition, this group had delayed plasma clearance of LPL-released 13C-NEFA (P=0.052, RM ANOVA; Figure 2B) and higher AUC₆₉₆ (high=10.2±4.2 μmol/L versus low=1.6±0.9 μmol/L; P=0.050). Whereas plasma 13C-NEFA in women with low-WAT apoC-I peaked at 4 hours and returned to baseline values after 6 hours, plasma 13C-NEFA accumulated continually in women with high-WAT and remained elevated at 6 hours. Finally, women with high-WAT apoC-I had a higher oxidation...
rate of the $^{13}$C high-fat meal ($P=0.040$, RM ANOVA), which may be secondary to the higher $^{13}$C-NEFA substrate availability in this group.

As described in the Methods section, we fractionated fasting and postprandial lipoproteins based on their size by FPLC, and 3 lipoprotein subclasses were identified: TRL, IDL/LDL, and HDL (Figure 3). As would be expected, there were significant intrasubject increases in the postprandial concentrations of TG (Figure 3A and 3B) and cholesterol (Figure 3C and 3D) in the 3 subclasses examined, whereas total apoB (apoB48+apoB100) increased in the TRL subclass only (Figure 3F; +185%; $P=0.0001$). Plasma apoB48, reflecting TRL of intestinal origins, also increased in both groups by $\approx 123\%$ at 4 hours and $\approx 94\%$ at 6 hours postprandially. There were no significant group differences in the TRL, IDL/LDL or HDL-TG, cholesterol, or apoB in the fasting or postprandial states. In the whole group, WAT apoC-I secretion did not correlate with TRL or IDL/LDL-TG, cholesterol, or apoB but correlated with both the AUC$_{6h}$ of apoB48 ($r=0.48; P=0.043$) and the increase in AUC$_{6h}$ of apoB48 ($r=0.56; P=0.017$). Taken together, the data thus far indicate that increased WAT apoC-I secretion associates with delayed plasma clearance of dietary, but not total, TG and of dietary, but not total, TRL and TRL remnant particle number. Because both dietary particle number (apoB48) and TG ($^{13}$C-TG) represent a minor fraction of total apoB and TG even in the postprandial state (<2%), the differences in dietary TRL clearance are likely insufficient to influence total plasma TRL clearance.

The secretion of apoC-I from WAT over 4 hours correlated with fasting total and non-HDL apoC-I (Figure 4A and 4D). On the other hand, although $>80\%$ of total apoC-I were recovered in the HDL fractions, there were no association between WAT apoC-I with fasting HDL-apoC-I (Figure 4G). In the postprandial state, WAT apoC-I remained correlated with total apoC-I (Figure 4B), and its correlation with non-HDL apoC-I was increased (Figure 4E) and significant in relation to both TRL apoC-I and IDL/LDL apoC-I ($r=0.61; P=0.007$ for both). There was a significant increase in postprandial non-HDL apoC-I (+70.7±16.1%; $P=0.001$), resulting from the significant increase in TRL apoC-I (fasting=1.41±0.40 µmol/L versus postprandial=4.02±0.85 µmol/L, +221±35%; $P<0.001$) but not IDL/LDL apoC-I (fasting=2.76±0.34 µmol/L versus

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**Figure 1.** Plasma concentrations of total triglyceride (A), glucose (B), nonesterified fatty acids (NEFA) (C), and insulin (D) after the ingestion of the $^{13}$C-triolein–labeled high-fat meal in women with high (n=9, solid line) vs low (n=10, dashed line) white adipose tissue (WAT) apolipoprotein (apo) C-I secretion. Group differences are analyzed by repeated measures ANOVA.

