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

Leanne Hodson, PhD; Rajarshi Banerjee, DPhil; Belén Rial, PhD; Wiebke Arlt, MD, DSc; Martin Adiels, PhD; Jan Boren, MD, PhD; Kyriakoula Marinou, MD, PhD; Ciaran Fisher, PhD; Ingrid L. Mostad, PhD; Irene M. Stratton, PhD; P. Hugh R. Barrett, PhD; Dick C. Chan, PhD; Gerald F. Watts, DSc, PhD, DM; Karin Harnden, BSc; Fredrik Karpe, PhD; Barbara A. Fielding, PhD

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, abdominally obese women were more insulin resistant with enhanced hepatic secretion of large (404±30 versus 268±26 mg/kg lean mass, P<0.001) but not small VLDL (160±11 versus 142±13). However, postmenopausal status had a pronounced effect on the characteristics of small VLDL particles, which were considerably triglyceride-enriched (production ratio of VLDL triglyceride:apolipoprotein B 30±5.3 versus 19±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 (r2=−0.49, P=0.006), de novo lipogenesis (r2=0.55, P=0.003), and desaturation (r2=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.1 Many different factors contribute, including marked hormonal changes,2 changes in metabolic profile associated with increased risk of the metabolic syndrome,3 and relative increase in intra-abdominal fat with age.4 Accumulation of intra-abdominal fat is associated with increased waist circumference and liver fat,5 overproduction of very low-density lipoprotein (VLDL), and decreased catabolism of apolipoprotein (apo)B-containing particles in men.5 The catabolism of apoB-containing particles is partly

From the Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK (L.H., K.M., K.H., F.K., B.A.F.); Division of Cardiovascular Medicine, Oxford Centre for Clinical Magnetic Resonance Research, John Radcliffe Hospital, Oxford, UK (R.B., B.R.); Centre for Endocrinology, Diabetes and Metabolism, School of Clinical & Experimental Medicine, University of Birmingham, UK (W.A.); Departments of Molecular and Clinical Medicine (M.A.) and Mathematical Sciences (M.A., J.B.), University of Gothenburg, Sweden; Department of Experimental Physiology, Athens University School of Medicine, Athens, Greece (K.M.); Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK (C.F., B.A.F.); Department of Clinical Nutrition, Clinic of Clinical Service, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway (I.L.M.); Department of Cancer Research and Molecular Medicine, Faculty of Medicine, NTNU, Trondheim, Norway (I.L.M.); Gloucestershire Diabetic Retinopathy Research Group, Cheltenham General Hospital, Gloucestershire, UK (I.M.S.); Metabolic Research Centre, School of Medicine and Pharmacology (P.H.R.B., D.C.C., G.F.W.) and Faculty of Engineering, Computing and Mathematics (P.H.R.B., D.C.C., G.F.W.) and Faculty of Engineering, Computing and Mathematics (P.H.R.B., D.C.C., G.F.W.) and Faculty of Engineering, Computing and Mathematics (P.H.R.B., D.C.C., G.F.W.) and Faculty of Engineering, Computing and Mathematics (P.H.R.B., D.C.C., G.F.W.); University of Western Australia, Perth, WA, Australia; National Institute for Health Research Oxford Biomedical Research Centre, Oxford University Hospital Trusts, Oxford, UK (F.K.).

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

Correspondence to: Barbara A. Fielding, PhD, University of Surrey, Guildford, GU2 7WG, UK. E-mail: b.fielding@surrey.ac.uk

Received June 19, 2015; accepted August 18, 2015.

