

## Menopausal Status and Abdominal Obesity Are Significant Determinants of Hepatic Lipid Metabolism in Women

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**Background**—Android fat distribution (abdominal obesity) is associated with insulin resistance, hepatic steatosis, and greater secretion of large very low-density lipoprotein (VLDL) particles in men. Since abdominal obesity is becoming increasingly prevalent in women, we aimed to investigate the relationship between android fat and hepatic lipid metabolism in pre- and postmenopausal women.

**Methods and Results**—We used a combination of stable isotope tracer techniques to investigate intrahepatic fatty acid synthesis and partitioning in 29 lean and 29 abdominally obese women (android fat/total fat 0.065 [0.02 to 0.08] and 0.095 [0.08 to 0.11], respectively). Thirty women were premenopausal aged 35 to 45 and they were matched for abdominal obesity with 28 postmenopausal women aged 55 to 65. As anticipated, abdominal obese women were more insulin resistant with enhanced hepatic secretion of large ( $404 \pm 30$  versus  $268 \pm 26$  mg/kg lean mass,  $P < 0.001$ ) but not small VLDL ( $160 \pm 11$  versus  $142 \pm 13$ ). However, postmenopausal status had a pronounced effect on the characteristics of small VLDL particles, which were considerably triglyceride-enriched (production ratio of VLDL<sub>2</sub>-triglyceride:apolipoprotein B  $30 \pm 5.3$  versus  $19 \pm 1.6$ ,  $P < 0.05$ ). In contrast to postmenopausal women, there was a tight control of hepatic fatty acid metabolism and triglyceride production in premenopausal women, whereby oxidation ( $r_s = -0.49$ ,  $P = 0.006$ ), de novo lipogenesis ( $r_s = 0.55$ ,  $P = 0.003$ ), and desaturation ( $r_s = 0.48$ ,  $P = 0.012$ ) were closely correlated with abdominal obesity-driven large VLDL-triglyceride secretion rate.

**Conclusions**—In women, abdominal obesity is a major driver of hepatic large VLDL particle secretion, whereas postmenopausal status was characterized by increased small VLDL particle size. These data provide a mechanistic basis for the hyperlipidemia observed in postmenopausal obesity. (*J Am Heart Assoc.* 2015;4:e002258 doi: 10.1161/JAHA.115.002258)

**Key Words:** apolipoproteins • cholesterol • lipids • lipoproteins • menopause • women

Menopause is associated with increased cardiovascular disease and once women develop acute coronary symptoms, they have worse short- and long-term outcomes than men.<sup>1</sup> Many different factors contribute, including marked hormonal changes,<sup>2</sup> changes in metabolic profile associated with increased risk of the metabolic syndrome,<sup>3</sup>

and relative increase in intra-abdominal fat with age.<sup>4</sup> Accumulation of intra-abdominal fat is associated with increased waist circumference and liver fat,<sup>5</sup> overproduction of very low-density lipoprotein (VLDL), and decreased catabolism of apolipoprotein (apo)B-containing particles in men.<sup>6</sup> The catabolism of apoB-containing particles is partly

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Accompanying Tables S1 through S7 and Figures S1 through S3 are available at <http://jaha.ahajournals.org/content/4/9/e002258/suppl/DC1>

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determined by plasma apoC-III concentrations, and higher plasma apoC-III has been associated with dyslipidemia in obese men.<sup>7</sup> Although abdominal obesity tends to be associated with obesity in men, data from the United States have been used to estimate that 40% of women have an abdominal fat distribution pattern as defined by waist:hip ratio.<sup>8</sup>

Normally, fasting plasma triglycerides (TG) are determined by 2 distinct subclasses of VLDL<sup>9</sup>; VLDL<sub>1</sub> is larger and more TG-rich than VLDL<sub>2</sub>, the latter can either be secreted directly from the liver, or formed by the peripheral hydrolysis of VLDL<sub>1</sub>. Hypertriglyceridemia is associated with atherogenic dyslipidemia including the production of small dense LDL, lower HDL cholesterol, and accumulation of postprandial TG-rich lipoproteins.<sup>10</sup> In men with type 2 diabetes, the secretion of VLDL<sub>1</sub> is associated with liver fat, hypertriglyceridemia, and increased atherogenic risk.<sup>11</sup>

Impaired hepatic fatty acid oxidation has been reported to be related to obesity and insulin resistance by some<sup>12,13</sup> but not all.<sup>14</sup> Few detailed studies have investigated VLDL<sub>1</sub> and VLDL<sub>2</sub> kinetics in women, and none have compared the kinetics of VLDL or apoC-III in pre- and postmenopausal women. We hypothesized that VLDL<sub>1</sub>-TG and -apoB secretion would be higher in abdominally obese compared with abdominally lean women and aimed to investigate the effect of menopause status on this relationship by measuring hepatic de novo fatty acid synthesis (DNL), oxidation, and desaturation in relation to VLDL<sub>1</sub> and VLDL<sub>2</sub> kinetics in pre- and postmenopausal women.

## Materials and Methods

### Subjects

We recruited 60 healthy white women from local advertising and the Oxford Biobank as previously reported<sup>15</sup> equally into pre- and postmenopausal groups aged 35 to 45 and 55 to 65, respectively. The age groups ensured that perimenopausal women were not included and postmenopausal status was defined as absence of menses for at least 12 months and follicle-stimulating hormone >30 IU/L. Since we also wished to investigate the effect of android fat (abdominal obesity), we used waist circumference, a marker of android fat, to facilitate recruitment of women into groups with low or high android fat. For simplicity, we have referred to the group with low android fat as “lean.” A waist circumference of  $\geq 80$  cm was selected as the proxy measure of high android fat, with increased risk of cardiovascular disease in Europid women defined by the International Diabetes Federation<sup>16</sup> and additionally, we recruited women into small waist (<80 cm, n=30), or large waist (80 to 84 cm, n=5; 85 to 91 cm, n=5; and 92 to 110 cm, n=5) categories

in both menopausal groups. This was to ensure a good range of android fat in our cohort, and ensure exact matching of abdominal obesity between menopausal groups. Other inclusion and exclusion criteria have been previously described in a study relating to energy intake in a subset of the participants<sup>15</sup> but briefly, women were excluded if they had any condition or treatment that would affect metabolic or hormonal status (including smoking, diabetes, or hormone replacement therapy), or had body mass index (BMI) <18.5 or >34.9. Smokers or women exceeding alcohol consumption guidelines of 2 to 3 units per day were also excluded.<sup>17</sup> All participants gave informed, written consent and the study was approved by the Oxfordshire Clinical Research Ethics Committee. Participants attended the Clinical Research Unit prior to the metabolic day in order to be given deuterated water for consumption the evening before the study day, and to give a blood sample for background isotopic enrichment measurements relating to the measurement of DNL (see below).

### Measurement of Liver, Subcutaneous, and Visceral Fat and Body Composition

Intrahepatic fat was measured by magnetic resonance spectroscopy, visceral and subcutaneous fat were measured by magnetic resonance imaging after an overnight fast and within 2 weeks of the study day,<sup>18</sup> and whole body composition and fat distribution (eg, android and gynoid fat) were measured using DEXA.<sup>15</sup>

### Metabolic Study Day

Participants arrived after an overnight fast and after consuming deuterated water (<sup>2</sup>H<sub>2</sub>O, in order to measure de novo lipogenesis, DNL) (3 g/kg body water) at 8 and 10 PM the evening before the study day and then continued to consume enriched water (2.5 g per 500 mL water), in order to achieve and maintain a plasma water enrichment of 0.3%.<sup>19</sup> A cannula was placed in an antecubital vein in order to take blood samples for the estimation of DNL in VLDL<sub>1</sub>- and VLDL<sub>2</sub>-palmitate, and background isotopic enrichments for the kinetic studies. Another cannula was placed in the contralateral arm to administer intravenous boluses of [<sup>2</sup>H<sub>3</sub>]leucine (7 mg/kg) and [<sup>2</sup>H<sub>5</sub>]glycerol (500 mg), while an intravenous infusion of [U-<sup>13</sup>C]palmitic acid, potassium salt complexed with albumin<sup>20</sup> at 0.03  $\mu$ mol/kg per minute, was started. Blood samples were taken for a further 8 hours and VLDL<sub>1</sub> and VLDL<sub>2</sub> were isolated from plasma using density gradient ultracentrifugation.<sup>20</sup> Due to technical problems, 1 participant did not receive the palmitate infusion, 1 participant's infusion was stopped early, and 1 participant did not complete the metabolic study day.