**Figure 2.** Postprandial plasma concentrations of $^{13}$C-triglyceride (TG) (A), plasma concentration of $^{13}$C- nonesterified fatty acids (NEFA) (B), and percent of $^{13}$C recovered in breath (C) after the ingestion of the $^{13}$C-triolein–labeled high-fat meal in women with high (n=9, solid line) vs low (n=10, dashed line) white adipose tissue (WAT) apolipoprotein (apo) C-I secretion. Group differences are analyzed by repeated measures ANOVA.
postprandial=2.89±0.36 µmol/L; NS). Finally, WAT apoC-I secretion over 4 hours correlated with the postprandial increase in non-HDL apoC-I over the same time period (Figure 4F), secondary to its correlation with the increase in TRL-apoC-I only (r=0.57; P=0.014). The postprandial changes in total or HDL apoC-I were not significant nor did they correlate with WAT apoC-I (Figure 4C and 4I).

The postprandial increase in TRL apoC-I is believed to be secondary to the transfer of apoC-I from HDL to TRL. However, there was no correlation between fasting and postprandial changes in HDL apoC-I with postprandial changes in TRL apoC-I. To determine whether WAT apoC-I contributes to postprandial TRL apoC-I, we conducted a stepwise forward regression analysis using fasting WAT apoC-I secretion and fasting concentrations, postprandial concentrations, and postprandial changes in HDL apoC-I as independent predictors. WAT apoC-I secretion over 4 hours was the primary predictor of postprandial TRL apoC-I, explaining 34% of its interindividual variations, followed by postprandial HDL apoC-I, explaining an additional 15% (regression model; postprandial TRL apoC-I=−0.45+10.4 WAT apoC-I−0.25 postprandial HDL apoC-I, total R²=0.55; P=0.002). Using a similar regression model to predict the postprandial changes in TRL apoC-I revealed that WAT apoC-I secretion was the sole predictor (R²=0.28; P=0.014).

Fasting WAT apoC-I secretion was correlated with the AUC₆₆₆₆hr of plasma ¹³C-TG (r=0.63; P=0.007). However, the AUC₆₆₆₆hr of plasma ¹³C-TG also correlated with TRL apoC-I (Figure 5A) but not with IDL/LDL, HDL, or total apoC-I (Figure 5B–5D) (NB AUC₆₆₆₆hr ¹³C-TG correlated with IDL/LDL apoC-I once the outlier point of 6.68 µmol/L apoC-I was removed). To examine whether the association of the AUC₆₆₆₆hr of ¹³C-TG with WAT apoC-I was mediated by its association with fasting TRL-apoC-I, we conducted a partial correlation analysis. Correction for TRL apoC-I eliminated the association of WAT apoC-I with the AUC₆₆₆₆hr of ¹³C-TG. There was no correlation between any fasting indices of insulin sensitivity (insulin, glucose, homeostasis model assessment, or quantitative insulin sensitivity check index) or inflammation (high-sensitivity C-reactive protein, orosomucoid, or haptoglobin) measured with the AUC₆₆₆₆hr of ¹³C-TG. However, because women with high-WAT also had evidence for higher postprandial insulin resistance, we corrected the association of WAT apoC-I secretion with the AUC₆₆₆₆hr of ¹³C-TG for the ratio of AUC₆₆₆₆hr glucose/AUC₆₆₆₆hr insulin. Correction for this postprandial index of insulin resistance did not eliminate the association of WAT apoC-I secretion with the AUC₆₆₆₆hr of ¹³C-TG (r=0.48; P=0.045). Similarly, correction for fasting inflammatory status did not eliminate this association (correction for all 3 inflammatory markers; r=0.55; P=0.023).