© 2015 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley Blackwell. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1161/JAHA.115.002258
determined by plasma apoC-III concentrations, and higher plasma apoC-III has been associated with dyslipidemia in obese men.\(^7\) 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.\(^8\)

Normally, fasting plasma triglycerides (TG) are determined by 2 distinct subclasses of VLDL;\(^9\) VLDL\(_1\) is larger and more TG-rich than VLDL\(_2\), the latter can either be secreted directly from the liver, or formed by the peripheral hydrolysis of VLDL\(_1\). Hypertriglyceridemia is associated with atherogenic dyslipidemia including the production of small dense LDL, lower HDL cholesterol, and accumulation of postprandial TG-rich lipoproteins.\(^10\) In men with type 2 diabetes, the secretion of VLDL\(_1\) is associated with liver fat, hypertriglyceridemia, and increased atherogenic risk.\(^11\)

Impaired hepatic fatty acid oxidation has been reported to be related to obesity and insulin resistance by some\(^12,13\) but not all.\(^14\) Few detailed studies have investigated VLDL\(_1\) and VLDL\(_2\) kinetics in women, and none have compared the kinetics of VLDL or apoC-III in pre- and postmenopausal women. We hypothesized that VLDL\(_1\)-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\(_1\) and VLDL\(_2\) 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\(^15\) 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 ≥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\(^16\) 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\(^15\) 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.\(^17\) 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,\(^18\) and whole body composition and fat distribution (eg, android and gynoid fat) were measured using DEXA.\(^15\)

Metabolic Study Day

Participants arrived after an overnight fast and after consuming deuterated water (\(^2\)H\(_2\)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%.\(^19\) A cannula was placed in an antecubital vein in order to take blood samples for the estimation of DNL in VLDL\(_{1}\) and VLDL\(_{2}\)-palmitate, and background isotopic enrichments for the kinetic studies. Another cannula was placed in the contralateral arm to administer intravenous boluses of [\(^1\)H\(_3\)]leucine (7 mg/kg) and [\(^1\)H\(_3\)]glycerol (500 mg), while an intravenous infusion of [U-\(^13\)C]palmitic acid, potassium salt complexed with albumin\(^20\) at 0.03 \(\mu\)mol/kg per minute, was started. Blood samples were taken for a further 8 hours and VLDL\(_1\) and VLDL\(_2\) were isolated from plasma using density gradient ultracentrifugation.\(^20\) 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, 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. 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. [2H5] glycerol in plasma, VLDL1 and VLDL2-TG (to trace TG) and [2H3]leucine in plasma, and VLDL1-and VLDL2-apoB (to trace whole particles) were measured by gas chromatography–mass spectrometry. [U-13C]palmitic acid was measured in plasma NEFA and VLDL1 and VLDL2-TG by gas chromatography–mass spectrometry and the proportion of fatty acids (FAs) in VLDL-TG that were derived from nonsystemic sources was calculated, assuming that 16:0 is representative of all FAs. Mathematical modeling of VLDL kinetics (VLDL1-TG, VLDL2-TG, VLDL1-apoB, VLDL2-apoB production and clearance) was calculated from [2H5]glycerol and [2H3]leucine enrichments in plasma and lipoprotein fractions. 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 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 Hydragel 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 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-13C]16:1n-7/[U-13C]16:0 in VLDL1 and VLDL2-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. FA methyl esters prepared from VLDL1 and VLDL2-TG FAs were analyzed by GC to quantify 16:0 and 16:1n-7, and by GC-Isotope Ratio Mass Spectrometer to measure isotopic enrichment. Hepatic DNL was measured on the study day, based on the incorporation of [2H] in plasma water (Finnigan GasBench-II; ThermoFisher Scientific, UK) and into VLDL1- and VLDL2-TG palmitate using gas chromatography–mass spectrometry. For simplicity, this is referred to as “%DNL” and represents synthesis of FAs from precursors such as sugars and amino acids.

FA rate of appearance (RaNEFA) was calculated from the [U-13C]16:0 infusion rate and enrichment in the plasma NEFA fraction and RaNEFA was assumed to equal R0,NEFA.

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.” 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/VLDL1 or VLDL2). 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 VLDL1 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, and DNL), 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 α 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.

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
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 abdominal obesity (Table 1). Liver fat was generally low, although 6% of 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/m² in abdominally obese and 19.5 to 27.6 kg/m² 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, VLDL₁ and VLDL₂-TG concentrations). One postmenopausal woman had impaired fasting glucose.

The rate of disappearance of FAs (RₕNEFA), expressed per kg lean mass was significantly higher in post- compared to premenopausal women (Table 3). Release of FAs into plasma (RₕNEFA) per unit weight of adipose tissue was lower in women with abdominal obesity, but VLDL₁-TG and VLDL₁-apoB production were significantly higher. The ratio of VLDL₂-TG direct production:VLDL₂-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, VLDL₁-TG and VLDL₂-TG direct production, VLDL₁ and VLDL₂ direct apoB production, or VLDL₁ and VLDL₂-TG:apoB production ratios within menopausal groups. Plasma apoC-III concentrations positively correlated with apoC-III production rate (rₛ=0.59, P=9.0×10⁻⁷) 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 VLDL₁ and VLDL₂-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 VLDL₁-TG

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<sub>menopause</sub> | P<sub>abdom obesity</sub> |
|--------------------------|----------------------|------------------------|-------------|--------------------------|-----------------------|---------------------------|
| Waist, cm                | 83.5 (1.8)           | 82.1 (1.4)             | 77.1 (0.86) | 88.6 (1.5)               | NS                    | <0.001                    |
| BMI, kg/m²               | 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<sub>menopause</sub>, statistical significance for an effect of menopausal status; P<sub>abdom obesity</sub>, 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.
production was significantly higher in abdominally obese women, in line with higher VLDL$_1$-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$_2$ 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$_1$ and VLDL$_2$-TG and plasma 3OHB area under the curve (Figure 2E and 2F). VLDL-ApoB and -TG production were highly correlated for VLDL$_1$ and less so for VLDL$_2$ (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. 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 2.** Biochemical and Metabolic Variables in Women According to Menopausal Status and Abdominal Obesity

|                               | Premenopausal (n=30) | Postmenopausal (n=30)* | Lean (n=29) | Abnormally Obese (n=29) | $P_{\text{meno}}$ | $P_{\text{Abd obesity}}$ |
|-------------------------------|----------------------|------------------------|------------|------------------------|------------------|---------------------|
| 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$_1$-TG, μmol/L          | 142 (33 to 2083)     | 226 (82 to 1090)       | 181 (26)   | 369 (72)               | NS               | 0.001               |
| VLDL$_2$-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$_1$-apoB, g/L           | 0.006 (0.001 to 0.05)| 0.007 (0.004 to 0.03)  | 0.005 (0.001 to 0.03) | 0.006 (0.004 to 0.05) | NS               | <0.05               |
| VLDL$_2$-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: $P_{\text{meno}}$, statistical significance for an effect of menopausal status; $P_{\text{Abd obesity}}$, 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 $n=29$. 

DOI: 10.1161/JAHA.115.002258
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<sub>menop</sub> | P<sub>abdom obesity</sub> |
|----------------------|---------------|----------------|------|-------------------|--------------------|--------------------------|
| R<sub>N</sub>NEFA, μmol min<sup>-1</sup> 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<sub>N</sub>NEFA, μmol min<sup>-1</sup> 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 |
| VLDL<sub>1</sub>-TG Prod, mg/kg lean mass | 193 (12) n=30 | 220 (12) n=28 | 197 (13) n=28 | 219 (11) n=28 | 0.05 | NS |
| VLDL<sub>2</sub>-TG dirProd, 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>1</sub>-TG indirProd, 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 | <0.05 | NS |
| 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 |
| Apo-C<sub>III</sub> 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 |
| Apo-C<sub>III</sub> 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<sub>menop</sub> statistical significance for an effect of menopausal status; P<sub>abdom obesity</sub> 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<sub>NEFA</sub>, rate of appearance; R<sub>apoB</sub>, 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-apoB<sub>100</sub> secretion, 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.

DOI: 10.1161/JAHA.115.002258
### Table 4. Variables Relating to FA Metabolism in Women According to Menopausal Status and Abdominal Obesity

|                              | Premenopausal | Postmenopausal | Lean | Abnormally Obese | $P_{\text{menop}}$ | $P_{\text{Abd obesity}}$ |
|------------------------------|---------------|----------------|------|------------------|---------------------|------------------------|
| Nonsystemic FA—VLDL$_1$-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$_2$-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$_1$-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$_2$-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$_1$-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$_2$-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$_1$-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$_2$-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$_1$: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 | 0.06                | NS                     |
| VLDL$_2$: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                  |
| 3OHB/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: $P_{\text{menop}}$, statistical significance for an effect of menopausal status; $P_{\text{Abd obesity}}$, statistical significance for an effect of abdominal obesity. 3OHB 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 ($r_3$) for Premenopausal Women Between Selected Variables Relating to VLDL$_1$ Metabolism, Liver Fat, and Intra-Abdominal Fat

|                              | Total Body Fat, kg | Android/ Tot Fat, kg | Visceral Fat, cm$^2$ | Subcut Fat, cm$^2$ | HOMA-IR | Plasma NEFA*, $\mu$mol/L | Plasma 3OHB*, $\mu$mol/L | VLDL$_1$:TG SCD Iso | VLDL$_1$:TG SCD 16 | VLDL$_1$:TG DNL (%) | VLDL$_1$:TG Prod/apoB Prod |
|------------------------------|-------------------|----------------------|----------------------|-------------------|--------|-------------------------|--------------------------|---------------------|-------------------|------------------|------------------------|
| Liver fat, %                 | 0.57              | 0.63                 | −0.39                | 0.71              | 0.47   | −0.07                   | −0.42                    | 0.40                | 0.41              | −0.05               | −0.33                  |
|                              | $P<0.001$         | $P<0.001$            | $P<0.001$            | $P<0.001$         | $P<0.001$ | $P<0.001$               | $P<0.001$               | $P<0.001$           | $P<0.001$         | $P<0.001$               | $P<0.001$               |
| Total body fat, kg           | 0.68              | 0.63                 | −0.63                | 0.80              | 0.90   | −0.00                   | −0.33                    | 0.61                | 0.39              | −0.13               | −0.49                  |
|                              | $P<0.001$         | $P<0.001$            | $P<0.001$            | $P<0.001$         | $P<0.001$ | $P<0.001$               | $P<0.001$               | $P<0.001$           | $P<0.001$         | $P<0.001$               | $P<0.001$               |
| Android/ Tot fat, kg         | −0.74             | 0.78                 | 0.63                 | 0.53              | −0.10  | −0.37                   | 0.53                     | 0.53                | 0.35              | −0.20               | −0.72                  |
|                              | $P<0.001$         | $P<0.001$            | $P<0.001$            | $P<0.001$         | $P<0.001$ | $P<0.001$               | $P<0.001$               | $P<0.001$           | $P<0.001$         | $P<0.001$               | $P<0.001$               |

See Supplemental 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; SCD 16, ratio of 16:1n-7/16:0; SCD 18, ratio 18:1n-9/18:0; Subcut, subcutaneous; TG, triglyceride; tot, total; VLDL, very-low-density lipoprotein.

* AUC.
### Table 6. Correlation Coefficients ($r_p$) for Premenopausal Women Between Selected Variables Relating to VLDL$_2$ Metabolism, Liver Fat, and Intra-Abdominal Fat

|                   | Total Body Fat, kg | Android/ Tot Fat, kg | Gynoid/ Tot Fat, kg | Visceral Fat, cm$^2$ | Subcut Fat, cm$^2$ | HOMA-IR | Plasma NEFA*, µmol/L | Plasma 3OHBA*, µmol/L | VLDL$_2$-TG Prod/d | VLDL$_2$-TG SCD Iso Index | VLDL$_2$-TG SCD16 | VLDL$_2$-TG DNL (%) | VLDL$_2$-TG 18:2n-6 (%) | VLDL$_2$-TG Prod/apoB Prod |
|-------------------|-------------------|---------------------|--------------------|----------------------|---------------------|---------|----------------------|------------------------|----------------|------------------------|----------------|----------------|------------------------|------------------------|
| Liver fat, %      | 0.57              | 0.63                | 0.39 P<0.001       | 0.71 P<0.001         | 0.47 P<0.009        | 0.63 P<0.001 | −0.07 P=0.702       | −0.42 P<0.022         | 0.21 P=0.306 | 0.38 P<0.040           | 0.01 P=0.952 | −0.37 P=0.042     | −0.19 P<0.001       | −0.29 P<0.150       |
| Total body fat, kg| 0.68 P<0.001      | 0.63 P<0.001        | 0.80 P<0.001       | 0.90 P<0.001         | 0.36 P<0.052        | 0.00 P=0.982 | −0.33 P<0.077       | 0.51 P=0.007          | 0.41 P=0.023 | 0.21 P=0.257           | 0.24 P<0.020 | 0.41 P<0.002      | 0.25 P<0.177        | 0.23 P<0.255       |
| Android/ tot fat, kg | −0.74 P<0.001    | 0.78 P<0.001        | 0.63 P<0.001       | 0.53 P<0.003         | −0.10 P=0.596       | −0.37 P<0.046 | 0.29 P=0.145        | 0.36 P=0.052          | 0.12 P=0.516 | −0.31 P=0.092          | 0.22 P<0.240 | 0.05 P<0.791      | 0.11 P<0.580        |

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.

### Table 7. Correlation Coefficients ($r_p$) for Post-Menopausal Women Between Selected Variables Relating to VLDL$_1$ Metabolism, Liver Fat and Intra-Abdominal Fat

|                   | Total Body Fat, kg | Android/ Tot Fat, kg | Gynoid/ Tot Fat, kg | Visceral Fat, cm$^2$ | Subcut Fat, cm$^2$ | HOMA-IR | Plasma NEFA*, µmol/L | Plasma 3OHBA*, µmol/L | VLDL$_1$-TG Prod/d | VLDL$_1$-TG SCD Iso Index | VLDL$_1$-TG SCD16 | VLDL$_1$-TG DNL (%) | VLDL$_1$-TG 18:2n-6 (%) | VLDL$_1$-TG Prod/apoB Prod |
|-------------------|-------------------|---------------------|--------------------|----------------------|---------------------|---------|----------------------|------------------------|----------------|------------------------|----------------|----------------|------------------------|------------------------|
| Liver fat, %      | 0.28 P=0.150      | 0.26 P=0.184        | −0.42 P=0.028      | 0.61 P<0.001         | 0.26 P=0.192        | 0.23 P=0.232 | 0.00 P=0.994         | 0.09 P=0.647          | 0.05 P=0.797 | −0.11 P=0.059          | −0.42 P=0.023 | −0.26 P=0.173     | 0.11 P=0.578        | 0.26 P=0.200       |
| Total body fat, kg| 0.78 P=0.001      | 0.73 P=0.001        | 0.86 P<0.001       | 0.29 P=0.133         | −0.09 P=0.648       | 0.19 P=0.326 | 0.34 P=0.093         | −0.01 P=0.512         | −0.13 P=0.279 | 0.22 P=0.027           | 0.01 P=0.967 | 0.32 P=0.106      | 0.35 P=0.077        |
| Android/ tot fat, kg | −0.76 P=0.001    | 0.72 P<0.001        | 0.69 P<0.001       | 0.35 P=0.064         | −0.35 P=0.072       | −0.14 P=0.475 | 0.57 P=0.003         | 0.23 P=0.246          | −0.21 P=0.287 | −0.05 P=0.788          | 0.19 P=0.342 | 0.29 P=0.143      | 0.25 P=0.225        |

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.
Table 8. Correlation Coefficients ($r$) for Postmenopausal Women Between Selected Variables Relating to VLDL$_2$ Metabolism, Liver Fat, and Intra-Abdominal Fat

| Variable                  | Total Body Fat, kg | Android/Total Fat, kg | Gynoid/Total Fat, kg | Visceral Fat, cm$^2$ | Subcut Fat, cm$^2$ | HOMA-IR | Plasma NEFA*, $\mu$mol/L | Plasma 3OHBA*, $\mu$mol/L | VLDL$_2$-TG Prod/d | VLDL$_2$-TG SCD Iso Index | VLDL$_2$-TG SCD16 | VLDL$_2$-TG SCD18 | VLDL$_2$-TG DNL (%) | VLDL$_2$-TG 18:2n-6 (%) | VLDL$_2$-TG Prod/apoB Prod |
|--------------------------|-------------------|----------------------|---------------------|----------------------|---------------------|--------|--------------------------|--------------------------|---------------------|--------------------------|----------------|----------------|----------------|----------------|----------------|
| Liver fat, %             | 0.28              | 0.26                 | -0.42               | -0.028               | 0.61                | 0.00   | 0.09                      | 0.09                      | -0.15               | -0.05                    | -0.46          | -0.44          | 0.11           | 0.15           | 0.11           |
| Total body fat, kg       | 0.78              | 0.57                 | 0.57                | 0.001                | 0.73                | 0.66   | 0.29                      | 0.09                      | -0.09               | 0.19                      | 0.18           | 0.13           | 0.13           | 0.27           | 0.27           |
| Android/tot fat, kg      | -0.76             | 0.72                 | 0.69                | 0.001                | 0.35                | -0.35  | -0.14                     | -0.14                     | -0.35               | 0.02                      | 0.20           | 0.20           | 0.16           | 0.22           | 0.31           |

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.
isotopic desaturation index in VLDL1- and VLDL2-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.48 However, we found no correlation between serum cortisol concentrations and intra-abdominal fat area. In agreement with previous studies of aging,49 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.4 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.50 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,32 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.50 One study found that VLDL-TG secretion rate was
Figure 2. Correlations between VLDL₁-TG production (mg/day) and the proportion (%) of DNL fatty acids VLDL₁-TG (A), VLDL₂-TG direct production (mg/day) and the proportion (%) of DNL fatty acids VLDL₂-TG (B), the proportion (%) of DNL fatty acids VLDL₁-TG and the AUC for plasma 3-hydroxybutyrate (μmol/L) (C), the proportion (%) of DNL fatty acids VLDL₂-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 ([U¹³C₁₆:1n-7/U¹³C₁₆:0]*1000) in VLDL₁-TG (E) and VLDL₂-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.
significantly higher in pre-menopausal women than men, whereas another found that VLDL$_2$-TG but not VLDL$_1$-TG secretion rate was higher in post-menopausal women than men. We have previously reported no difference in the postprandial contribution of dietary and nonsystemic FA to VLDL-TG between insulin-sensitive men and women. 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. 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$_1$ and VLDL$_2$ metabolism is complex in women, and hive plots illustrate that the patterns of associations with metabolic variables are different between menopausal groups. A lack of significant correlation between hepatic VLDL$_2$-TG and VLDL$_2$-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$_1$-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$_1$-TG secretion is significantly higher in abdominally obese women and accounts for increased plasma VLDL$_1$-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. Weight gain in postmenopausal women is likely to impact on both VLDL$_1$-TG and VLDL$_2$-TG secretory pathways with consequent implications for cardiovascular disease risk.

**Acknowledgments**

Thanks to Jane Cheeseman, Louise Dennis, Marjorie Gilbert, Pauline Sutton, Catriona McNeil, Sandy Humphreys, Keith Frayn for help, and Costas Christodoulides who was the duty clinician. We also thank the enthusiastic participants.

**Sources of Funding**

The study was funded by the British Heart Foundation (Project Grant PG/09/003) and Hodson is a British Heart Foundation Intermediate Fellow in Basic Science (FS/11/18/28633). The British Heart Foundation had no role in the design, analysis, or writing of this article. Marinou was funded by the European Commission under the Marie Curie Programme (FP7-PEOPLE-2011-IEF). The contents reflect only the author’s views and not the views of the European Commission. ApoC-III kinetics were funded by a grant from the National Heart Foundation of Australia (G 11 P 5739). PHRB is a senior research fellow of the National Health and Medical Research Council (NHMRC) of Australia. Chan is a career development fellow of the NHMRC.

**Disclosures**

None.
1. Ng MK. New perspectives on Mars and Venus: unravelling the role of androgens in gender differences in cardiovascular biology and disease. Heart Lung Circ. 2007;16:185–192.

2. Pasquali R, Vicenati V, Bertazzo D, Casimiri F, Pascal G, Tortelli O, Labate AM. Determinants of sex hormone-binding globulin blood concentrations in premenopausal and postmenopausal women with different estrogen status. Virgilio-Menopause-Health Group. Metabolism. 1997;46:5–9.

3. Carr MC. The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab. 2003;88:2404–2411.

4. Pou KM, Massaro JM, Hoffmann U, Lieb K, Vasan RS, O’Donnell CJ, Fox CS. Patterns of abdominal fat distribution: the Framingham Heart Study. Diabetes Care. 2009;32:481–485.

5. Kotronen A, Westerbacka J, Vehkavaara S, Hakkinen A, Olofsson SO, Yki-Jarvinen H, Boren J. A new combined multicompartmental model for apolipoprotein B-100 and triglyceride metabolism in VLDL subfractions. J Lipid Res. 2005;46:58–67.

23. Adiels M, Packard C, Caslake MJ, Stewart P, Soro A, Westerbacka J, Wennberg B, Kotronen A, Taskinen MR, Boren J. A new combined multicompartmental model for apolipoprotein B-100 and triglyceride metabolism in VLDL subfractions. J Lipid Res. 2005;46:58–67.

24. Sarac I, Backhouse K, Shojaee-Moradie F, Stolinski M, Robertson MD, Bell JD, Thomas EL, Hovrka R, Wright J, Umplyfe AM. Gender differences in VLDL1 and VLDL2 triglyceride kinetics and fatty acid kinetics in obese postmenopausal women and obese men. J Clin Endocrinol Metab. 2012;97:2475–2481.

25. Chan DC, Nguyen MN, Watts GF, Barrett PH. Plasma apolipoprotein C-III transport in centrally obese men: associations with very low-density lipoprotein apolipoprotein B and high-density lipoprotein apolipoprotein A-I metabolism. J Clin Endocrinol Metab. 2008;93:537–544.

26. Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? Prog Lipid Res. 2013;52:15–42.

27. Semple RK, Aellig S, Muragutroyd PR, Adams CA, Bluck L, Jackson S, Vottero A, Kanabar D, Charlton-Menys V, Durrington P, Soos MA, Carpenter TA, Lomas DC, Cochran EK, Gorden P, O’Rahilly S, Savage DB. Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. J Clin Invest. 2009;119:315–322.

28. Moore JB, Gunn PJ, Fielding BA. The role of dietary sugars and de novo lipogenesis in non-alcoholic fatty liver disease. Nutrients. 2014;6:5769–5793.

29. Kryzwinski M, Birol I, Jones SJ, Marra MA. Hive plots—rational approach to visualizing networks. Brief Bioinform. 2012;13:627–644.

30. Magkos F, Patterson BW, Mittendorfer B. Reproducibility of stable isotope-labeled tracer measures of VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics. J Lipid Res. 2007;48:1204–1211.

31. Magkos F, Patterson BW, Mohammed BS, Klein S, Mittendorfer B. Women produce fewer but triglyceride-richer very low-density lipoproteins than men. J Clin Endocrinol Metab. 2007;92:1311–1318.

32. Fabbrini E, Magkos F, Mohammed BS, Piotka T, Abumrad NA, Patterson BW, Okunade A, Klein S. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. Proc Natl Acad Sci USA. 2009;106:15430–15435.

33. Nielsen S, Karpe F. Determinants of VLDL-triglycerides production. Curr Opin Lipidol. 2012;23:321–326.

34. Gill JM, Brown JC, Bedford D, Wright DM, Cooney J, Hughes DA, Packard CJ, Caslake MJ. Hepatic production of VLDL1 but not VLDL2 is related to insulin resistance in normoglycaemic middle-aged subjects. Atherosclerosis. 2004;174:49–56.

35. Mittendorfer B, PattersonBW, Klein S. Effect of weight loss on VLDL-triglyceride and apoB-100 kinetics in women with abdominal obesity. Am J Physiol Endocrinol Metab. 2003;284:E549–E556.

36. Mittendorfer B, Patterson BW, Klein S, Sidowski LS. VLDL-triglyceride kinetics during hyperglycemia-hyperinsulinemia: effects of sex and obesity. Am J Physiol Endocrinol Metab. 2003;284:E708–E715.

37. Gormsen LC, Nelemann B, Sorensen LP, Jensen MD, Christiansen JS, Nielsen S. Impact of body composition on very-low-density lipoprotein-triglyceride kinetics. Am J Physiol Endocrinol Metab. 2009;296:E165–E173.

38. Nordestgaard BG, Tybjaerg-Hansen A, DL, VLDL, chylomicrons and atherosclerosis. Eur J Epidemiol. 1992;8(suppl 1):92–98.

39. Gav A, Packard CJ, Lindsay GM, Griffin BA, Caslake MJ, Lorimer AR, Shepherd J. Overproduction of small very low density lipoproteins (SI 20–60) in hypercholesterolaemia: relationships between apolipoprotein B kinetics and plasma lipoproteins. J Lipid Res. 1995;36:158–171.

40. Mittendorfer B, Patterson BW, Klein S. Effect of sex and obesity on basal VLDL-triaclyglycerol kinetics. Am J Clin Nutr. 2003;77:573–579.

41. Smith GI, Reeds DN, Okunade AL, Patterson BW, Mittendorfer B. Systematic delivery of estradiol, but not testosterone or progesterone, alters VLDL-triglyceride kinetics in postmenopausal women. J Clin Endocrinol Metab. 2014;99:E1306–E1310.

42. Matthews KA, Crawford SL, Chae CJ, Everson-Rose SA, Sowers MF, Sternfeld B, Sutton-Tyrrell K. Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? J Am Coll Cardiol. 2009;54:2366–2373.

43. Jensen MD, Martin ML, Cryer PE, Roust LR. Effects of estrogen on free fatty acid metabolism in humans. Am J Physiol. 1994;266:E914–E920.

44. McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, Ruige T, Gilbert M, Fielding BA, Frayn KN, Karpe F. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? Diabetes. 2011;60:47–55.

45. Karpe F, Dickmann JR, Frayn KN. Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes. 2011;60:2441–2449.
46. Vedala A, Wang W, Neese RA, Christiansen MP, Hellerstein MK. Delayed secretory pathway contributions to VLDL-triglycerides from plasma NEFA, diet, and de novo lipogenesis in humans. *J Lipid Res.* 2006;47:2562–2574.

47. Matikainen N, Adiels M, Soderlund S, Stennabb S, Ahola T, Hakkarainen A, Boren J, Taskinen MR. Hepatic lipogenesis and a marker of hepatic lipid oxidation, predict postprandial responses of triglyceride-rich lipoproteins. *Obesity (Silver Spring)*. 2014;22:1854–1859.

48. Pedersen SB, Jonler M, Richelsen B. Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab.* 1994;78:1354–1359.

49. Larsson CA, Gullberg B, Rastam L, Lindblad U. Salivary cortisol differs with age and sex and shows inverse associations with WHR in Swedish women: a cross-sectional study. *BMC Endocr Disord.* 2009;9:16.

50. Wang X, Magkos F, Mittendorfer B. Sex differences in lipid and lipoprotein metabolism: it’s not just about sex hormones. *J Clin Endocrinol Metab.* 2011;96:885–893.

51. Sarwar N, Sandhu MS, Ricketts SL, Butterworth AS, Di Angelantonio E, Boekholdt SM, Ouwehand W, Watkins H, Samani NJ, Saleheen D, Lawlor D, Reilly MP, Hingorani AD, Talmud PJ, Danesh J. Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies. *Lancet.* 2010;375:1634–1639.