## Biochemical Analyses

Blood samples were drawn into heparinized syringes (Sarstedt, Leicester, UK) and plasma was rapidly separated at 4°C. Plasma metabolites were analyzed enzymatically,<sup>20</sup> insulin was measured by radioimmunoassay (Millipore [UK] Ltd, Watford, UK), and homeostatic model assessment of insulin resistance was calculated as an index of insulin resistance.<sup>21</sup> A time-averaged area-under-the-curve for plasma 3-hydroxybutyrate (3OHB) and nonesterified fatty acids (NEFA) was calculated from hourly values taken during the study. Serum steroids (cortisol, dehydroepiandrosterone, and androstenedione) were measured by liquid chromatography/tandem mass spectrometry using a Waters Xevo mass spectrometer with Acquity uPLC system as described previously.<sup>22</sup> [<sup>2</sup>H<sub>5</sub>]glycerol in plasma, VLDL<sub>1</sub> and VLDL<sub>2</sub>-TG (to trace TG) and [<sup>2</sup>H<sub>3</sub>]leucine in plasma, and VLDL<sub>1</sub>- and VLDL<sub>2</sub>-apoB (to trace whole particles) were measured by gas chromatography–mass spectrometry.<sup>23</sup> [U-<sup>13</sup>C]palmitic acid was measured in plasma NEFA and VLDL<sub>1</sub> and VLDL<sub>2</sub>-TG by gas chromatography–mass spectrometry<sup>20</sup> and the proportion of fatty acids (Fas) in VLDL-TG that were derived from nonsystemic sources was calculated,<sup>20</sup> assuming that 16:0 is representative of all FAs. Mathematical modeling of VLDL kinetics (VLDL<sub>1</sub>-TG, VLDL<sub>2</sub>-TG, VLDL<sub>1</sub>-apoB, VLDL<sub>2</sub>-apoB production and clearance) was calculated from [<sup>2</sup>H<sub>5</sub>]glycerol and [<sup>2</sup>H<sub>3</sub>]leucine enrichments in plasma and lipoprotein fractions.<sup>11</sup> See Figures S1 and S2 for examples of raw data used for modeling. VLDL-TG production rates were corrected for lean mass in order to consider delivery of TG to muscle as previously described<sup>24</sup> but not corrected when considering hepatic FA trafficking. Total plasma apoC-III and apoC-III in plasma devoid of apoB-containing particles were measured using a Hydrigel LP CIII Electroimmunodiffusion kit (Sebia, France) with appropriate standards and quality controls according to the manufacturer's instructions. By difference, we calculated apoC-III concentrations in apoB-containing particles (apoC-III LpB). ApoCIII kinetic modeling was carried out as previously described<sup>25</sup> and assumes (consistent with previous studies, and earlier radiotracer studies) that apoCIII exchanges between VLDL and HDL particles, and therefore that measuring apoCIII kinetics in plasma is valid.

The ratio of [U-<sup>13</sup>C]16:1n-7/[U-<sup>13</sup>C]16:0 in VLDL<sub>1</sub> and VLDL<sub>2</sub>-TG was determined as a short-term index of hepatic stearoyl-CoA desaturase (SCD) activity (the “isotopic desaturation index”) and also the SCD16 and SCD18 FA ratios.<sup>26</sup> FA methyl esters prepared from VLDL<sub>1</sub> and VLDL<sub>2</sub>-TG FAs<sup>20</sup> were analyzed by GC<sup>13</sup> to quantify 16:0 and 16:1n-7, and by GC-Isotope Ratio Mass Spectrometer to measure isotopic enrichment.<sup>26</sup>

Hepatic DNL was measured on the study day, based on the incorporation of <sup>2</sup>H in plasma water (Finnigan GasBench-II;

ThermoFisher Scientific, UK) and into VLDL<sub>1</sub>- and VLDL<sub>2</sub>-TG palmitate using gas chromatography–mass spectrometry.<sup>27</sup> For simplicity, this is referred to as “%DNL” and represents synthesis of FAs from precursors such as sugars and amino acids.<sup>28</sup>

FA rate of appearance (R<sub>a</sub>NEFA) was calculated from the [U-<sup>13</sup>C]16:0 infusion rate and enrichment in the plasma NEFA fraction and R<sub>a</sub>NEFA was assumed to equal R<sub>a</sub>NEFA.<sup>24</sup>

## Statistical Analysis

Statistical analysis was performed using SPSS Statistics 19 (IBM, SPSS products, Chertsey, UK). Two-way ANOVA was used to determine the effect of abdominal obesity and menopausal status (fixed factors) on each dependent variable, and interaction between the fixed factors. A significant interaction term indicated that the relationship between the dependent variable and abdominal obesity was significantly different in pre- and postmenopausal women. Associations between variables were carried out using Spearman's rank correlation coefficient (univariate analysis).

In order to visualize relationships between metabolic variables, we plotted significant correlations between metabolic and anthropometric variables related to hepatic FA partitioning using “hive plots.”<sup>29</sup> Each variable is represented by a node and the nodes are joined by blue (significant positive correlations) or red (significant negative correlations) lines. The nodes are placed on 3 duplicated radial axes, which represent grouped variables (anthropometric and metabolic variables/VLDL<sub>1</sub> or VLDL<sub>2</sub>). The axes are duplicated in order to allow for representation of correlations within the variable group (eg, there are lines joining the isotopic desaturation index and %DNL in VLDL<sub>1</sub> for pre- and postmenopausal women, representing significant positive correlations).

## Power Calculation

Using data from a study of the reproducibility of relevant kinetic parameters (VLDL TG and apoB100 secretion rates, VLDL-TG clearance rate, rate of appearance NEFA,<sup>30</sup> and DNL),<sup>19</sup> separate power calculations were carried out and the numbers in pre- and postmenopausal groups to detect a 40% difference with power of 0.80 at  $\alpha$  of 0.05 were 4, 8, 9, 10, and 15, respectively (in each group). A difference of 40% was considered to be clinically significant and was within the range of differences previously reported in other studies.<sup>11</sup>

## Results

Sixty women were recruited: mean age was 41.0 years (range 35 to 45) for premenopausal and 58.1 years (55 to 64) for postmenopausal women. Mean age when divided according to

**Table 1.** Body Composition in Women According to Menopausal Status and Abdominal Obesity

	Premenopausal (n=30)	Postmenopausal (n=30)*	Lean (n=29)	Abdominally Obese (n=29)	$P_{\text{meno}}$	$P_{\text{Abd obesity}}$
Waist, cm	83.5 (1.8)	82.1 (1.4)	77.1 (0.86)	88.6 (1.5)	NS	<0.001
BMI, kg/m <sup>2</sup>	24.9 (0.6)	24.8 (0.4)	23.2 (0.36)	26.6 (0.54)	NS	<0.001
WHR	0.85 (0.01)	0.84 (0.01)	0.82 (0.009)	0.87 (0.01)	NS	0.001
Gynoid fat	5.3 (0.25)	5.0 (0.14)	4.6 (0.17)	5.6 (0.20)	NS	<0.001
Gynoid fat <sup>†</sup>	0.24 (0.07)	0.21 (0.05)	0.25 (0.006)	0.20 (0.004)	<0.01	<0.001
Android:gynoid ratio	0.35 (0.02)	0.39 (0.02)	0.27 (0.015)	0.47 (0.015)	<0.05	<0.001
Intra-ab fat, cm <sup>2</sup>	40.7 (4.1)	53.3 (5.5)	26.5 (2.0)	67 (4.6)	<0.05	<0.001
Subcut fat, cm <sup>2</sup>	225.0 (17.0)	241.0 (14.0)	181 (11)	282 (14)	NS	<0.001
Fat mass, kg	23.2 (1.3)	23.4 (0.9)	19.0 (0.72)	27.6 (0.88)	NS	<0.001
Lean mass, kg	42.4 (0.9)	39.4 (0.8)	39.5 (0.89)	42.4 (0.87)	<0.05	<0.001
Fat:lean mass	0.54 (0.03)	0.60 (0.02)	0.49 (0.21)	0.65 (0.015) <sup>‡</sup>	<0.05	<0.001
Liver fat, %	0.78 (0.25 to 11.5)	0.97 (0.44 to 6.8)	0.61 (0.25 to 2.1)	1.3 (0.32 to 11.5)	NS	<0.001

Data presented as mean (SEM) or median (range). Statistical significance based on 2-way ANOVA:  $P_{\text{meno}}$ , statistical significance for an effect of menopausal status;  $P_{\text{Abd obesity}}$ , statistical significance for an effect of abdominal obesity. BMI indicates body mass index; Intra-ab fat, intra-abdominal fat; NS, not significant; Subcut fat, subcutaneous fat; WHR, waist-to-hip ratio. \*n=30 for postmenopausal women apart from data derived from DEXA measurements, which were n=28 (gynoid fat, android: gynoid ratio, fat mass, lean mass and fat:lean mass).