We then examined whether, in addition to apoC-I, WAT secretes apoC-II, apoC-III, and apoE, known to regulate TRL clearance, and explored whether their 24-hour WAT secretion associated with plasma ¹³C-TG and ¹³C-NEFA clearance. WAT apoC-I secretion over 24 hours was slightly higher than its secretion over 4 hours (24 hours=0.89±0.09 µmol/L per g versus 4 hours=0.80±0.09 µmol/L per g; P=0.05), and its secretion levels over 4 and 24 hours were highly correlated (r=0.90; P<0.001). The secretion of WAT apoC-I at 24 hours was significantly higher than that of apoC-III and apoE but not apoC-II (apoC-II=0.60±0.11 µmol/L per g; apoC-III=0.46±0.07 µmol/L per g and apoE=0.10±0.02 µmol/L per g; Figure 6A). There was no association between the WAT secretion of the apolipoproteins with each other, except for that of apoC-II and apoC-III (Figure 6B). Although all measured transferable apolipoproteins have well-documented roles in TRL metabolism, the AUC₆₆₆₆hr of plasma ¹³C-TG was correlated with WAT apoC-I secretion only (Figure 6C) and not with apoC-II, apoC-III, or apoE. In parallel, when examining the molar ratio of WAT secretion of the LPL inhibitors (apoC-I or apoC-III) to the LPL activator (apoC-II), the AUC₆₆₆₆hr of plasma ¹³C-TG correlated strongly with the ratio of WAT secretion of apoC-I to that of apoC-II (Figure 6D) but not of apoC-III to apoC-II. There was no correlation between the AUC₆₆₆₆hr of plasma ¹³C-NEFA with the 24-hour WAT secretion of any of the transferable apolipoproteins. Furthermore, there were no differences in the 24-hour WAT secretion of apoC-II, apoC-III, and apoE between women with high versus low 4-hour WAT apoC-I secretion, but there were differences for 24-hour WAT apoC-I secretion (P<0.001).

Discussion

This is the first report of the secretion of apoC-I, along with apoC-II, apoC-III, and apoE, from human subcutaneous...
WAT. Major findings from this study demonstrate that, in postmenopausal obese women, fasting WAT apoC-I secretion over 4 hours (1) associated with increased fasting TRL apoC-I and delayed dietary TRL and NEFA clearance, (2) was the primary predictor of postprandial concentrations and increases in TRL apoC-I, and (3) was no longer associated with delayed dietary TG clearance once fasting TRL apoC-I was accounted for.

It should be noted at first that although the correlative nature of our study does not allow causal connections to be determined, it allows the generation of hypotheses regarding the underlying mechanisms and clinical impact of our findings. Furthermore, a great advantage of this study was the use of FPLC to fractionate the lipoproteins instead of...

Figure 4. Correlation of fasting white adipose tissue (WAT) apolipoprotein (apo) C-I secretion over 4 hours with fasting concentrations, 4-hour postprandial concentrations, and 4-hour postprandial changes in total plasma apoC-I (A–C), non–high-density lipoprotein (HDL) apoC-I (D–F), and HDL apoC-I (G–I) in the 19 women examined (NB non-HDL apoC-I represents the sum of triglyceride-rich lipoprotein apoC-I and intermediate-density lipoprotein/low-density lipoprotein apoC-I).

Figure 5. Correlation of 6-hour area under the curve (AUC_{6hours}) of plasma ^13C-triglyceride (TG) with fasting plasma triglyceride-rich lipoprotein (TRL) apolipoprotein (apo) C-I (A), intermediate-density lipoprotein (IDL)/low-density lipoprotein (LDL) apoC-I (B), high-density lipoprotein (HDL) apoC-I (C), and total apoC-I (D) in the 19 women examined.

Figure 6. Fasting white adipose tissue (WAT) secretion of apolipoprotein (apo) C-I, apoC-II, apoC-III, and apoE over 24 hours (A), correlation of 24-hour WAT secretion of apoC-III with apoC-II (B), correlation of 6-hour area under the curve (AUC_{6hours}) of plasma ^13C-triglyceride (TG) with 24-hour WAT apoC-I secretion (C), and correlation of AUC_{6hours} of plasma ^13C-TG with the ratio of 24-hour WAT secretion of apoC-I:apoC-II (D) in the 19 women examined. **P<0.01 and ***P<0.001.
the traditionally used ultracentrifugation. The FPLC fractionates the lipoproteins solely based on size, thus reducing possible detachment of their content of transferable apolipoproteins as a result of high centrifugal forces, repeated dilutions, and high-salt gradients, particularly needed for HDL isolation. Finally, the use of stable isotope, the gold standard technique for the tracing of substrate metabolism in vivo in humans, allowed for a higher sensitivity to detect the reported group differences in dietary fat clearance. These differences would have been missed should the analysis have depended solely on total plasma TG, which is a pool of both intestinal and hepatic TG with variable intersubject contributions.

