Low density lipoprotein delays clearance of triglyceride-rich lipoprotein by human subcutaneous adipose tissue

Simon Bissonnette, 1, * a,† Huda Salem, 1, * b,† Hanny Wassef, 1, * c,8 Nathalie Saint-Pierre, 1, * Annie Tardif, † Alexis Baass, 1, * Robert Dufour, 1, * and May Faraj, 1, * a, b,† e-mail: may.faraj@umontreal.ca

Faculty of Medicine, Université de Montréal, 1 Montréal, Québec, Canada; Institut de Recherches cliniques de Montréal (IRCM), 1 Montréal, Québec, Canada; Division of Experimental Medicine, 1 McGill University, Montréal, Québec, Canada; and Montreal Diabetes Research Center (MDRC), 2 Montréal, Québec, Canada

Abstract Delayed clearance of triglyceride-rich lipoprotein (TRL) by white adipose tissue (WAT) promotes hypertriglycerideremia and elevated apoB-lipoproteins, which are primarily in the form of LDL. This study examines whether LDL promotes delayed clearance of TRL by WAT. Following the ingestion of a 13C-triolein-labeled high-fat meal, obese women with high plasma apoB (> median 0.93 g/l, N = 11, > 98% as IDL/LDL) had delayed clearance of postprandial 14C-triglyceride and 13C-NEFA over 6 h compared with controls. AUC6 h of plasma 13C-triglyceride and 13C-NEFA correlated with plasma apoB but not with LDL diameter or adipocyte area. There was no group difference in 13C-triolein oxidation rate, which suggests lower 13C-NEFA storage in peripheral tissue in women with high apoB. Ex vivo/in vitro plasma apoB correlated negatively with WAT 3H-lipid following a 4 h incubation of women’s WAT with synthetic 3H-triolein-TRL. LDL-differentiated 3T3-L1 adipocytes had lower 3H-TRL hydrolysis and 3H-NEFA storage. Treatment of women’s WAT with their own LDL decreased 3H-TRL hydrolysis and 3H-NEFA uptake. Finally, LDL, although not an LPL substrate, reduced LPL-mediated 3H-TRL hydrolysis as did VLDL and HDL. Exposure to LDL decreases TRL clearance by human WAT ex vivo. This may promote production of apoB-lipoproteins and hypertriglyceridermia through a positive-feedback mechanism in vivo.—Bissonnette, S., H. Salem, H. Wassef, N. Saint-Pierre, A. Tardif, A. Baass, R. Dufour, and M. Faraj. Low density lipoprotein delays clearance of triglyceride-rich lipoprotein by human subcutaneous adipose tissue. J. Lipid Res. 2013. 54: 1466–1476.

Supplementary key words dyslipidemia • hyperapoB • apolipoprotein B48 • apolipoprotein B100 • dietary triglyceride clearance

Postprandial hypertriglyceridermia is an independent risk factor for cardiometabolic disease (1). Many factors have been implicated in the etiology of hyperlipidemia; among the most common is reduced triglyceride-rich lipoprotein (TRL) clearance by peripheral tissue. White adipose tissue (WAT) is a major regulator of TRL clearance, particularly in the postprandial state (2–6). Following a meal, dietary fat enters the circulation in the form of chylomicrons, TRL with apoB48. Efficient clearance of chylomicrons by WAT requires three sequential steps: i) the hydrolysis of chylomicrons by endothelial lipoprotein lipase (LPL); ii) the uptake of LPL-generated nonesterified fatty acid (NEFA) by underlying adipocytes; and iii) the utilization or storage of NEFA (3, 5). Dietary TRL remnants and NEFA that are not cleared by peripheral tissue are then taken up by the liver for utilization and resecretion as VLDL (TRL with apoB100).

Healthy WAT is able to respond promptly to postprandial signals, such as insulin increasing the hydrolysis of dietary TRL and the uptake and storage of generated NEFA, thus reestablishing the homeostasis in plasma lipids. The storage versus the release of TRL-generated NEFA in human subcutaneous WAT was reported to be almost absent in the fasting state, to increase to 100% 1 h after the ingestion of a meal, and to decrease to 10–30% 6 h after the meal (5). Accordingly, delayed plasma clearance of postprandial TRL by WAT is believed to increase the influx of dietary TRL remnants and NEFA into nonadipose peripheral tissues, including muscle, pancreas, and liver, inducing lipotoxicity and insulin resistance (6–8). In the liver, this also leads to increased synthesis and secretion of VLDL, which further reduces chylomicron clearance due to competitive binding to LPL (9–14). Altogether, this increases the plasma concentrations of apoB-lipoproteins, which is measured as plasma apoB and represents mostly LDL particles (>90%) (14–16). Dysfunctional WAT is thus
closely associated with hypertriglyceridemia and hyperapoB in humans (6, 14, 17–19). Inherent abnormalities in WAT function are also believed to lead to the most common primary dyslipoproteinemia, familial combined hyperlipidemia (FCHL), which is characterized by hypertriglyceridemia, hypercholesterolemia, hyperapoB, small dense LDL, and insulin resistance (19, 20).