<sup>†</sup>Corrected for total fat mass in order to investigate differences in body fat distribution.

<sup>‡</sup> $P < 0.05$  for interaction between abdominal obesity and menopausal status.

abdominal obesity was 49.3 (35 to 64) and 49.3 (35 to 63) for lean and abdominally obese women, respectively. Plasma follicle-stimulating hormone concentrations ranged from 3.0 to 21.3 and 46.5 to 125 IU/L in pre- and postmenopausal women, respectively (confirming menopausal status). Fifty-eight women from whom DEXA scans were available are included in this study, divided according to menopause status and android fat measurement (corrected for total fat), for statistical analyses. Lean women had a mean android fat of 0.065 (SD 0.013) and abdominally obese women had a mean value of 0.094 (SD 0.008). Gynoid fat was significantly lower, and intra-abdominal fat was significantly higher in postmenopausal women, despite being matched for abdominal obesity (Table 1). Liver fat was generally low, although 6 women, all abdominally obese, had values of >5%. BMI was not significantly different between menopausal groups and ranged from 21.5 to 33.0 kg/m<sup>2</sup> in abdominally obese and 19.5 to 27.6 kg/m<sup>2</sup> in abdominally lean women. Thus, some abdominally lean women would be classified as overweight by BMI, and some abdominally obese women would be classified as lean by BMI.

Postmenopausal women had significantly higher concentrations of plasma total, LDL, non-HDL cholesterol, apoCIII-LpB, plasma apoB, and systolic BP than premenopausal women (Table 2). Women with abdominal obesity were more insulin resistant with a more adverse lipid profile (higher non-HDL cholesterol, plasma TG, VLDL<sub>1</sub>- and VLDL<sub>2</sub>-TG concentrations). One postmenopausal woman had impaired fasting glucose.

The rate of disappearance of FAs ( $R_d$ NEFA), expressed per kg lean mass was significantly higher in post- compared to premenopausal women (Table 3). Release of FAs into plasma ( $R_a$ NEFA) per unit weight of adipose tissue was lower in women with abdominal obesity, but VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB production were significantly higher. The ratio of VLDL<sub>2</sub>-TG direct production: VLDL<sub>2</sub>-apoB production was significantly higher in post- compared to premenopausal women, indicating production of larger particles.

There were no significant positive correlations between age and liver fat, VLDL<sub>1</sub>-TG and VLDL<sub>2</sub>-TG direct production, VLDL<sub>1</sub> and VLDL<sub>2</sub> direct apoB production, or VLDL<sub>1</sub> and VLDL<sub>2</sub>-TG:apoB production ratios within menopausal groups.

Plasma apoC-III concentrations positively correlated with apoC-III production rate ( $r_s=0.59$ ,  $P=9.0 \times 10^{-7}$ ) but not clearance rate, indicating that plasma apoC-III concentrations were determined by production rate. Plasma, HDL- and apoC-III associated with lipoprotein B-containing particle (apoC-III LpB) concentrations were not affected by abdominal obesity, but plasma apoC-III LpB concentrations were higher in postmenopausal women.

Overall, mean %DNL was less than 10% in VLDL<sub>1</sub>- and VLDL<sub>2</sub>-palmitate (data not shown) and when corrected for flux from the liver, was not significantly different between menopausal groups, but was higher with abdominal obesity. Menopause status affected the relationship between abdominal obesity and 3OHB:NEFA, and abdominal obesity per se had a strong influence on factors related to FA partitioning (Table 4). Of note, the systemic FA contribution to VLDL<sub>1</sub>-TG

**Table 2.** Biochemical and Metabolic Variables in Women According to Menopausal Status and Abdominal Obesity

	Premenopausal (n=30)	Postmenopausal (n=30) *	Lean (n=29)	Abdominally Obese (n=29)	<i>P</i> <sub>meno</sub>	<i>P</i> <sub>Abd obesity</sub>
Total chol, mmol/L	4.9 (0.1)	5.9 (0.2)	5.3 (0.2)	5.5 (0.2)	<0.001	NS
LDL chol, mmol/L	2.9 (0.1)	3.8 (0.2)	3.1 (0.1)	3.5 (0.2)	<0.001	<0.05
HDL chol, mmol/L	1.6 (0.1)	1.7 (0.1)	1.8 (0.07)	1.4 (0.06)	NS	<0.001
Non-HDL chol, mmol/L	3.3 (0.1)	4.2 (0.2)	3.5 (0.15)	4.0 (0.18)	<0.001	<0.01
TG, mmol/L	0.9 (0.1)	0.9 (0.1)	0.72 (0.05)	1.09 (0.15)	NS	<0.01
VLDL <sub>1</sub> -TG, μmol/L	142 (33 to 2083)	226 (82 to 1090)	181 (26)	369 (72)	NS	0.001
VLDL <sub>2</sub> -TG, μmol/L	142 (39 to 1061)	189 (78 to 488)	154 (17)	247 (34)	NS	<0.01
Plasma apoC-III, mg/L	28.7 (1.9)	33.8 (1.5)	31 (1.7)	31 (2.0)	0.06	NS
apoCIII-LpB, mg/L	11.9 (0.9)	15.5 (1.0)	13 (0.9)	14 (1.1)	<0.01	NS
ApoC-III Lp nonB, mg/L	16.8 (1.4)	18.4 (1.0)	18 (1.2)	17 (1.2)	NS	NS
Plasma apoB, g/L	0.73 (0.51 to 1.27)	0.89 (0.51 to 1.37)	0.78 (0.51 to 1.23)	0.88 (0.51 to 1.27)	0.001	<0.05
VLDL <sub>1</sub> -apoB, g/L	0.006 (0.001 to 0.05)	0.007 (0.004 to 0.03)	0.005 (0.001 to 0.03)	0.008 (0.004 to 0.05)	NS	<0.05
VLDL <sub>2</sub> -apoB, g/L	0.018 (0.004 to 0.13)	0.023 (0.01 to 0.06)	0.018 (0.004 to 0.04)	0.023 (0.01 to 0.13)	NS	<0.05
Insulin, mU/L	11.5 (0.9)	10.5 (0.4)	9.2 (0.44)	12.8 (0.79)	NS	<0.001
Glucose, mmol/L	5.0 (0.1)	5.1 (0.1)	4.9 (0.08)	5.2 (0.07)	NS	<0.05
HOMA-IR	3.1 (0.3)	2.8 (0.1)	2.4 (0.14)	3.5 (0.24)	NS	<0.01
NEFA, μmol (AUC)	591 (33)	626 (25)	633 (32)	579 (27)	NS	NS
Plasma 3OHB, μmol (AUC)	156 (13.5)	130 (13.5)	160 (14)	126 (13)	NS	<0.05
Systolic BP, mm Hg	114 (2.2)	126 (2.5)	119 (2.8)	121 (2.5)	0.001	NS
Diastolic BP, mm Hg	75 (1.4)	75 (2.3)	75 (1.7)	76 (1.8)	NS	NS

Data presented as mean (SEM) or median (range). Statistical significance based on 2-way ANOVA: *P*<sub>meno</sub>, statistical significance for an effect of menopausal status; *P*<sub>Abd obesity</sub>, statistical significance for an effect of abdominal obesity; NS, not significant; no significant interaction between abdominal obesity and menopausal status was found. 3OHB indicates plasma 3-hydroxybutyrate; apoB, apolipoprotein B; apoC-III LpB, apoC-III associated with lipoprotein B containing particles; AUC, area under the curve; BP, blood pressure; Chol, cholesterol; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; NEFA, nonesterified fatty acids; TG, triglyceride; VLDL, very low-density lipoprotein.

\*n=30 for postmenopausal women apart from AUC measurements for NEFA and 3OHB which are n=29.

production was significantly higher in abdominally obese women, in line with higher VLDL<sub>1</sub>-TG secretion.

To explore FA partitioning in relation to metabolic and anthropometric measurements, we tabulated univariate correlations between relevant variables in pre- and postmenopausal women (selected data in Tables 5 through 8 and complete analysis in Tables S1 through S4). The data are illustrated in hive plots (Figure 1), which clearly show that the patterns of correlations are quite different in pre- and postmenopausal women. In particular, the density of correlations in the top left of the figure for premenopausal women (Figure 1A, anthropometric and metabolic variables with VLDL<sub>2</sub> metabolism) is markedly less for postmenopausal women (Figure 1B). Correlations relating to %DNL and VLDL production are shown in Figure 2A and 2B and between %DNL and plasma 3OHB in Figure 2C and 2D. The most marked univariate correlations relating to hepatic FA partitioning were between the isotopic desaturation index in VLDL<sub>1</sub> and VLDL<sub>2</sub>-TG and plasma 3OHB area under the curve (Figure 2E and 2F). VLDL-ApoB and -TG production were highly correlated for

VLDL<sub>1</sub> and less so for VLDL<sub>2</sub> (Figure 3). Serum cortisol concentrations were negatively correlated with waist-to-hip ratio in pre- ( $r_s=-0.38$ ,  $P=0.04$ ) but not postmenopausal women. There was a significant correlation between abdominal fat and liver fat ( $r_s=0.50$ ,  $P<0.001$ ,  $n=60$ ). The importance of menopausal status in this relationship is shown in the hive plots and Tables 5 through 8 which showed, remarkably, that a significant correlation between liver fat and abdominal obesity was observed only in premenopausal women.