The postprandial increase in the concentrations of transferable apolipoproteins on non-HDL is reported to be secondary to their transfer from HDL. Current work from our laboratory has demonstrated that although the increased concentrations of apoC-III, apoC-III, and apoE on non-HDL were reciprocated by a decrease in their concentrations on the HDL particles, the postprandial increase in non-HDL apoC-I was not (Wassef H, unpublished data, 2012). These findings led us to believe that other mechanisms may be underlying increased postprandial TRL apoC-I, such as the direct acquisition of apoC-I from hepatic and extrahepatic source. Subcutaneous WAT is the most physiologically relevant tissue for the clearance of dietary fat, particularly in the early postprandial state. The trapping versus the release of LPL-generated dietary NEFA in human subcutaneous WAT was reported to be almost absent at fasting, to increase to 100% at 1 hour, and to return to 10% to 30% at 6 hours, after the ingestion of a meal. The secretion of apoC-I from human WAT has never been evaluated, and like many other apolipoproteins, apoC-I is believed to be primarily produced by the liver. However, the tissue origins of apoC-I were defined based on the mRNA expression of apoC-I, with the highest expression being in the liver, whereas WAT was never a part of these arrays. Yet, the apoE/ apoC-I apoCIII/aapoCII gene cluster found on chromosome 19 contains multienhancer regions that regulate gene expression in extracellular, such as macrophages and adipocytes. In line with this, our previous work using a human adipocyte model (SW872 liposarcoma cells) provided the first published evidence that human adipocytes can synthesize and secrete both apoC-I and apoE. More recently, the secretion of apoC-I was confirmed in differentiated 3T3-L1 adipocytes and mice WAT. Similarly, the mRNA expression of apoC-III increases during the differentiation of 3T3-L1 adipocytes. Thus, in light of these findings, the secretion of apoC-I, apoC-II, apoC-III, and apoE from human WAT was suspected. Our primary aim was to establish whether, in humans, WAT apoC-I secretion was related to the postprandial increase in TRL apoC-I and clearance of dietary TRL.

Our data demonstrate that human subcutaneous WAT secretes considerable amounts of apoC-I, apoC-II, and apoE over 24 hours. However, although apoC-I secretion at 24 hours was associated with delayed dietary TG clearance, WAT secretion of apoC-II, apoC-III, and apoE at the same time point were not. Furthermore, WAT apoC-I secretion over 4 hours correlated with postprandial concentrations and changes in TRL apoC-I over the same time period. Although postprandial HDL apoC-I associated negatively with postprandial TRL apoC-I, supporting the transfer of apoC-I from HDL to TRL, this only became significant once WAT apoC-I was accounted for, highlighting WAT as the primary source of TRL apoC-I. The acquisition of WAT-secreted apoC-I by TRL may occur while the TRL are docked on the endothelial surface of WAT for postprandial clearance. This may be facilitated by the C-terminal amphipathic α-helix of human apoC-I, particularly by the 2 aromatic residues F42 and F46 that are believed to be important for structural stability and phospholipid interaction. A similar mechanism was proposed for the binding of apoC-I secreted from skin fibroblasts to NEFA impeding their skin uptake. Naturally, our findings do not exclude the contribution of hepatic apoC-I to postprandial increase in TRL apoC-I. Indeed, hepatic endoplasmic reticulum–derived VLDL transport vesicles in rats were reported to contain apoC-I. ApoC-I was, however, not found in the endoplasmic reticulum–derived prechylomicron transport vesicles, which supports the presence of other sources for chylomicron apoC-I. Our data suggest that WAT, a nonlipoprotein secreting tissue, may be a major source of TRL apoC-I in the postprandial state, secreting ~534 pmol apoC-I/g.WAT per day. Because the production rate of apoC-I in vivo in normolipidemic subjects was estimated at ~272 pmol/g body weight per day (1.8 mg/kg per day) with a plasma resident time of 3.2 days, the amount of apoC-I secretion from women’s WAT in this study, although ex vivo, cannot be considered negligible. Supporting this, albeit in wild-type and ob/ob mice, both the liver and WAT were reported to be significant sources of apoC-I production. Because this is the first report of WAT apoC-I secretion in humans, future studies remain to explore the differential contribution of WAT and liver to circulating apoC-I and whether our findings are applicable in different populations.