The mechanisms responsible for delayed plasma clearance of postprandial TRL by WAT are not fully understood. However, the uptake of LDL, albeit oxidized, by 3T3-L1 adipocytes was reported to increase cell proliferation and decrease cell differentiation (21–23). Moreover, multiple clinical studies have demonstrated that statin therapy, which reduces plasma concentrations of apoB-lipoproteins, also improves plasma clearance of triglyceride (TG) (24–27). Therefore, it is possible that elevated concentrations of apoB-lipoproteins not only are a consequence of dysfunctional WAT and hypertriglyceridemia but also play an active role in their pathology.

We thus examined the relation of elevated numbers of apoB-lipoproteins, specifically LDL, to postprandial TRL clearance and WAT function in healthy postmenopausal overweight and obese women. Our hypotheses were that elevated levels of apoB-lipoproteins in the form of LDL associates with reduced plasma clearance of dietary TRL and NEFA in vivo and directly reduces TRL clearance by women’s WAT and 3T3-L1 adipocytes ex vivo and in vitro.

**METHODS**

**Study design and population**

Twenty-two postmenopausal women were studied. The inclusion criteria were the following: body mass index (BMI) ≥ 27 kg/m²; age = 45–74 years; nonsmoker; sedentary (<2 h of structured physical exercise/week); and low alcohol consumption (<2 drinks/day). The exclusion criteria were the following: elevated risk of cardiovascular disease (≥20% calculated Framingham Risk Score) (28); prior history of chronic disease (untreated thyroid disease, cardiovascular disease, diabetes); inflammatory disease or cancer within the last 3 years; claustrophobia; abnormal plasma values (Hb < 120 g/l, creatinine > 100 μmol/l, ALT or AST >3 times normal limit); abnormal blood coagulation; concomitant medications [hormone replacement therapy (except thyroid hormone at a stable dose), lipid-lowering hypotensive agents, systemic corticosteroids, antipsychotic medication, psychoactive medication, anti-coagulant treatment, weight-loss agents, or adrenergic agonist]; known substance abuse; and lack of time to participate in the full length of the study (five weeks). The 22 women were separated into two groups based on median plasma apoB, which represents an average of two values measured three weeks apart. All participants gave written informed consent prior to initiation of the study, which had been approved by the Ethics Board at IRCM.

**Anthropometric and metabolic measurements**

All analyses were conducted at the end of a four-week weight stabilization period (±2 kg) to eliminate the effects of weight fluctuation on the measured outcomes (29). Body composition was measured by dual energy X-ray absorptiometry (General Electric Lunar Corp. version 6.10.019) (30, 31). Resting metabolic rate (RMR) and substrates oxidation rates were measured over 20 min by indirect calorimetry (Vmax encore, Cardinal Health) (31–33). Serum lipids and apoB were measured by an automated analyzer and LDL-C was calculated by the Friedewald equation (34) (COBAS INTEGRA 400, Roche Diagnostic). Plasma apoB48 was measured by ELISA (BioVendor) (35), serum glucose was measured by an automated analyzer (YSI 2300 STAT Plus, Life Sciences), and insulin was measured by a human radioimmunoassay (Millipore Corp.).

**Postprandial in vivo fat clearance**

The clearance and oxidation of a 13C-labeled high-fat meal was assessed as previously published (33, 36). At T = 0 h, fasting subcutaneous WAT samples were obtained by the physicians of the study from the right hip by needle biopsy under local anesthesia (Xylocaine 20 mg/ml, AstraZeneca) (37, 38). Fasting RMR, breath, and blood samples were collected at T = 0 h, followed by the consumption of a high-fat meal that was labeled with 13C-triolein (glycerol tri(oleate-1-13C), 99 atom% 13C, Sigma-Aldrich, Canada) standardized to body surface area (600 kcal/m², 0.017 g 13C-triolein/g fat, 68% fat, 18% carbohydrate). Serial measurements of respiration and collection of breath and blood samples were conducted at 1, 2, 4, and 6 h postprandially. The 13C enrichment in the breath 13CO2 and plasma 13C-TG and 13C-NEFA were analyzed by isotopic ratio mass spectrometer with a continuous flow module (Vario Micro CHNs Cube, Elementar Americas Inc.). The mass spectrometry was calibrated using two international L-glu tamate standards (USGS40 and USGS41, International Atomic Energy Agency http://nucleus.iaea.org) with 13C-enrichment of −26.389 and +37.626 ppm. The 13C-enrichment of the samples was calculated as previously described (33):

\[
\delta^{13}C_{\text{\%at} = t = i} = \left( \frac{R_{\text{VPDB}} - R_{\text{VPDB}}}{R_{\text{VPDB}}} \right) \times 10^3 \quad (\text{Eq. 1})
\]

where \(\delta^{13}C_{\text{\%at} = t = i}\) = delta at time = i h in ppm; \(R_{\text{VPDB}} = ^{13}C/^{12}C\) of the sample at time = i h; and \(R_{\text{VPDB}} = ^{13}C/^{12}C\) of the international standard VPDB = 0.0112372.