We also took the opportunity to examine metabolic variables according to liver fat content because of the importance of liver fat with respect to the metabolic complications of obesity.<sup>32</sup> The median value in the cohort of 60 women was 0.85%. Of the 50% of women with lower liver fat, 17 were premenopausal and 13 were postmenopausal. In general, significant effects reflected those found by considering women according to abdominal obesity (Tables S5 through S7). However, the effect of liver fat on LDL, HDL, and non-HDL cholesterol as well as VLDL-TG production was less than for abdominal obesity.

**Table 3.** Kinetic Estimates Relating to NEFA, VLDL, and apoC-III Metabolism in Women According to Menopausal Status and Abdominal Obesity

	Premenopausal	Postmenopausal	Lean	Abdominally Obese	$P_{\text{meno}}$	$P_{\text{Abd obesity}}$
$R_a\text{NEFA}$ , $\mu\text{mol min}^{-1}$ per kg fat mass	9.1 (0.8) n=30	10.0 (0.6) n=26	11 (0.8) n=28	8.2 (0.6) n=28	NS	0.01
$R_d\text{NEFA}$ , $\mu\text{mol min}^{-1}$ per kg lean mass	4.6 (0.3) n=30	5.8 (0.3) n=26	5.1 (0.4) n=28	5.3 (0.3) n=28	<0.055	NS
$R_a\text{NEFA}$ , $\mu\text{mol min}^{-1}$	193 (12) n=30	220 (12) n=28	197 (13) n=28	219 (11) n=28	0.05	NS
VLDL <sub>1</sub> -TG Prod, mg/kg lean mass	332 (35) n=26	350 (27) n=26	268 (26) n=24	404 (30) n=28	NS	0.001
VLDL <sub>2</sub> -TG dirProd, mg/kg lean mass	129 (12) n=26	174 (12) n=26	142 (13) n=24	160 (11) n=28	<0.01	0.07
VLDL <sub>2</sub> -TG indirProd, mg/kg lean mass	100 (21) n=25	110 (14) n=26	88 (17) n=24	120 (18) n=27	NS	NS
VLDL <sub>1</sub> -TG FCR, pools/day	29 (3.2) n=26	26 (2.5) n=27	31 (2.9) n=24	25 (2.8) n=28	NS	NS
VLDL <sub>2</sub> -TG FCR, pools/day	22 (2.3) n=26	26 (2.7) n=27	26 (2.6) n=24	22 (2.4) n=28	NS	NS
VLDL <sub>1</sub> -TG FTR, pools/day	6.8 (1.0) n=25	7.5 (1.0) n=27	7.9 (1.2) n=24	6.6 (0.8) n=27	NS	NS
VLDL <sub>1</sub> -TG FDC, pools/day	22 (3.2) n=26	18 (2.3) n=27	23 (3.0) n=24	18 (2.7) n=28	NS	NS
VLDL <sub>1</sub> -apoB FDC, pools/day	9.2 (1.7) n=28	7.5 (1.5) n=28	8.5 (1.5) n=26	8.5 (1.7) n=29	NS	NS
VLDL <sub>1</sub> -apoB FTR, pools/day	8.6 (1.0) n=27	8.2 (0.8) n=28	8.5 (1.0) n=26	8.1 (0.8) n=28	NS	NS
VLDL <sub>1</sub> -apoB FCR, pools/day	18 (1.9) n=28	16 (1.5) n=28	17 (1.6) n=26	16 (1.8) n=29	NS	NS
VLDL <sub>2</sub> -apoB FCR, pools/day	8.6 (0.8) n=28	7.7 (0.8) n=28	8.7 (0.8) n=26	7.8 (0.8) n=29	NS	NS
VLDL <sub>1</sub> -apoB Prod, mg/day	344 (38) n=28	316 (27) n=28	253 (26) n=26	397 (34)* n=29	NS	0.001
VLDL <sub>2</sub> -apoB dirProd, mg/day	304 (21) n=28	278 (20) n=28	269 (19) n=26	311 (22)* n=29	NS	NS
VLDL <sub>2</sub> -apoB indirProd, mg/day	194 (34) n=28	163 (19) n=28	130 (18) n=25	215 (31) n=28	NS	<0.05
VLDL <sub>1</sub> -TG Prod:VLDL <sub>1</sub> apoB Prod, mg/day	42 (2.8) n=26	49 (5.9) n=27	45 (4.2) n=24	47 (5.2) n=28	NS	NS
VLDL <sub>2</sub> -TG dirProd:VLDL <sub>2</sub> apoB Prod, mg/day	19 (1.6) n=26	30 (5.3) n=27	21 (2.1) n=24	28 (5.1) n=28	<0.05	NS
ApoC-III FCR, pools/day	1.1 (0.1) n=30	1.1 (0.1) n=30	1.1 (0.1) n=29	1.0 (0.09) n=29	NS	NS
ApoC-III PR, mg/kg per day	1.5 (0.2) n=30	1.7 (0.1) n=30	1.6 (0.2) n=29	1.4 (0.1) n=29	NS	NS

Data presented as mean (SEM). Statistical significance based on 2-way ANOVA:  $P_{\text{meno}}$ , statistical significance for an effect of menopausal status;  $P_{\text{Abd obesity}}$ , statistical significance for an effect of abdominal obesity. apoB indicates apolipoprotein B100; dirprod, direct production; FCR, fractional clearance rate; FDC, fractional direct clearance; FTR, fractional transfer rate; indirprod, indirect production; lean, lean tissue; NEFA, nonesterified fatty acids; NS, not significant; Prod, production; PR, production rate;  $R_a$ , rate of appearance;  $R_d$ , rate of disappearance; TG, triacylglycerol; VLDL, very low-density lipoprotein.

\* $P<0.05$  for interaction between abdominal obesity and menopausal status.

## Discussion

Using a combination of stable isotope tracer techniques, we investigated kinetic parameters of apoB, apoC-III, and TG metabolism in pre- and postmenopausal women. We report for the first time that menopausal status is a determinant of hepatic TG flux through enhancement of adipose tissue NEFA flux, altered intrahepatic FA partitioning, and secretion of larger VLDL<sub>2</sub>. VLDL-TG secretion is normally dependent on VLDL-apoB100 secretion,<sup>33</sup> but we found that VLDL<sub>2</sub>-TG secretion after the menopause was dissociated from VLDL<sub>2</sub>-apoB production. Systemic FAs were the major source of VLDL<sub>2</sub>-TG in all women, but both systemic and nonsystemic FAs contributed to greater VLDL<sub>2</sub>-TG secretion in postmenopausal women. We also report for the first time that VLDL<sub>1</sub>-TG secretion is higher in abdominally obese women. Our main findings are summarized in Figure S3.

VLDL<sub>1</sub> and VLDL<sub>2</sub> metabolism have not previously been measured in relation to menopausal and abdominal obesity

status in women. We measured 2 aspects of VLDL secretion: VLDL-apoB secretion rate, which measures whole particle secretion; and VLDL-TG secretion, which tracks the lipid component. Using these 2 parameters we were also able to estimate the relative sizes of VLDL<sub>1</sub> and VLDL<sub>2</sub> at the point of hepatic secretion. VLDL<sub>1</sub> and VLDL<sub>2</sub> secretion rates were correlated but in agreement with previous findings, their metabolism was independent<sup>34</sup> as shown in hive plots.

VLDL<sub>1</sub>-TG and VLDL<sub>1</sub>-apoB production rates were significantly higher in the abdominally obese compared to abdominally lean women. Higher VLDL<sub>1</sub>-TG secretion was attributable to both systemic and nonsystemic FA. There are no previous comparable studies, but in lean and obese premenopausal women there were no differences in total VLDL-apoB or VLDL-TG secretion.<sup>35,36</sup> Another study in premenopausal women found higher total VLDL-TG production in upper-body obese compared with lean women,<sup>37</sup> although production was not corrected for any measure of body mass.

**Table 4.** Variables Relating to FA Metabolism in Women According to Menopausal Status and Abdominal Obesity

	Premenopausal	Postmenopausal	Lean	Abdominally Obese	<i>P</i> <sub>meno</sub>	<i>P</i> <sub>Abd obesity</sub>
Nonsystemic FA—VLDL <sub>1</sub> -TG, %	15.5 (3.2) n=27	14.2 (2.6) n=23	9.5 (1.9) n=24	20 (3.4) n=26	NS	<0.05
Nonsystemic FA—VLDL <sub>2</sub> -TG, %	17.2 (3.4) n=27	18.6 (2.6) n=23	11 (2.1) n=24	24 (3.3) n=26	NS	<0.01
Nonsystemic FA contribution to VLDL <sub>1</sub> -TG production, mg/day	2943 (803) n=24	1678 (367) n=21	1180 (349) n=20	3290 (747) n=25	NS	<0.001
Nonsystemic FA contribution to VLDL <sub>2</sub> -TG direct production, mg/day	1028 (197) n=24	1192 (181) n=21	668 (146) n=20	1453 (185)* n=25	<0.05	<0.001
Systemic FA contribution to VLDL <sub>1</sub> -TG production, mg/day	11 814 (1307) n=24	11 449 (956) n=21	9613 (946) n=20	13 269 (1183) n=25	NS	<0.05
Systemic FA contribution to VLDL <sub>2</sub> -TG direct production, mg/day	4563 (511) n=24	5594 (389) n=21	4709 (441) n=20	5312 (486) n=25	0.06	NS
VLDL <sub>1</sub> -TG isotopic desaturation index	9.2 (3.0 to 29.2) n=30	10.5 (3.6 to 68.2) n=28	8.2 (3 to 29) n=28	10.6 (36 to 68) n=29	NS	0.06
VLDL <sub>2</sub> -TG isotopic desaturation index	9.0 (3.2 to 28.5) n=30	10.2 (4.9 to 60.7) n=28	8.5 (3.5 to 28) n=28	10.6 (4.9 to 61) n=29	NS	<0.05
VLDL <sub>1</sub> -16:0 TG synthesized de novo, mg/day	118 (4.9 to 1444) n=26	283 (27.2 to 2564) n=26	114 (4.9 to 1444) n=23	309 (12 to 2564) n=28	NS	0.06
VLDL <sub>2</sub> -16:0 TG synthesized de novo, mg/day	62 (0.42 to 351) n=26	102 (7.4 to 583) n=26	61 (42 to 351) n=23	117 (7.4 to 583) n=28	NS	<0.05
30HB/NEFA	0.24 (0.012) n=30	0.22 (0.02) n=29	0.25 (0.02) n=29	0.21 (0.02) n=29**	NS	NS

Data presented as mean (SEM) or median (range). Statistical significance based on 2-way ANOVA: *P*<sub>meno</sub>, statistical significance for an effect of menopausal status; *P*<sub>Abd obesity</sub>, statistical significance for an effect of abdominal obesity. 30HB indicates 3-hydroxybutyrate; FA, fatty acid; NEFA, nonesterified fatty acids; NS, not significant; TG, triglyceride; VLDL, very low-density lipoprotein. \**P*<0.05, \*\**P*<0.01 for a statistically significant interaction between abdominal obesity and menopausal status.

**Table 5.** Correlation Coefficients (*r*<sub>s</sub>) for Premenopausal Women Between Selected Variables Relating to VLDL<sub>1</sub> Metabolism, Liver Fat, and Intra-Abdominal Fat

	Total Body Fat, kg	Android/Tot Fat, kg	Gynoid/Tot Fat, kg	Visceral Fat, cm <sup>2</sup>	Subcut Fat, cm <sup>2</sup>	HOMA-IR	Plasma NEFA*, μmol/L	Plasma 30HB*, μmol/L	VLDL <sub>1</sub> -TG Prod/d	VLDL <sub>1</sub> -TG SCD Iso Index	VLDL <sub>1</sub> -TG SCD16	VLDL <sub>1</sub> -TG SCD18	VLDL <sub>1</sub> -TG DNL (%)	VLDL <sub>1</sub> -TG 18:2n-6 (%)	VLDL <sub>1</sub> -TG Prod/apoB Prod
Liver fat, %	0.57 <i>P</i> =0.001	0.63 <i>P</i> <0.001	-0.39 <i>P</i> =0.034	0.71 <i>P</i> <0.001	0.47 <i>P</i> =0.009	0.63 <i>P</i> <0.001	-0.07 <i>P</i> =0.702	-0.42 <i>P</i> =0.022	0.40 <i>P</i> =0.045	0.41 <i>P</i> =0.025	-0.05 <i>P</i> =0.803	-0.33 <i>P</i> =0.078	0.47 <i>P</i> =0.009	-0.19 <i>P</i> =0.323	-0.29 <i>P</i> =0.150
Total body fat, kg		0.68 <i>P</i> <0.001	-0.63 <i>P</i> <0.001	0.80 <i>P</i> <0.001	0.90 <i>P</i> <0.001	0.36 <i>P</i> =0.052	-0.00 <i>P</i> =0.982	-0.33 <i>P</i> =0.077	0.61 <i>P</i> =0.001	0.39 <i>P</i> =0.039	0.17 <i>P</i> =0.370	-0.13 <i>P</i> =0.480	0.49 <i>P</i> =0.006	-0.35 <i>P</i> =0.055	-0.07 <i>P</i> =0.739
Android/tot fat, kg			-0.74 <i>P</i> <0.001	0.78 <i>P</i> <0.001	0.63 <i>P</i> <0.001	0.53 <i>P</i> =0.003	-0.10 <i>P</i> =0.596	-0.37 <i>P</i> =0.046	0.53 <i>P</i> =0.005	0.35 <i>P</i> =0.057	0.09 <i>P</i> =0.629	-0.20 <i>P</i> =0.300	0.32 <i>P</i> =0.085	-0.07 <i>P</i> =0.699	-0.07 <i>P</i> =0.719

See Supplemental Material for full statistical analysis. 30HB indicates 3-hydroxybutyrate; apoB, apolipoprotein B; AUC, area under the curve; d, day; DNL, de novo lipogenesis; HOMA-IR, homeostatic model assessment of insulin resistance; iso, isotopic; NEFA, nonesterified fatty acids; prod, production; SCD, stearoyl-CoA desaturase 1; SCD16, ratio of 16:1n-7/16:0; SCD18, ratio 18:1n-9/18:0; Subcut, subcutaneous; TG, triglyceride; tot, total; VLDL, very low-density lipoprotein. \*AUC.

**Table 6.** Correlation Coefficients ( $r_s$ ) for Premenopausal Women Between Selected Variables Relating to VLDL<sub>2</sub> Metabolism, Liver Fat, and Intra-Abdominal Fat

	Total Body Fat, kg	Android/Tot Fat, kg	Gynoid/Tot Fat, kg	Visceral Fat, cm <sup>2</sup>	Subcut Fat, cm <sup>2</sup>	HOMA-IR	Plasma NEFA*, μmol/L	Plasma 3OHBA*, μmol/L	VLDL <sub>2</sub> -TG Prod/d	VLDL <sub>2</sub> -TG SCD Iso Index	VLDL <sub>2</sub> -TG SCD16	VLDL <sub>2</sub> -TG SCD18	VLDL <sub>2</sub> -TG DNL (%)	VLDL <sub>2</sub> -TG 18:2n-6 (%)	VLDL <sub>2</sub> -TG Prod/apoB Prod
Liver fat, %	0.57 <i>P</i> <0.001	0.63 <i>P</i> <0.001	-0.39 <i>P</i> <0.034	0.71 <i>P</i> <0.001	0.47 <i>P</i> <0.009	0.63 <i>P</i> <0.001	-0.07 <i>P</i> <0.702	-0.42 <i>P</i> <0.022	0.21 <i>P</i> <0.306	0.38 <i>P</i> <0.040	0.01 <i>P</i> <0.952	-0.37 <i>P</i> <0.042	0.42 <i>P</i> <0.021	-0.19 <i>P</i> <0.323	-0.29 <i>P</i> <0.150
Total body fat, kg		0.68 <i>P</i> <0.001	-0.63 <i>P</i> <0.001	0.80 <i>P</i> <0.001	0.90 <i>P</i> <0.001	0.36 <i>P</i> <0.052	-0.00 <i>P</i> <0.982	-0.33 <i>P</i> <0.077	0.51 <i>P</i> <0.007	0.41 <i>P</i> <0.023	0.21 <i>P</i> <0.257	-0.24 <i>P</i> <0.202	0.41 <i>P</i> <0.023	-0.25 <i>P</i> <0.177	0.23 <i>P</i> <0.255
Android/tot fat, kg			-0.74 <i>P</i> <0.001	0.78 <i>P</i> <0.001	0.63 <i>P</i> <0.001	0.53 <i>P</i> <0.003	-0.10 <i>P</i> <0.596	-0.37 <i>P</i> <0.046	0.29 <i>P</i> <0.145	0.36 <i>P</i> <0.052	0.12 <i>P</i> <0.516	-0.31 <i>P</i> <0.092	0.22 <i>P</i> <0.240	-0.05 <i>P</i> <0.791	0.11 <i>P</i> <0.580

See Supplementary Material for full statistical analysis. 3OHBA indicates 3-hydroxybutyrate; apoB, apolipoprotein B; AUC, area under the curve; d, day; DNL, de novo lipogenesis; HOMA-IR, homeostatic model assessment of insulin resistance; iso, isotopic; NEFA, nonesterified fatty acids; prod, production; SCD, stearoyl-CoA desaturase 1; SCD16, ratio of 16:1n-7/16:0; SCD18, ratio 18:1n-9/18:0; Subcut, subcutaneous; TG, triglyceride; tot, total; VLDL, very low-density lipoprotein. \*AUC.

**Table 7.** Correlation Coefficients ( $r_s$ ) for Post-Menopausal Women Between Selected Variables Relating to VLDL<sub>1</sub> Metabolism, Liver Fat and Intra-Abdominal Fat

	Total Body Fat, kg	Android/Tot Fat, kg	Gynoid/Tot Fat, kg	Visceral Fat, cm <sup>2</sup>	Subcut Fat, cm <sup>2</sup>	HOMA-IR	Plasma NEFA*, μmol/L	Plasma 3OHBA*, μmol/L	VLDL <sub>1</sub> -TG Prod/d	VLDL <sub>1</sub> -TG SCD Iso Index	VLDL <sub>1</sub> -TG SCD16	VLDL <sub>1</sub> -TG SCD18	VLDL <sub>1</sub> -TG DNL (%)	VLDL <sub>1</sub> -TG 18:2n-6 (%)	VLDL <sub>1</sub> -TG Prod/apoB Prod
Liver fat, %	0.28 <i>P</i> <0.150	0.26 <i>P</i> <0.184	-0.42 <i>P</i> <0.028	0.61 <i>P</i> <0.001	0.26 <i>P</i> <0.192	0.23 <i>P</i> <0.232	0.00 <i>P</i> <0.994	0.09 <i>P</i> <0.647	0.05 <i>P</i> <0.797	-0.11 <i>P</i> <0.595	-0.42 <i>P</i> <0.023	-0.26 <i>P</i> <0.173	0.04 <i>P</i> <0.838	0.11 <i>P</i> <0.578	0.26 <i>P</i> <0.200
Total body fat, kg		0.78 <i>P</i> <0.001	-0.57 <i>P</i> <0.002	0.73 <i>P</i> <0.001	0.86 <i>P</i> <0.001	0.29 <i>P</i> <0.133	-0.09 <i>P</i> <0.648	0.19 <i>P</i> <0.326	0.34 <i>P</i> <0.093	-0.01 <i>P</i> <0.949	-0.13 <i>P</i> <0.512	0.22 <i>P</i> <0.279	0.01 <i>P</i> <0.967	0.32 <i>P</i> <0.106	0.35 <i>P</i> <0.077
Android/tot fat, kg			-0.76 <i>P</i> <0.001	0.72 <i>P</i> <0.001	0.69 <i>P</i> <0.001	0.35 <i>P</i> <0.064	-0.35 <i>P</i> <0.072	-0.14 <i>P</i> <0.475	0.57 <i>P</i> <0.003	0.23 <i>P</i> <0.246	-0.21 <i>P</i> <0.287	-0.05 <i>P</i> <0.788	0.19 <i>P</i> <0.342	0.29 <i>P</i> <0.143	0.25 <i>P</i> <0.225

See Supplementary Material for full statistical analysis. 3OHBA indicates 3-hydroxybutyrate; apoB, apolipoprotein B; AUC, area under the curve; d, day; DNL, de novo lipogenesis; HOMA-IR, homeostatic model assessment of insulin resistance; iso, isotopic; NEFA, nonesterified fatty acids; prod, production; SCD, stearoyl-CoA desaturase 1; SCD16, ratio of 16:1n-7/16:0; SCD18, ratio 18:1n-9/18:0; Subcut, subcutaneous; TG, triglyceride; tot, total; VLDL, very low-density lipoprotein. \*AUC.



**Table 8.** Correlation Coefficients ( $r_s$ ) for Postmenopausal Women Between Selected Variables Relating to VLDL<sub>2</sub> Metabolism, Liver Fat, and Intra-Abdominal Fat

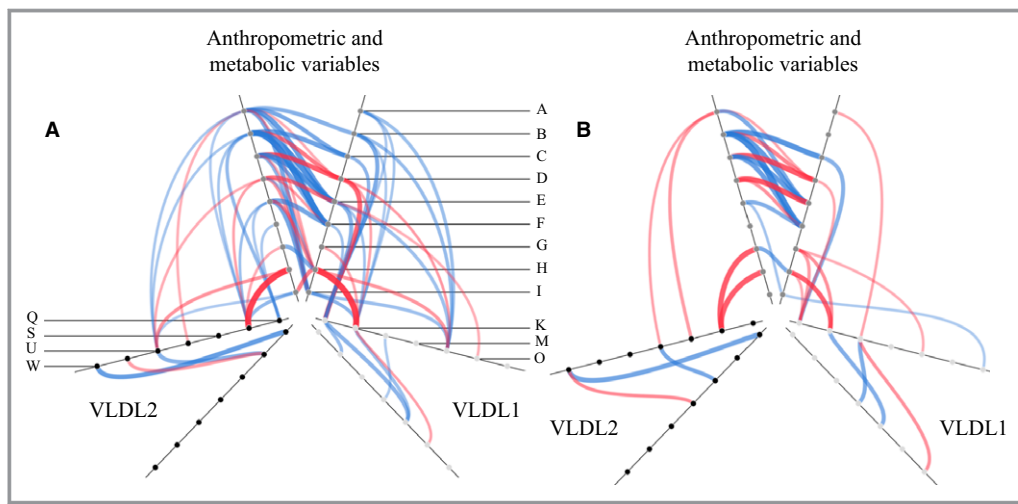
	Total Body Fat, kg	Android/Tot Fat, kg	Gynoid/Tot Fat, kg	Visceral Fat, cm <sup>2</sup>	Subcut Fat, cm <sup>2</sup>	HOMA-IR	Plasma NEFA*, μmol/L	Plasma 3OHB*, μmol/L	VLDL <sub>2</sub> -TG Prod/d	VLDL <sub>2</sub> -TG SCD Iso Index	VLDL <sub>2</sub> -TG SCD16	VLDL <sub>2</sub> -TG SCD18	VLDL <sub>2</sub> -TG DNL (%)	VLDL <sub>2</sub> -TG 18:2n-6 (%)	VLDL <sub>2</sub> -TG Prod/apoB Prod
Liver fat, %	0.28 <i>P</i> =0.150	0.26 <i>P</i> =184	-0.42 <i>P</i> =0.028	0.61 <i>P</i> <0.001	0.26 <i>P</i> =0.192	0.23 <i>P</i> =0.232	0.00 <i>P</i> =0.994	0.09 <i>P</i> =0.647	-0.15 <i>P</i> =0.470	-0.05 <i>P</i> =0.797	-0.46 <i>P</i> =0.013	-0.44 <i>P</i> =0.016	0.11 <i>P</i> =0.564	0.15 <i>P</i> =0.450	0.11 <i>P</i> =0.594
Total body fat, kg		0.78 <i>P</i> <0.001	-0.57 <i>P</i> =0.002	0.73 <i>P</i> <0.001	0.86 <i>P</i> <0.001	0.29 <i>P</i> =0.133	-0.09 <i>P</i> =0.648	0.19 <i>P</i> =0.326	0.18 <i>P</i> =0.380	-0.03 <i>P</i> =0.875	-0.12 <i>P</i> =0.554	-0.04 <i>P</i> =0.842	0.13 <i>P</i> =0.518	0.27 <i>P</i> =0.171	0.30 <i>P</i> =0.133
Android/tot fat, kg			-0.76 <i>P</i> <0.001	0.72 <i>P</i> <0.001	0.69 <i>P</i> <0.001	0.35 <i>P</i> =0.064	-0.35 <i>P</i> =0.072	-0.14 <i>P</i> =0.475	0.20 <i>P</i> =0.341	0.20 <i>P</i> =0.329	-0.23 <i>P</i> =0.244	-0.13 <i>P</i> =0.506	0.16 <i>P</i> =0.423	0.22 <i>P</i> =0.275	0.31 <i>P</i> =0.123

See Supplementary Material for full statistical analysis. 3OHB indicates 3-hydroxybutyrate; apoB, apolipoprotein B; AUC, area under the curve; d, day; DNL, de novo lipogenesis; HOMA-IR, homeostatic model assessment of insulin resistance; iso, isotopic; NEFA, nonesterified fatty acids; prod, production; SCD, stearoyl-CoA desaturase 1; SCD16, ratio of 16:1n-7/16:0; SCD18, ratio 18:1n-9/18:0; Subcut, subcutaneous; TG, triglyceride; tot, total; VLDL, very low-density lipoprotein. \*AUC.

VLDL<sub>2</sub>-TG direct production was higher in post- compared to premenopausal women, due to production of TG-enriched VLDL<sub>2</sub>. This has not been reported previously and the relevance is not clear as yet, but small rather than large VLDL is implicated in atherosclerosis progression.<sup>38</sup> VLDL<sub>2</sub>-TG production was higher in postmenopausal women than men matched for plasma TG concentrations<sup>24</sup> and as discussed by the authors, VLDL<sub>2</sub> is more efficiently converted to LDL than VLDL<sub>1</sub>. Moderate hypercholesterolemia arises principally from overproduction of VLDL<sub>2</sub> particles in men<sup>39</sup>; our study was not designed to measure LDL, kinetics but higher plasma and LDL cholesterol concentrations after the menopause were not accompanied by an increase in VLDL<sub>2</sub>-apoB production.

Menopausal status did not affect VLDL<sub>1</sub> or VLDL<sub>2</sub> clearance, in contrast to the work of Mittendorfer.<sup>40</sup> VLDL kinetics are sensitive to intra-abdominal and liver fat content,<sup>32</sup> and discrepancies between published studies may be due to liver fat content, and/or kinetic parameters not corrected for adiposity or lean mass. Although estrogen per se may affect VLDL kinetics,<sup>41</sup> other factors such as the changes in body composition accompany estrogen deficiency. We found increased concentrations of plasma total and LDL cholesterol and apoB, which are typical features of dyslipidemia in postmenopausal women.<sup>42</sup> Additionally, we found significantly higher apoC-III LpB in postmenopausal women. Since apoC-III plays a pivotal role in the development of hypertriglyceridemia,<sup>7</sup> apoC-III may play a role in the development of dyslipidemia in older, postmenopausal women.

Higher R<sub>d</sub>NEFA in postmenopausal women is consistent with the finding that exogenous estrogen reduced NEFA flux in postmenopausal women.<sup>43</sup> This suggests that higher NEFA flux in postmenopausal women is a result of estrogen depletion. R<sub>d</sub>NEFA, when corrected for fat mass, was significantly lower with increasing abdominal obesity, in agreement with the concept of downregulated adipose tissue FA trafficking in obesity with reduced expression of lipolytic genes such as hormone-sensitive lipase and adipose triglyceride lipase.<sup>44</sup> The relationship between obesity, insulin resistance, and lipolysis is not clear in the literature and has been elegantly reviewed.<sup>45</sup> At a whole body level, lipolysis was similar in lean and abdominally obese groups, but the contribution of systemic FA to increased VLDL<sub>1</sub>-TG was significantly higher in abdominally obese women, as was the contribution of nonsystemic FA, with a tendency toward increased de novo hepatic FA secretion indicating an upregulation of secretion of FA from all sources. Higher secretion of de novo palmitate has previously been found in obese hypertriglyceridemic men compared with lean normolipidemic men and women.<sup>46</sup> Our findings of inverse correlations between plasma 3OHB and %DNL in VLDL<sub>1</sub>-TG are in agreement with studies in healthy men and women.<sup>47</sup> Moreover, we found strong inverse correlations between the



**Figure 1.** Significant correlations between variables relating to VLDL-TG metabolism represented as hive plots for premenopausal (A) and postmenopausal (B) women. Each variable is represented by a node and the nodes are joined by blue (significant positive correlations) or red (significant negative correlations) lines. The thickness of the line represents the strength of the correlation. The nodes are placed on 3 duplicated radial axes that represent grouped variables (anthropometric and metabolic variables/VLDL<sub>1</sub> or VLDL<sub>2</sub>). Individual nodes are coded as indicated and specific correlation coefficients are given in Supplemental Material. Node codes: A, liver fat (%); B, total body fat (kg); C, android fat/total fat; D, gynoid fat/total fat; E, visceral fat (cm<sup>2</sup>); F, subcut fat (cm<sup>2</sup>); G, NEFA (μmol/L); H, 3OHB (AUC); I, HOMA-IR; K, VLDL-TG SCD isotopic index; M, VLDL-TG SCD18 index; O, VLDL-TG 18:2n-6 (%); Q, VLDL-TG production per day; S, VLDL-TG SCD16; U, VLDL-TG DNL (%); W, VLDL-TG production/apoB production. 3OHB indicates plasma 3-hydroxybutyrate; apoB, apolipoprotein B; AUC, area under the curve; DNL, hepatic de novo lipogenesis; HOMA-IR, homeostatic model assessment of insulin resistance; NEFA, plasma nonesterified fatty acids; SCD, stearoyl-CoA desaturase; SCD16, 16:1 n-7/16:0 ratio in VLDL-TG; SCD18, 18:1 n-9/18:0 ratio in VLDL-TG; subcut, subcutaneous; TG, triglyceride; VLDL, very low-density lipoprotein.

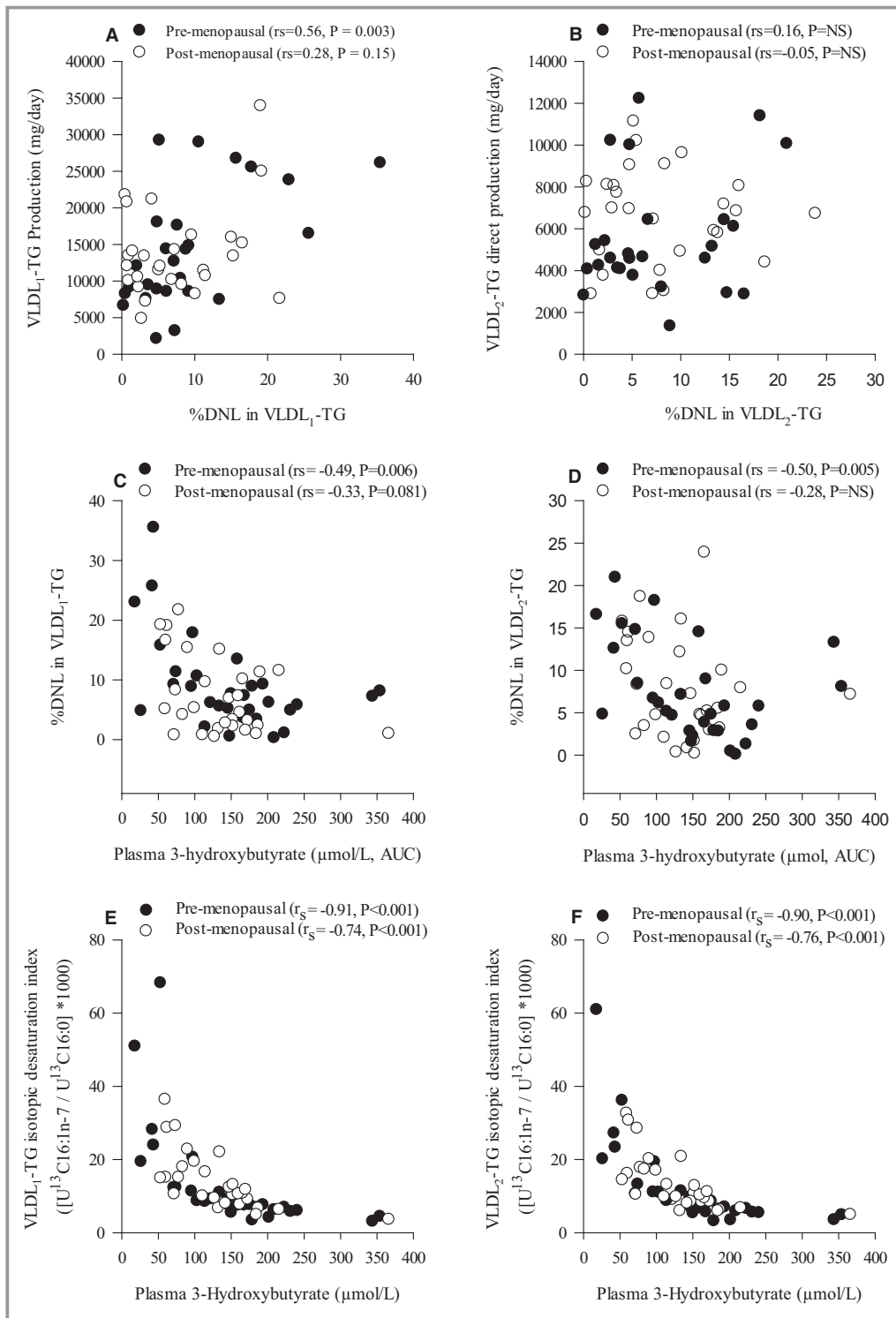
isotopic desaturation index in VLDL<sub>1</sub>- and VLDL<sub>2</sub>-TG and plasma 3OHB in the whole cohort. These 2 variables are not obviously related but provide the first evidence of a clear divergence of FA partitioning in humans in vivo such that hepatic desaturation of FAs was low when FA oxidation was high (and vice versa).

Serum cortisol concentrations were negatively correlated with waist-to-hip ratio in pre- but not postmenopausal women. Cortisol status has previously been inversely related to waist-to-hip ratio in women, although menopause status was not defined; this has been explained by a higher local clearance rate of cortisol in visceral fat, which has more glucocorticoid receptors than subcutaneous fat.<sup>48</sup> However, we found no correlation between serum cortisol concentrations and intra-abdominal fat area. In agreement with previous studies of aging,<sup>49</sup> serum cortisol concentrations were higher in postmenopausal women.

Strengths of our study include the unique combination of kinetic and anthropometric measurements in large groups of well-matched women, but a limitation is the cross-sectional design. Therefore, we cannot ascribe causality to any of the correlations found. Postmenopausal women were older and therefore any effects of “menopausal status” do not reflect a

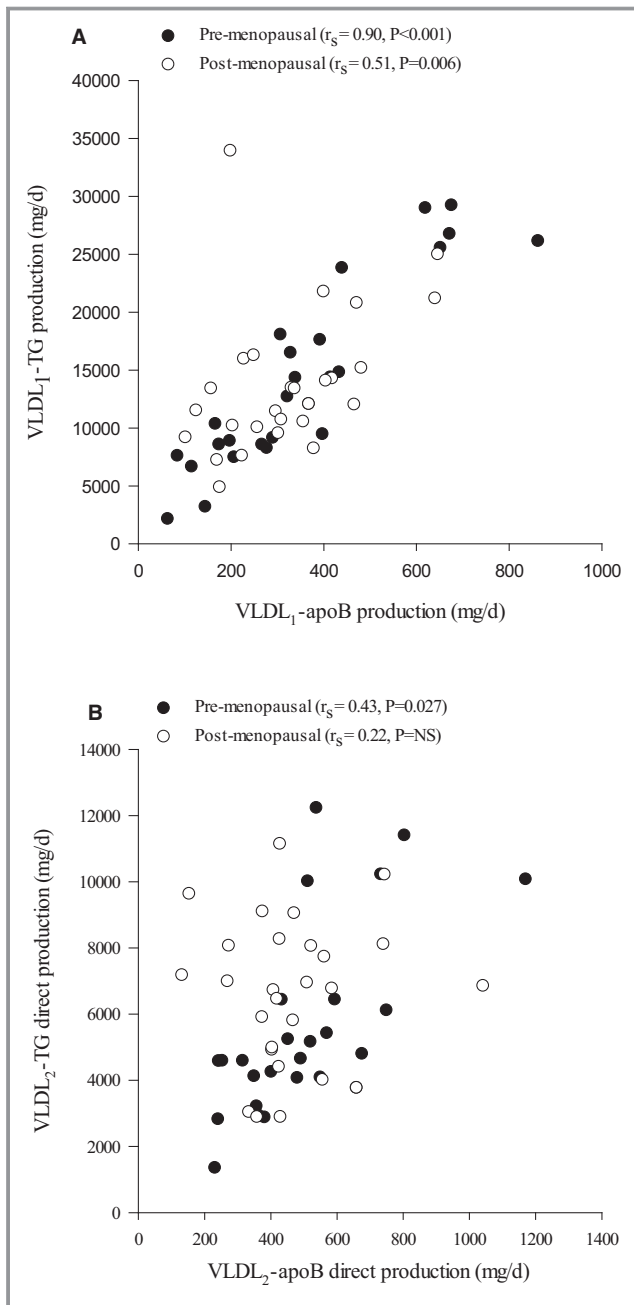
difference in hormone concentrations, but rather the natural course of events (menopause plus aging with accompanying changes in body fat distribution). Indeed, postmenopausal women had equal abdominal obesity but higher intra-abdominal fat and less gynoid fat, demonstrating a change in body fat distribution.<sup>4</sup> Given the importance of estrogen in determining body fat distribution and direct effects on lipid metabolism, it may have been insightful to measure serum estrogen concentrations, although it is clear that many factors beyond sex hormones contribute to lipid and lipoprotein metabolism.<sup>50</sup> The study design meant that we were able to look at correlations within each menopausal group. We also analyzed our data according to liver fat and we found that overall, the results were similar to when we divided according to abdominal obesity. This is in contrast to findings in individuals with a large range of liver fat,<sup>32</sup> where liver fat was found to be more discriminatory. However, we found that abdominal obesity in women was more related to impaired VLDL-TG secretion than liver fat. This suggests that other intrahepatic factors are contributing to VLDL-TG secretion.

We did not include a comparator group of men, but other groups have compared lipoprotein metabolism in men and women.<sup>50</sup> One study found that VLDL-TG secretion rate was



**Figure 2.** Correlations between VLDL<sub>1</sub>-TG production (mg/day) and the proportion (%) of DNL fatty acids VLDL<sub>1</sub>-TG (A), VLDL<sub>2</sub>-TG direct production (mg/day) and the proportion (%) of DNL fatty acids VLDL<sub>2</sub>-TG (B), the proportion (%) of DNL fatty acids VLDL<sub>1</sub>-TG and the AUC for plasma 3-hydroxybutyrate (μmol/L) (C), the proportion (%) of DNL fatty acids VLDL<sub>2</sub>-TG and the AUC for plasma 3-hydroxybutyrate (μmol/L) (D), and the association between plasma 3-hydroxybutyrate concentrations (μmol/L) and the isotopic desaturation index ( $[U^{13}C16:1n-7 / U^{13}C16:0] * 1000$ ) in VLDL<sub>1</sub>-TG (E) and VLDL<sub>2</sub>-TG (F) in pre- (●) and post- (○) menopausal women. AUC indicates area under the curve; DNL, hepatic de novo lipogenesis; NS, not significant; TG, triglyceride; VLDL, very low-density lipoprotein.

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**Figure 3.** Correlations between VLDL<sub>1</sub>-apoB production (mg/day) and VLDL<sub>1</sub>-TG production (mg/day) (A), VLDL<sub>2</sub>-apoB production (mg/day) and VLDL<sub>2</sub>-TG direct production (mg/day) (B) in pre- (●) and post- (○) menopausal women. apoB, apolipoprotein B; NS, not significant; TG, triglyceride; VLDL, very low-density lipoprotein.

significantly higher in pre-menopausal women than men,<sup>31</sup> whereas another found that VLDL<sub>2</sub>-TG but not VLDL<sub>1</sub>-TG secretion rate was higher in post-menopausal women than men.<sup>24</sup> We have previously reported no difference in the postprandial contribution of dietary and nonsystemic FA to VLDL-TG between insulin-sensitive men and women.<sup>13</sup> However, lipoprotein metabolism is dependent on many factors, and accumulation of excess body fat seems to affect lipid

kinetics differently in men and women as recently discussed.<sup>50</sup> Total body fat and body fat distribution are obvious differences between men and women, and this study has highlighted the importance of body fat distribution in women.

## Conclusions

VLDL<sub>1</sub> and VLDL<sub>2</sub> metabolism is complex in women, and five plots illustrate that the patterns of associations with metabolic variables are different between menopausal groups. A lack of significant correlation between hepatic VLDL<sub>2</sub>-TG and VLDL<sub>2</sub>-apoB production in post-menopausal women is intriguing and requires further study. Abdominal obesity was characterized by increased cardiovascular disease risk factors such as VLDL<sub>1</sub>-TG and -apoB production, liver fat, and non-HDL cholesterol. Interestingly, this was observed despite a considerable overlap in BMI between abdominally lean and abdominally obese groups. Our study is the first to report that VLDL<sub>1</sub>-TG secretion is significantly higher in abdominally obese women and accounts for increased plasma VLDL<sub>1</sub>-TG and plasma TG concentrations. This is important because there is increasing evidence that there is a causal relationship between TG-mediated pathways and coronary heart disease.<sup>51</sup> Weight gain in postmenopausal women is likely to impact on both VLDL<sub>1</sub>-TG and VLDL<sub>2</sub>-TG secretory pathways with consequent implications for cardiovascular disease risk.

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## Disclosures

None.

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