Despite no apparent differences in body composition, adipocyte size, lipoprotein profile, insulin sensitivity, or inflammation at baseline, when challenged with a high-fat meal, women with high-WAT apoC-I secretion had delayed postprandial dietary TRL and NEFA clearance. This group also had evidence for postprandial insulin resistance, both systemic and WAT-specific, which may have promoted decreased TRL clearance, given the pivotal role of insulin in WAT LPL activity and TRL clearance in the postprandial state. However, our previous work has demonstrated that treatment of human adipocyte model with insulin reduced adipocyte apoC-I secretion. Thus, although higher plasma insulin and insulin resistance may have contributed per se to delayed postprandial TRL clearance in women with high-WAT apoC-I, it is unlikely that it had contributed to increased WAT apoC-I secretion in this group, at least not from the adipocytes. Adipocytes are, however, not the only source of apoC-I from WAT, because apoC-I is also produced by macrophages. Furthermore, correction for postprandial insulin resistance did not eliminate the association between WAT apoC-I secretion and AUCsob of 13C-TG. This further argues for increased WAT apoC-I secretion being upstream from insulin resistance in our subjects, because delayed TRL and NEFA clearance is known to promote lipotoxicity and insulin resistance in nonadipose tissue. Increased WAT apoC-I secretion in obese subjects
may thus be an early abnormality promoting WAT dysfunction, which, though not apparent in the fasting steady state, becomes evident in the dynamic state after the challenge of a high-fat meal.

Although it cannot be determined from the present study whether high-WAT apoC-I secretion is a promoter or a consequence of WAT dysfunction in the postprandial state or why some obese women had ≈4-fold higher WAT apoC-I secretion (Figures 4 and 6), correction for TRL apoC-I eliminated the association of WAT apoC-I with AUC6hours of 13C-TG. This suggests that increased TRL apoC-I may be the mechanism linking WAT apoC-I secretion to delayed dietary TG clearance, which is supported by the detrimental role of TRL apoC-I.13,19,24 This cross-talk between WAT and TRL, through apoC-I, may fuel a vicious cycle of reduced WAT clearance of TRL, increased hepatic NEFA uptake and TRL production, and increased systemic insulin resistance. Of high clinical relevance is the finding that WAT apoC-I correlated solely with TRL apoC-I but not with HDL apoC-I. This is vital, given the dual role of apoC-I in lipoprotein metabolism; whereas TRL apoC-I promotes delayed TRL clearance, HDL apoC-I promotes increased HDL cholest erol.14–17 We therefore propose that the reduction of WAT apoC-I secretion may provide a mechanism for ameliorating WAT function and dietary fat clearance without affecting the cardioprotective qualities of HDL apoC-I.

In summary, our data provide the first published human evidence for the secretion of apoC-I, along with apoC-II, apoC-III, and apoE, from subcutaneous WAT. Furthermore, it indicates that increased WAT apoC-I secretion associates with higher TRL apoC-I and delayed TRL clearance in the postprandial state. We hypothesize that reducing WAT apoC-I secretion ameliorates WAT dysfunction and associated cardiometabolic abnormalities in obese subjects.

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Disclosures

None.