The percentage of 13C-recovered in breath 13CO2 per hour was calculated as:

\[
\%^{13}C_{\text{rec}\text{h}_{t = i}} = \left( \frac{m\text{mol excess }^{13}C/\text{mm CO}_{2\text{rec}}}{m\text{mol }^{13}C_{\text{admin}}\text{rec}} \right) \times m\text{mol CO}_{2\text{rec}}/\text{hex}_{t = i} \times \frac{1.3}{100} \times 10^3 \quad (\text{Eq. 2})
\]

where m mol excess 13C/ mm CO2rec = (\(\delta^{13}C_{\text{\%at} = t = i} - \delta^{13}C_{\text{\%at} = 0}\)) × RVPDB \times 10^−3,

\[
\text{m mol }^{13}C_{\text{admin} = t = i} = \frac{mg \ 13C\text{-triolein/M}}{\text{X} \times (P \times n)/100} \quad (\text{Eq. 3})
\]

\[
\text{m mol }^{13}C_{\text{excreted/hr}} = \frac{\text{CO}_{2\text{excreted}} \times (\text{mL/hr})}{22.4} \quad (\text{Eq. 4})
\]

where \(mg \ 13C\text{-triolein} = \text{weight of administered }^{13}C\text{-triolein}; \ M = \text{molecular weight of }^{13}C\text{-triolein or 885.4 g/mol}; P = \text{13C-isotope purity or 99%}; n = \text{number of labeled carbon position or 3}; L = \text{correction factor to adjust for the uptake of label into the HCO3}^{-} \text{pool with bolus feeding} \ (33, 39–41); \text{and 22.4 is the volume in liters of 1 mol of CO}_2\text{.}

The concentrations of 13C-TG and 13C-NEFA in the plasma pools at each time point were calculated as follows (33, 39–41):

\[
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where \( R_{51/1+} = \frac{^{13}C/^{12}C}{} \) of the sample at time = i, and \( ^{13}C \) represents percentage increase in the \( ^{13}C \)-enrichment in plasma samples taken after the ingestion of the \( ^{13}C \)-labeled meal compared with fasting background \( ^{13}C \)-enrichment.

**Lipoprotein profiling and LDL isolation**

LDL diameter was measured by an automated polyacrylamide gel electrophoresis system that separates apoB-lipoproteins based on size into one TRL, three IDL, and seven LDL fractions (FDA-approved, Lipoprint system, Quantimetrix) (42). Fresh plasma lipoproteins were also fractioned by size using fast protein liquid chromatography (FPLC, Sepharose 6 column) (36). As a quality control, we measured and compared the concentrations of cholesterol in the fasting TRL and LDL/LDL fractions as separated by the FPLC and by the Lipoprint. The cholesterol concentrations measured by these two techniques were highly correlated (TRL, \( r = 0.82 \); LDL/LDL, \( r = 0.92 \); \( P < 0.0001 \)), indicating the reliability of the FPLC fractionation of the lipoproteins. ApoB and apoE concentrations in 80 FPLC fractions were quantified by in-house ELISA using polyclonal antibodies against human apolipoproteins (Academy Biomedical) as published (36, 43). LDL was isolated from fresh fasting plasma by sequential ultracentrifugation using KBr density solution (1.019–1.063 g/ml, 0.01% EDTA) and Beckman Ti-50 rotor (45,000 rpm, 32 h, 5°C), after removal of TRL and KBr density solution (1.019–1.063 g/ml, 0.01% EDTA) and Beckman Ti-50 rotor (45,000 rpm, 32 h, 5°C), after removal of TRL and KBr density solution (1.019–1.063 g/ml, 0.01% EDTA) and Beckman Ti-50 rotor (45,000 rpm, 32 h, 5°C), after removal of TRL and KBr.

**Acute effect of LDL on women’s WAT function and LPL activity**

The direct effect of LDL on WAT in situ LPL activity and NEFABSA uptake over 4 h was assessed as described above but in the presence of each woman’s own LDL (1.2 g apoB/l). The average of three to six WAT samples/woman/condition is reported for each set of experiments.

To examine the direct effect of LDL on LPL activity, adipocyte-associated LPL was collected following the differentiation of 3T3-L1-adipocytes for seven days and their incubation with 100 µg/ml heparin for 45 min (control). Heparin-releasable LDL activity was assessed using the \( ^{3}H \)-TRL (1.41 mM TG) as previously published (44) in the presence or absence of physiologically concentrations of lipoproteins. The LDL concentrations used were enough to determine only those of apoB expressed in apoA-I units (1.2 and 1.8 g apoB/l), and VLDL concentrations were set as ~10% of LDL (0.12 and 0.15 g apoB/l). Of note, HDL and VLDL were enough to generate only two experimental doses per woman; thus, the highest doses possible were used. The activity of LDL dimers naturally released into the medium over 45 min was also measured as background. Moreover, the activity of a standard curve of purified LDL (Sigma-Aldrich, Canada) was measured over 45 min using the \( ^{3}H \)-TRL substrate (44) in the presence or absence of similar concentrations of the same woman’s lipoproteins as above. A representative experiment of \( N = 4 \) is presented.

**Adipocyte area**

WAT samples obtained by the needle biopsy were immediately fixed overnight at 4°C in 4% paraformaldehyde, embedded in paraffin, and cut into 4 µm slices. Adipocyte area was measured in a blinded fashion by digital-imaging analysis. The average surface area of 1,111 ± 96 adipocytes in six fields of view in three WAT slides is reported per woman. The three slides were at least 48 µm apart to avoid multilayered images of one cell. The adipocyte area represents the area of the pixels within intact cell membranes measured using MATLAB software (MathWorks) at the microscopy core at IRCM. Images were obtained using Leica DMRB microscope (Leica, Montreal, Canada) and a Retiga EXi camera (Q-Imaging, Burnaby, Canada) on a 0.67× mount over a 10×/NA 0.3 phase contrast objective.

**Statistical analysis**

Data are presented as mean ± SEM. Group differences over the 6 h time curves were analyzed by repeated-measures two-way ANOVA with interaction. When interaction was significant, intra-subject differences were analyzed by paired t-test, whereas intersubject differences were analyzed by unpaired t-test. Non-parametric Wilcoxon-rank sum test was used when equal variance, measured by Mauchly’s sphericity test, failed (plasma \( ^{13}C \)-TG and \( ^{13}C \)-NEFA data). Spearman correlation (two-tailed) was used to examine the association between variables. Statistical analysis was performed using SPSS V15, and significance was set at \( P \leq 0.05 \).
RESULTS

Twenty-two healthy postmenopausal overweight and obese women were separated based on plasma apoB median (0.93 g/l) into two groups of low and high apoB. Women with high apoB had delayed plasma clearance of total TG (Fig. 1A) and higher increment increase in the area under the 6 h curve of plasma TG above baseline (iAUC_{6h}) (high versus low: 8.46 ± 1.43 versus 4.00 ± 0.69 mM, P = 0.011). The two groups had identical concentrations of NEFA with similar postprandial drop at 1 and 2 h, suggesting equivalent insulin-induced inhibition of WAT lipolysis (Fig. 1B). There were no significant group differences in postprandial plasma insulin (Fig. 1C) or glucose (data not shown).

Specific to TRL of dietary origins, women with high apoB also had delayed plasma clearance of 13C-TG at 6 h (high versus low: 44.8 ± 17.4 versus 7.81 ± 3.33 µM, P = 0.039, Fig. 1D) and greater AUC 6 h of plasma 13C-TG (high versus low: 147.8 ± 54.5 versus 51.4 ± 19.8 µM, P = 0.045). Plasma 13C-NEFA remained elevated at the end of the 6 h in the women with high apoB, whereas it returned to fasting, non-enriched levels in women with low apoB (2.64 ± 1.56 versus 0.06 ± 0.22 µM, P = 0.014, Fig. 1E). As there was no group difference in the rate of 13C-NEFA oxidation (Fig. 1F), this suggests less uptake of LPL-released 13C-NEFA for storage by peripheral tissue in women with high apoB.

The types of apoB-lipoproteins were further characterized and compared in the two groups of women (Fig. 2). In the fasting state, ~1.0% of total plasma apoB (apoB48 and apoB100) was recovered in the TRL fractions (chylomicron and VLDL), with no significant group differences in TRL concentrations or percentage TRL of total apoB (Fig. 2A). The group difference in plasma apoB was secondary to differences in IDL/LDL (P < 0.0001), which represented ~99% of plasma apoB in both groups. Women with high apoB had higher fasting apoB48 (chylomicron remnants), which represented an equivalent minor percentage of total apoB in both groups (~0.6%, Fig. 2B, table insert). In line with the 13C-TG data, both TRL and apoB48-lipoprotein particle numbers increased in the postprandial state and to a higher extent in women with high apoB.

As apoE plays a major role in the hepatic clearance of TRL and IDL, we measured the concentration of apoE in TRL and IDL/LDL fractions. Women with high apoB had higher TRL and IDL/LDL apoE in both fasting (TRL = 0.27 ± 0.08 versus 0.09 ± 0.03 µM, IDL/LDL = 0.78 ± 0.06 versus 0.50 ± 0.05 µM, P < 0.05) and postprandial states (TRL = 0.72 ± 0.11 versus 0.28 ± 0.06 µM, IDL/LDL = 0.76 ± 0.07 versus 0.49 ± 0.04 µM, P < 0.01). However, correcting for total number of TRL (i.e., TRL apoB) eliminated the group differences in the fasting (high = 238.0 ± 35.8 versus low = 202.6 ± 27.8 µM apoE/µM apoB, not significant) and the postprandial state (high = 145.7 ± 17.1 versus low = 86.7 ± 24.4 µM apoE/µM apoB, not significant). The increase in the enrichment of fasting TRL with apoE in the postprandial state was equally significant in both groups. (Analysis of the IDL/LDL enrichment with apoE was not conducted, as most of apoB is bound to LDL, which is poor in apoE.)

As presented in Table 2, in the whole group, both total apoB and all forms of apoB measured correlated with iAUC_{6h} of plasma apoB48, TG, 13C-TG, and 13C-NEFA; however, fasting IDL/LDL showed stronger associations with these parameters than TRL (except 13C-NEFA). Despite correction for baseline levels, fasting plasma apoB48 remained strongly correlated to iAUC_{6h} of plasma apoB48 and fasting plasma TG to iAUC_{6h} of plasma TG. This, indicating that the inhibitory effect of elevated baseline remnants and TG on postprandial TRL clearance cannot be totally accounted for by a mathematical correction. Plasma IDL/LDL correlated best with the iAUC_{6h} of plasma TG and eliminated the association of LDL size with iAUC_{6h} of plasma TG once corrected for in regression models. Taken together, this data suggest that women with high apoB, mainly in the form of IDL/LDL, have delayed hydrolysis and clearance of postprandial dietary TRL by peripheral tissue and/or reduced uptake of TRL remnants by the liver, resulting in increased postprandial plasma TG and TRL particle number.

**Effect of chronic exposure to apoB-lipoproteins on women’s WAT and 3T3-L1 adipocyte function**

To examine whether WAT dysfunction may contribute to reduced dietary TRL clearance in women with high apoB delays triglyceride clearance by WAT 1469

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**TABLE 1.** Baseline characteristics of the postmenopausal women examined (N = 11/group)

| Characteristic | Low ApoB   | High ApoB  |
|---------------|------------|------------|
| ApoB (g/l)    | 0.73 ± 0.16| 1.20 ± 0.27**|
| Age (years)   | 59.1 ± 1.2 | 58.8 ± 1.9  |
| Weight (kg)   | 79.2 ± 3.0 | 71.8 ± 3.1  |
| BMI (kg/m²)   | 31.9 ± 1.2 | 30.2 ± 1.1  |
| BSA (m²)      | 1.80 ± 0.04| 1.69 ± 0.04|
| Waist (cm)    | 102.6 ± 3.2| 101.6 ± 2.2|
| LBM (kg)      | 40.0 ± 1.0 | 36.9 ± 1.1  |
| Fat mass (kg) | 36.1 ± 2.7 | 32.0 ± 2.2  |
| Android fat (kg) | 3.4 ± 0.3 | 3.1 ± 0.2 |
| Gynoid fat (kg) | 6.6 ± 0.5 | 5.8 ± 0.5 |
| Adipocyte area (µm²) | 3.145 ± 155 | 3.086 ± 287 |
| BMR (kcal/day) | 1,292 ± 40 | 1,205 ± 33 |
| SBP (mmHg)    | 113 ± 5    | 120 ± 5    |
| DBP (mmHg)    | 76 ± 3     | 77 ± 2     |
| Plasma glucose (mM) | 4.9 ± 0.1 | 5.1 ± 0.2 |
| Plasma insulin (µU/ml) | 14.5 ± 1.7 | 12.9 ± 1.8 |
| Plasma TC (mM) | 5.0 ± 0.1 | 6.8 ± 0.3**|
| Plasma LDL-C (mM) | 2.8 ± 0.1 | 4.5 ± 0.2**|
| Plasma HDL-C (mM) | 1.8 ± 0.2 | 1.4 ± 0.1 |
| Plasma TG (mM) | 1.0 ± 0.1 | 1.9 ± 0.3* |
| Plasma NEFA (mM) | 0.62 ± 0.04 | 0.56 ± 0.05 |
| Plasma apoB48 (mg/l) | 3.56 ± 0.42 | 8.34 ± 1.47**|
| LDL diameter (Å) | 271 ± 1 | 267 ± 2* |

*P < 0.05 and **P < 0.001 for group difference by unpaired t-test. Bold typeface indicates significant differences.
apoB, WAT samples were incubated for 4 h with synthetic
\(^3\)H-TRL, and tissue \(^3\)H-lipid was measured. As presented
in Fig. 3A, plasma apoB correlated negatively with WAT
\(^3\)H-lipid (r = -0.48, P = 0.049), indicating lower hydrolysis of
\(^3\)H-TRL and uptake and storage of generated \(^3\)H-NEFA
with increased plasma apoB. However, the correlation of
WAT \(^3\)H-lipid was with IDL/LDL apoB (r = -0.54; P = 0.048),
not with TRL apoB. As a control, we measured background
medium \(^3\)H-NEFA after incubating the \(^3\)H-TRL
substrate for 4 h in the absence of WAT. The negligible
amount measured (0.69 ± 0.01%) indicated that medium
\(^3\)H-NEFA was the result of \(^3\)H-TRL hydrolysis. Most of the
WAT \(^3\)H-lipid was in the form of WAT \(^3\)H-TG
(83.2 ± 6.5% of \(^3\)H-lipid). This suggests that chronic exposure
to a high concentration of IDL/LDL has a negative association with WAT-mediated TRL clearance and TG storage.

To explore that directly in adipocytes, we differentiated
3T3-L1 preadipocytes in the presence or absence of elevated yet physiological concentrations of LDL (1.4 g
apoB/l). Differentiation with LDL led to morphological changes, as LDL-differentiated adipocytes appeared
overconfluent compared with control adipocytes (Fig. 3B). Following adipocyte incubation with \(^3\)H-TRL for 4 h, LDL-
differentiated adipocytes had lower hydrolysis of \(^3\)H-TRL
and uptake and storage of \(^3\)H-NEFA measured as lower intracellular \(^3\)H-lipid (Fig. 3C and in percentage, -38.3 ± 19.9%, P < 0.05). The inhibitory effect of LDL was not secondary to the reduction in cell viability as LDH released from the adipocytes was not affected by LDL treatment over the seven days of differentiation (Fig. 3D).

Acute effect of LDL on women’s WAT function
To explore whether LDL may also have an acute inhibitory
effect on WAT function, WAT samples were incubated for 4 h with synthetic \(^3\)H-TRL ± each woman’s own LDL
(1.2 g apoB/l). Of importance, LDL alone was used instead of IDL/LDL, because unlike IDL, LDL is not an LPL
substrate since it is poor in apoC-II (an LPL activator)
(46). There were no group differences in the response of
WAT to LDL treatment; thus, the two groups were pooled
for analysis of LDL effects on WAT (Fig. 4A–C). LDL treat-
ment increased medium accumulation of \(^3\)H-TRL, indic-
ing reduced \(^3\)H-TRL hydrolysis by WAT, whether examined in absolute values (baseline versus LDL: 81.7 ± 5.1 versus 75.6 ± 5.0 nmol TG/mg WAT,
\(P = 0.006\)) or percentage changes (\(P = 0.03\), Fig. 4A). Moreover, LDL treatment increased medium accumulation of released
\(^3\)H-NEFA, whether examined in absolute values (baseline
versus LDL: 0.84 ± 0.20 versus 1.16 ± 0.26 nmol FA/mg WAT,
\(P = 0.015\)) or percentage changes (\(P = 0.006\), Fig. 4B).

Of note, there was no association of adipocyte size with
WAT \(^3\)H-lipid, \(^3\)H-TRL hydrolysis, or \(^3\)H-NEFA uptake
when the WAT samples were incubated with \(^3\)H-TRL or
\(^3\)H-NEFA:BSA.

Finally, LDL is known to bind to LPL (47, 48), and phosphatidylcholine-containing liposomes were reported to compete with LDL for binding to LPL (48). This suggests that LDL may directly compete with \(^3\)H-TRL binding to LPL; however, this effect may not be LDL-specific as other
lipoprotein contains phospholipids. Thus, we measured LPL activity in the presence or absence of women’s LDL, VLDL, and HDL. As presented in Fig. 4D, all lipoproteins, except for 0.6 g/L LDL, inhibited LPL activity compared with control (i.e., adipocyte-associated LPL released by heparin). This effect was dose-dependent for HDL and LDL but not VLDL, where both doses of VLDL blocked LPL activity, reaching similar levels as background. Similar to the LPL standard curve (Fig. 4E), all lipoproteins inhibited LPL activity at all doses used (except at 0.6 g/L LDL with 0.3 and 0.5 units of LPL). Lipoprotein-induced inhibition of LPL activity increased as a function of LPL concentrations.

![Graph showing FPLC-fractioned apoB-lipoproteins at fasting and postprandial states (graphs) and the sum of apoB concentrations in each faction (table).](image)

**Fig. 2.** FPLC-fractioned apoB-lipoproteins at fasting and postprandial states (graphs) and the sum of apoB concentrations in each faction (table) (A). Concentrations of apoB48 at fasting and postprandial states (graph and table) in women with high and low apoB (N = 11/group) (B). *P < 0.05, **P < 0.01 for group differences; $P < 0.01 for intragroup differences.

| TABLE 2. Spearman correlations between measured parameters in postmenopausal overweight and obese women (N = 22) |
|---------------------------------------------------------------|
| **Fasting Plasma Measure** | **ApoB48** | **TG** | **13C-TG** | **13C-NEFA** |
| ApoB48                        | 0.847**   | 0.628** | 0.571**   | 0.306  |
| Total apoB                    | 0.600**   | 0.697** | 0.571**   | 0.304* |
| TRL apoB                      | 0.604**   | 0.563*  | 0.412    | 0.295  |
| IDL/LDL apoB                  | 0.616**   | 0.821** | 0.519*   | 0.317  |
| LDL diameter                  | −0.366    | −0.644**| −0.354   | −0.163 |
| TG                             | 0.693**   | 0.695** | 0.686**   | 0.515* |
| NEFA                           | −0.179    | −0.299  | −0.164   | 0.068  |

*P < 0.05 and **P < 0.01 (two-tailed); all of the presented significant correlations (bolded values) with Spearman were also significant with Pearson correlation analysis.
reaching a maximum inhibition of −85.5%, −67.2%, and −90.7% at 0.18 g apoB/l VLDL, 1.8 g apoB/l LDL, and 1.8 g apoA-I/l HDL, respectively, \( P < 0.001 \). There was a dose-dependent inhibition of LPL activity for all VLDL and LDL doses at LPL concentrations of 0.8 and 1.0 units.

**DISCUSSION**

Data in this study demonstrate that plasma clearance of dietary TG and NEFA is delayed in postmenopausal obese women with high plasma apoB without any difference in the oxidation rate of NEFA, which points to reduced TG storage in peripheral tissue. Given the role of WAT in postprandial TRL clearance, we further examined the effects of apoB-lipoproteins on WAT function. A chronic inhibitory effect of apoB-lipoproteins/LDL was revealed ex vivo and in vitro as i) plasma apoB was inversely related to in situ LPL activity and NEFA storage in women’s WAT and ii) LDL-differentiated 3T3-L1 adipocytes had decreased in situ LPL activity and NEFA storage. In addition, LDL had a direct acute inhibitory effect on TRL clearance as i) LDL treatment of WAT for 4 h reduced the hydrolysis of synthetic TRL and increased the accumulation of LPL-derived NEFA, ii) LDL treatment of WAT for 4 h reduced the uptake of albumin-bound NEFA, and finally, iii) LDL directly inhibited LPL activity.

Before further discussion of the significance of this data, it is important to highlight certain strengths and limitations of our study. The use of the gold-standard stable-isotopes technique to trace dietary fat represented a major strength as it increased the sensitivity to detect group differences in dietary NEFA clearance. These differences would have been missed had the analysis been dependent solely on total plasma NEFA, which is a pool of both endogenous and exogenous NEFA with variable intersubject contributions. Although the correlative nature of our in vivo findings does not allow causal relations to be established between elevated apoB-lipoproteins and delayed TRL clearance, it provided the first evidence that this relationship exists in humans. Furthermore, it is not possible to dissect whether elevated postprandial TG in vivo was secondary to reduced LPL-induced TRL hydrolysis by peripheral tissue, reduced hepatic uptake of TRL remnants, or both. Whereas the in vitro and ex vivo models support an inhibitory effect of LDL on WAT function, an effect of LDL on other tissue cannot be excluded. Particularly in relation to the liver, hepatic clearance of TRL and IDL is

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**Fig. 3.** Chronic effect of apoB-lipoproteins/LDL. The correlation of plasma apoB with WAT \(^3\)H-lipids in 17 women (A), the morphology of 3T3-L1 adipocytes differentiated ± LDL (1.4 g apoB/l) (B), intracellular \(^3\)H-lipid following 4 h incubation with \(^3\)H-TRL in 3T3-L1 adipocytes differentiated ± LDL (C), and lactate dehydrogenase released during differentiation ± LDL (D).
facilitated by the binding of their content of apoE to hepatic LDL receptors (49). Although there were no group differences in the enrichment of TRL particles with apoE, elevated concentrations of LDL per se may have hindered hepatic TRL/LDL clearance by competitive binding to the LDL receptors. This is indeed supported by elevated fasting plasma apoB48 in women with high apoB.

Both chronic and acute effects of LDL examined in this study support an inhibitory role of LDL on TRL clearance by human WAT. This role of LDL in healthy subjects is in line with that published on patients with hyperapoB due to FCHL, in which adipocytes taken from these patients had reduced efficiency of exogenous NEFA esterification and TG synthesis (50). More recently, elegant carbon dating studies by Arner et al. demonstrated that WAT of non-obese subjects with FCHL also have reduced exogenous TG clearance and storage (51). Although the underlying mechanisms remain to be explored, our findings in LDL-differentiated 3T3-L1 adipocytes suggest that LDL may negatively impact adipocyte differentiation. This helps explain both reduced TRL clearance in women with high apoB and their reduced WAT function following WAT extraction from the body. Of note, oxidized LDL were shown to bind and be internalized by CD36 in mouse 3T3-L1 cells, causing the upregulation of preadipocyte factor-1 (Pref-1), whose suppression is key for the expression of peroxisome proliferator-activated receptor γ and adipocyte differentiation (21–23). Although higher oxidized LDL levels are likely more abundant in women with high apoB and their effects on adipocytes function cannot be excluded; our data demonstrate that LDL taken from women with both low and high apoB induced equivalent negative effects on WAT function (Fig. 4). Thus, the inhibitory effect of LDL on adipocyte function is likely mediated by native LDL per se. Like oxidized LDL however, the effect of native LDL may entail particle uptake and internalization. Supporting this is that adipocytes (3T3-L1 and human) interact with apoB-lipoproteins via several mechanisms, including VLDL receptor, LDL receptor-related protein, and cell surface proteoglycans, the expression of all of which increases with differentiation (52, 53). Moreover, the absence of hypertriglyceridemia in patients with familial hypercholesterolemia (54) suggests that LDL receptor in particular may be involved in the effects of LDL on WAT.

Although exposure of WAT to LDL for 4 h decreased TRL hydrolysis and NEFA uptake, this was not translated...
into decreased \(^3\text{H}\)-lipid in WAT. Although this may seem contradictory, it should be taken into account that plasma apoB was reported to be negatively associated with norepinephrine-induced lipolysis ex vivo in subcutaneous adipocytes of healthy obese men (55). Moreover, carbon dating data showed that WAT from nonobese FCHL patients had not only reduced exogenous TG clearance and storage but also reduced endogenous TG turnover and increased TG age (51). Thus LDL-incubated WAT may have reduced lipolysis of both exogenous TRL and endogenous TG, with a reverse direction in control WAT. Reduced exogenous \(^3\text{H}\)-TRL clearance and storage in LDL-incubated WAT will be offset by increased endogenous \(^3\text{H}\)-TG lipolysis in control WAT, resulting in no net group differences in WAT \(^3\text{H}\)-lipid, despite the accumulation of the \(^3\text{H}\)-TRL in the medium of LDL-incubated WAT. Moreover, large variability in the fate of \(^3\text{H}\)-NEFA once taken up by WAT between oxidation and storage may have existed. Although there was no group difference in the percentage of ingested \(^13\text{C}\) recovered in breath CO\(_2\), energy expenditure in vivo is better reflected by substrate oxidation in nonadipose than in adipose tissue. For example, it was reported that energy expenditure per gram of tissue in obese women was 98-fold higher in the heart and kidneys and 2.9-fold higher in skeletal muscles than in adipose tissue (56). However, endogenous TG lipolysis and NEFA oxidation were not assessed in the present study given WAT sample size limitations and should be investigated in future new studies.

Finally, there are a couple of mechanisms by which LDL may hinder TRL clearance by WAT. The work presented here demonstrates that LDL inhibits LPL activity via direct or indirect mechanisms. One particle of LDL is documented to bind up to 15 LPL dimers, which is believed to be the mechanism responsible for the retention of LDL by macrophages in an atherosclerotic lesion (47, 48). Moreover, phosphatidylcholine-containing liposomes were reported to bind and compete with LDL binding to LPL (48); thus, all lipoproteins may potentially compete with \(^3\text{H}\)-TRL binding to LPL. This indeed was the case as both VLDL and HDL inhibited LPL activity to a greater extent than did LDL. However, the higher inhibition of LPL activity by VLDL and HDL may be secondary to HDL, and particularly TG-rich VLDL, being an LDL substrate as they contain apoC-II. Although it cannot be determined in vivo which lipoprotein subclass provided a higher competitor for \(^3\text{C}\)-TRL binding to LPL, the fasting plasma concentration that distinguished women with high apoB was higher IDL/LDL particle number, not higher HDL or TRL apoB.

Alternatively, LDL may indirectly inhibit LPL activity by favoring the accumulation of NEFA in the vicinity of LPL. LDL is reported to associate with albumin-bound NEFA in a concentration-dependent manner (0.25–2 mM) following incubation for 4 h (57–59), which is similar to conditions used in our experiments. Binding of LDL to NEFA in vivo is believed to be responsible for the generation of electronegative or minimally modified/oxidized LDL that characterizes subjects with hypertriglyceridemia, type 2 diabetes, and coronary heart disease (60). Binding of LDL to NEFA may not only reduce its availability for uptake by WAT, resulting in its medium accumulation, but may also hinder TRL clearance. This is because NEFA are well documented to inhibit LPL activity by several mechanisms, including displacing LPL from the endothelial surface and interfering with LPL binding to apoC-II and to its anchors on the cell membrane (61–67). However, it should be underscored that delayed clearance of dietary TRL and remnants in women with high apoB may also have contributed to elevated plasma NEFA pool due to prolonged spill-over from TG hydrolysis. This, however, cannot explain LDL-induced reduction in albumin-bound-NEFA uptake ex vivo into WAT in the absence of TRL.

In conclusion, LDL promotes delayed plasma clearance of TRL and NEFA in subcutaneous WAT of postmenopausal obese women, which may favor the postprandial hypertriglyceridemia observed in this group. We hypothesize that targeted reduction of plasma apoB may ameliorate WAT function, TRL clearance, and associated cardiometabolic risks in humans.

The authors acknowledge the invaluable work of Dr. Remi Rabasa-Lhoret in subject screening, recruitment, and medical follow-up. The authors thank Miguel Chagnon (Statistics Department, Université de Montréal) for his advice on the statistical analysis of the data.

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