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White Adipose Tissue Apolipoprotein C-I Secretion in Relation to Delayed Plasma Clearance of Dietary Fat in Humans
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Dietary fat clearance:

The clearance rate of $^{13}$C-triolein-labelled high fat meal (600 kcal/m$^2$, 0.017 gm $^{13}$C-triolein/gm fat, 68% fat, 18% carbohydrate) was measured as plasma $^{13}$C-TG and $^{13}$C-NEFA, while the oxidation rate of the $^{13}$C-labeled meal was measured as breath $^{13}$CO$_2$ over 6 hours. The $^{13}$C enrichment of the samples were analysed by an isotopic ratio mass spectrometer connected to an elemental analyzer (Vario Micro CHNS Cube, Elementar Americas Inc). The mass spectrometer was calibrated using 2 international L-glutamate standards with $^{13}$C enrichment of -26.389 and +37.626 ppm (International Atomic Energy Agency). The concentrations of $^{13}$C-TG and $^{13}$C-NEFA and $^{13}$C recovered in breath CO$_2$ were calculated as previously described:\textsuperscript{1-4}:\[ \delta^{13}C \%_{t=i} = \left( \frac{R_{S_{t=i}} - R_{VPDB}}{R_{VPDB}} \right) \times 10^3 \]

where $\delta^{13}C \%_{t=i}$ = Delta at time = i hr in part per million (ppm), $R_{S_{t=i}}$ = $^{13}$C/$^{12}$C of the sample at time = i hr, $R_{VPDB}$= $^{13}$C/$^{12}$C of the international standard $V_{PDB}$ = 0.0112372.

The % of $^{13}$C-recovered in breath $^{13}$CO$_2$ per hour was calculated as:

\[ \%^{13}C_{rec/hr} = \left[ \frac{\text{mM excess}^{13}C/\text{mM CO}_2_{t=i}}{\text{mM}^{13}C_{admin}} \right] \times \frac{\text{mM CO}_2 \text{excreted/hr}}{1.3} \times 100 \]

where \text{mM excess}^{13}C/\text{mM CO}_2_{t=i} = \left( \delta^{13}C \%_{t=i} - \delta^{13}C \%_{t=0} \right) \times R_{VPDB} \times 10^{-3}

\[ \text{mM}^{13}C_{admin} = \left[ \text{mg}^{13}C\text{-triolein}/ M \right] \times \left[ \left( P \times n \right)/100 \right] \]

\[ \text{mM CO}_2 \text{excreted/hr} = \frac{\text{CO}_2 \text{excreted (mL/hr)}}{22.4} \]

Where mg $^{13}$C-triolein = weight of administered $^{13}$C-triolein, M = Molecular weight of $^{13}$C-triolein or 885.4 g/mol, P = $^{13}$C-isotope purity or 99%, n = number of labelled carbon position or 3, 1.3 = correction factor to adjust for the uptake of label into the HCO$_3$ pool with bolus feeding\textsuperscript{21,29-31}, 22.4 is the volume in liters of 1 mole of CO$_2$. 


The concentrations of $^{13}$C-TG and $^{13}$C-NEFA in the plasma pools at each time point were calculated as follows $^{21, 29-31}$:

$^{13}$C atom percent (AP) = \( \frac{R_{S_{t=i}}}{R_{S_{t=i}} + 1} \times 100 \)

$^{13}$C atom percent excess (APE) = AP \(_{t=i}\) – AP \(_{t=0}\)

Plasma $^{13}$C-NEFA \(_{t=i}\) (µM) = APE \(_{S_{t=i}}\) x plasma NEFA (µM) \(_{t=i}\)

Plasma $^{13}$C-TG \(_{t=i}\) (µM) = APE \(_{S_{t=i}}\) x plasma (µM) \(_{t=i}\)

Where $\delta^{13}$C (‰) \(_{t=i}\) = Delta at time = i hr in part per million (ppm), $R_{S_{t=i}}$ = $^{13}$C/$^{12}$C of the sample at time = i hr, $R_{VPDB}$ = $^{13}$C/$^{12}$C of the international standard V\(_{PDB}\) = 0.0112372. Atomic percent excess represents the % increase in the enrichment of $^{13}$C tracer in plasma samples taken after ingestion of the $^{13}$C-labelled meal as compared to background fasting $^{13}$C-enrichment.

References:

**Dietary fat clearance:**

The clearance rate of $^{13}$C-triolein-labelled high fat meal (600 kcal/m$^2$, 0.017 gm $^{13}$C-triolein/gm fat, 68% fat, 18% carbohydrate) was measured as plasma $^{13}$C-TG and $^{13}$C-NEFA, while the oxidation rate of the $^{13}$C-labeled meal was measured as breath $^{13}$CO$_2$ over 6 hours. The $^{13}$C enrichment of the samples were analysed by an isotopic ratio mass spectrometer connected to an elemental analyzer (Vario Micro CHNS Cube, Elementar Americas Inc). The mass spectrometer was calibrated using 2 international L-glutamate standards with $^{13}$C enrichment of -26.389 and +37.626 ppm (International Atomic Energy Agency). The concentrations of $^{13}$C-TG and $^{13}$C-NEFA and $^{13}$C recovered in breath CO$_2$ were calculated as previously described:

$$\delta^{13}C \%_{t=i} = \left[ (R_{S_{t=i}} - R_{VPDB}) / R_{VPDB} \right] \times 10^3$$

where $\delta^{13}C \%_{t=i}$ = Delta at time $t=i$ hr in part per million (ppm), $R_{S_{t=i}}$ = $^{13}$C/$^{12}$C of the sample at time $t=i$ hr, $R_{VPDB}$ = $^{13}$C/$^{12}$C of the international standard V$PDB$ = 0.0112372.

The % of $^{13}$C-recovered in breath $^{13}$CO$_2$ per hour was calculated as:

$$%^{13}C_{rec/hr} = \left[ \left( mM \text{ excess }^{13}C/mM \text{ CO}_2_{t=i} \right) / mM \text{ }^{13}C_{admin} \right] \times mM \text{ CO}_2 \text{ excreted/hr} \times 1.3 \times 100$$

where $mM \text{ excess }^{13}C/mM \text{ CO}_2_{t=i} = (\delta^{13}C \%_{t=i} - \delta^{13}C \%_{t=0}) \times R_{VPDB} \times 10^{-3}$

$$mM \text{ }^{13}C_{admin} = [mg \text{ }^{13}C\text{-triolein/ M}] \times [(P \times n) / 100]$$

$$mM \text{ CO}_2 \text{ excreted/hr} = \text{ CO}_2 \text{ excreted (mL/hr) / 22.4}$$

Where mg $^{13}$C-triolein = weight of administered $^{13}$C-triolein, M = Molecular weight of $^{13}$C-triolein or 885.4 g/mol, $P = ^{13}$C-isotope purity or 99%, $n =$ number of labelled carbon position or 3, 1.3 = correction factor to adjust for the uptake of label into the HCO$_3$ pool with bolus feeding $^{21,29-31}$, 22.4 is the volume in liters of 1 mole of CO$_2$. 


The concentrations of $^{13}$C-TG and $^{13}$C-NEFA in the plasma pools at each time point were calculated as follows $^{21, 29-31}$:

$$^{13}\text{C atom percent (AP)} = \frac{R_{S_{\text{t=i}}}}{(R_{S_{\text{t=i}}} + 1)} \times 100$$

$$^{13}\text{C atom percent excess (APE)} = \text{AP}_{\text{t=i}} - \text{AP}_{\text{t=0}}$$

Plasma $^{13}$C-NEFA$_{\text{t=i}}$ (µM) = APE$_{S_{\text{t=i}}}$ x plasma NEFA (µM)$_{\text{t=i}}$

Plasma $^{13}$C-TG$_{\text{t=i}}$ (µM) = APE$_{S_{\text{t=i}}}$ x plasma (µM)$_{\text{t=i}}$

Where $\delta^{13}$C ($%$)$_{\text{t=i}}$ = Delta at time = i hr in part per million (ppm), $R_{S_{\text{t=i}}}$ = $^{13}\text{C}/^{12}\text{C}$ of the sample at time = i hr, $R_{VPDB}$ = $^{13}\text{C}/^{12}\text{C}$ of the international standard $V_{PDB} = 0.0112372$. Atomic percent excess represents the % increase in the enrichment of $^{13}$C tracer in plasma samples taken after ingestion of the $^{13}$C-labelled meal as compared to background fasting $^{13}$C-enrichment.

References: