

The Contribution of Splanchnic Fat to VLDL Triglyceride Is Greater in Insulin-Resistant Than Insulin-Sensitive Men and Women

Studies in the Postprandial State

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OBJECTIVE—We aimed to determine differences in the postprandial contributions of different fatty acid sources to VLDL triglycerides (TGs) in healthy men and women with varying degrees of insulin resistance.

RESEARCH DESIGN AND METHODS—Insulin-resistant ($n = 11$) and insulin-sensitive ($n = 11$) men and women ($n = 6$) were given an intravenous infusion of [$^2\text{H}_2$]palmitic acid to investigate systemic nonesterified fatty acid (NEFA) incorporation into VLDL TGs. Participants were also fed a mixed meal containing [$\text{U-}^{13}\text{C}$]palmitic acid to investigate the contribution of dietary fatty acids to VLDL TG production. Blood samples were taken over the following 6 h. Separation of VLDL was performed by density gradient ultracentrifugation and immunoaffinity techniques specific to apolipoprotein B-100.

RESULTS—Insulin-resistant and insulin-sensitive men had similar postprandial chylomicron and chylomicron remnant TG concentrations, but insulin-resistant men had higher postprandial VLDL TG concentrations (median [range]; area under the curve $485 \mu\text{mol/l}$ [123–992] vs. $287 \mu\text{mol/l}$ [162–510]; $P < 0.05$). At 360 min, most of the difference in VLDL TGs was accounted for by an additional contribution from splanchnic fat (means \pm SE; $331 \pm 76 \mu\text{mol/l}$ vs. $89 \pm 25 \mu\text{mol/l}$; $P < 0.01$). The contribution of fatty acids from endogenous systemic NEFAs was similar across the groups, as were dietary fatty acids. There was no difference in the VLDL TG concentration or the contribution of different fatty acid sources between insulin-sensitive men and women.

CONCLUSIONS—In the postprandial period, the only sources of fatty acids for VLDL TG production to differ in the insulin-resistant compared with the insulin-sensitive men are those derived from splanchnic sources. *Diabetes* 56:2433–2441, 2007

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3-OHB, 3-hydroxybutyrate; ALT, alanine aminotransferase; apo, apolipoprotein; DNL, de novo lipogenesis; NEFA, nonesterified fatty acid; TG, triglyceride; TRL, TG-rich lipoprotein; TTR, tracer-to-tracee ratio.

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There are two classes of triglyceride (TG)-rich lipoproteins (TRLs), and they differ in structure, function, site of synthesis, and metabolism. VLDLs are synthesized in the liver and contain apolipoprotein (apo) B-100, while apoB-48-containing chylomicrons are produced by the intestine after the consumption of fat. Despite the suppression of VLDL production in response to insulin (1) during the postprandial period, there are ~10 times more VLDLs than chylomicron particles, and the VLDLs carry >50% of the TRL TGs (2).

Investigating VLDL TG metabolism during the postprandial period is complex, as multiple sources of fatty acids are entering the hepatic TG and fatty acid pool simultaneously, with dietary fatty acids entering via two distinct pathways. One is the receptor-mediated uptake of chylomicron remnants (3). The other is via "spillover" nonesterified fatty acids (NEFAs), which are derived from the peripheral lipoprotein lipase-mediated lipolysis of chylomicron TGs (4–7). Other sources that may provide fatty acids for the production of VLDL TGs during the postprandial period include NEFAs that are derived from lipolysis of peripheral or visceral adipose tissue, hepatic fatty acids that are produced by de novo lipogenesis (DNL), or stored TGs in the cytosol of hepatocytes (8). The mobilization of stored cytosolic TGs for VLDL TG production involves lipolysis followed by reesterification of the fatty acids, with only part of the mobilized TGs being utilized for VLDL assembly; the remainder is recycled back to the cytosolic pool (8). Recently, increased hepatic lipid accumulation has been associated with insulin resistance and increased secretion of TG-rich VLDL (9). However, an alternative fate and important route of disposal for fatty acids entering the liver is β -oxidation. The ketone body 3-hydroxybutyrate (3-OHB), which is only produced in the liver, can be used as a proxy marker of hepatic fatty acid oxidation. Of note, the cytosolic TG fatty acids may not provide substrate for ketone body production, suggesting compartmentalization of the precursor pool of ketone bodies (10). Recent animal work (11) has reported that mice with higher levels of liver fat have lower hepatic fatty acid β -oxidation and systemic concentrations of 3-OHB compared with animals with low liver fat. Additionally, we have shown that insulin-resistant patients with familial combined hyperlipidemia have lower levels of 3-OHB, fasting and postprandially, than healthy control subjects (12).

Traditionally, VLDL TG metabolism has been investi-

gated in a fasting state. More recently, various models of postprandial lipemia have been used to investigate the contributions of fatty acids to TRL TGs (13–17) and VLDL-TGs (4,5). At the end of a postprandial period, a number of metabolic responses mediated by the postprandial rise of insulin will influence the fatty acid flux to the liver. Insulin-resistant subjects have disturbed fatty acid metabolism, and since NEFA supply to the liver is a major determinant of VLDL TG production (18,19), an increased flux of NEFAs could contribute to the hypertriglyceridemia of insulin resistance. Changes in the supply of other fatty acids arising from increased liver fat (9), meal fatty acids (20), and DNL (17) could also be important. We therefore aimed to determine the postprandial contribution of different fatty acid sources to VLDL TGs, in groups with varying degrees of insulin resistance, after the consumption of a mixed meal. In addition, there are differences between men and women in atherogenic risk, which may be related to alterations in VLDL TG metabolism (21). We therefore used combined simultaneous stable isotope labeling of dietary and endogenous fatty acids with specific isolation of VLDL to investigate differences in the hepatic partitioning of fatty acids in insulin-sensitive men and women and insulin-resistant men, after the consumption of a mixed meal. We hypothesized that splanchnic fat would be a particularly prominent contributor to VLDL TG production in insulin-resistant subjects.

RESEARCH DESIGN AND METHODS

Twenty-eight healthy subjects were recruited from the Oxford BioBank database (22) and by advertisement. Eleven men were considered insulin resistant, with a fasting plasma insulin concentration greater than the 75th centile (11.2 mU/l) of the Oxford BioBank. These subjects were matched for age and BMI with 11 insulin-sensitive men. Six insulin-sensitive women were matched for age and BMI with the insulin-sensitive men. All subjects were apparently healthy, and none of the subjects took regular medication of any kind. Other data presented from some of these subjects have been reported separately (23,24). All subjects gave written informed consent after all procedures, and potential risks were explained. The protocol was approved by the Oxfordshire Clinical Research Ethics Committee.

Before the study day, subjects were asked to avoid foodstuffs naturally enriched in ^{13}C for 48 h and refrain from strenuous exercise and alcohol for 24 h before the study. Subjects arrived at the clinical research unit after an overnight fast. Arterial (23) or arterialized venous (25) blood samples were obtained for measurement of background isotopic enrichment. After the background blood sample was collected, an intravenous infusion of [$^2\text{H}_2$]palmitic acid (16:0) (isotope purity 97%; CK Gas Products, Hook, U.K.) complexed with albumin (blood transfusion service; John Radcliffe Hospital, Oxford, U.K.) was started (palmitate infusion rate $0.04 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). After at least 90 min equilibration, a time 0 blood sample was taken and participants were fed a standard test meal consisting of 40 g Rice Krispies (Kellogg, Manchester, U.K.), 200 g skimmed milk, and a chocolate milkshake containing 40 g of olive oil. One hundred milligrams of [^{13}C]palmitic acid was added to the chocolate milkshake to trace dietary fatty acids. Serial blood samples were taken at regular intervals throughout the 360-min postprandial period.

Analytical methods. Whole blood was collected into heparinized syringes (Starstedt, Leicester, U.K.), and plasma was rapidly separated by centrifugation at 4°C for the measurement of metabolite and insulin concentrations. Plasma insulin, glucose, NEFAs, TGs, and lipoprotein TG concentrations were determined as previously described (23). Plasma alanine aminotransferase (ALT) was measured using an IL ALT/GPT kit (Instrumentation Laboratory, Cheshire, U.K.). A sample of whole blood was added to perchloric acid for analysis of 3-OHB, as described previously (26).

Separations of chylomicrons of Svedberg floatation rate (S_v) >400 and VLDL-rich fraction (S_v 20–400) were made by sequential floatation using density gradient ultracentrifugation (27). Ultracentrifugation was performed in a SW40Ti swinging bucket rotor (Beckman Instruments, Palo Alto, CA) at 40,000 rpm at 14°C . The gradients were run for 32 min to float S_v >400 lipoproteins and for a further 16 h to float S_v 20–400 lipoproteins.

The S_v 20–400 fraction was then further separated by immunoaffinity chromatography, as previously described by Heath et al. (4). The bound and

unbound fractions were collected. The bound fraction (containing lipoproteins bound by anti-apoB-100) was completely devoid of apoB-48 and will hereafter be called VLDL. The unbound fraction was composed of $\sim 70\%$ apoB-48 and 30% apoB-100 particles (4). This fraction will be referred to as the chylomicron remnant-rich fraction, and the chylomicron remnant TG concentration was calculated from isotopic enrichment values (in calculations and found in the online appendix [available at <http://dx.doi.org/10.2337/db07-0654>]) in the unbound fraction. Samples were taken at -30 , 0 , 30 , 60 , 90 , 120 , 180 , 240 , 300 , and 360 min after the mixed meal for plasma TGs, NEFAs, and blood 3-OHB analysis and at 0 , 120 , 180 , 240 , 300 , and 360 min for the analysis of S_v >400 , S_v 20–400, and VLDL.

Fatty acid analysis and isotopic enrichment. To determine specific fatty acid composition and isotopic enrichment, total lipids were extracted from plasma and lipoproteins. Fatty acid methyl esters were prepared from NEFA and TG fractions, as previously described (4). Fatty acid compositions ($\mu\text{mol}/100 \mu\text{mol}$ total fatty acids) in these fractions were determined by gas chromatography (28), and palmitate concentrations were calculated by multiplying the proportion of palmitate by the corresponding plasma concentrations of NEFAs or VLDL TGs determined enzymatically.

Analysis of [$^2\text{H}_2$] and [^{13}C]palmitate enrichments. [$^2\text{H}_2$]palmitate enrichments in the fatty acid methyl ester derivatives of plasma NEFAs, S_v >400 , and VLDL and [^{13}C]palmitate enrichments in plasma NEFAs were determined by gas chromatography–mass spectrometry, as previously described (23). Tracer-to-tracee ratios (TTRs) for [$^2\text{H}_2$]palmitate ($M + 2)/(M + 0)$) and [^{13}C]palmitate ($M + 16)/(M + 0)$) were calculated. $^{13}\text{C}/^{12}\text{C}$ ratios in [^{13}C]palmitate were measured in the S_v >400 , S_v 20–400, and VLDL TG fatty acid methyl ester derivatives using a Delta Plus XP gas chromatography–combustion isotope ratio mass spectrometry (Thermo Electron, Bremen, Germany) (29).

The TTR of a baseline measurement (before administration of the stable isotope tracer) was subtracted from each sample TTR to account for natural abundance. The TTRs for [^{13}C]palmitate and [$^2\text{H}_2$]palmitate were multiplied by the corresponding palmitate NEFA or palmitate TG concentrations to give plasma and lipoprotein tracer concentrations.

Calculations. Chylomicron remnant TG concentrations were calculated from unbound concentrations of [^{13}C]palmitate TGs using a model (30) that assumes no fatty acid selectivity during remnant formation (31) and that all [^{13}C]palmitate TGs in the unbound fraction represent chylomicron remnants, as described in the online appendix.

Calculations of the contribution of different sources of fatty acids to VLDL TGs were performed based on the principles described by Barrows and Parks (13) and Vedala et al. (17) and were calculated at 360 min as described in the online appendix. The contribution from splanchnic lipolysis (mainly visceral adipose tissue) together with hepatic sources (such as DNL and stored hepatic TGs) will be referred to as splanchnic sources. The whole-body rate of appearance of NEFAs ($R_{a\text{NEFA}}$) ($\mu\text{mol}/\text{min}$) was calculated at times when the TTR for plasma NEFAs was reasonably stable: [$^2\text{H}_2$]palmitate TTR (in plasma NEFAs)/infusion rate ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) \times body weight (in kg).

Statistical methods. Data were analyzed using SPSS for Windows version 15 (SPSS, Chertsey, U.K.). Statistical significance was set at $P < 0.05$. All data are presented as means \pm SE, unless otherwise stated. Comparisons of groups were made between the insulin-resistant versus insulin-sensitive men as well as women versus insulin-sensitive men. Subject characteristics were analyzed for group differences using ANOVA, with groups as a fixed factor. Areas under the curve were calculated by the trapezoid method. Areas under the curve have been divided by the relevant period to give a time-averaged value. All datasets were tested for normality according to the Shapiro-Wilk test. Parameters that were not normally distributed were log transformed before analysis. Repeated-measures ANOVA, with time and group as factors, was used to investigate the change between groups over time for systemic plasma TGs, NEFAs, blood 3-OHB, and [$^2\text{H}_2$]palmitate concentrations of VLDL TGs and plasma. The contributions from the different fatty acid sources were assessed using a Kruskal-Wallis. Correlations between the contributions from different fatty acid sources and plasma metabolites were performed using a Spearman rank correlation.

RESULTS

Subject characteristics. Insulin-sensitive and insulin-resistant subjects were well matched for age, BMI, waist circumference, and body fat (Table 1). Women were well matched to the insulin-sensitive men for age, BMI, and waist circumference. However, they had a significantly higher proportion of body fat ($31 \pm 6\%$ vs. $20 \pm 5\%$; $P < 0.001$) (Table 1). Fasting plasma insulin concentrations were $>50\%$ higher ($P < 0.05$) in the insulin-resistant

TABLE 1
Subject characteristics

	Women	Insulin-sensitive men	Insulin-resistant men
<i>n</i>	6	11	11
Age (years)	32 (23–64)	42 (23–54)	43 (22–45)
BMI (kg/m ²)	22 (22–27)	26 (21–31)	25 (22–35)
Waist circumference (cm)	75 (69–91)	88 (75–102)	98 (74–111)
Body fat (%)	31 (24–38) [†]	21 (9–27)	21 (9–31)
Systolic blood pressure (mmHg) ^a	111 (97–126)	125 (95–131)	125 (105–142)
Diastolic blood pressure (mmHg) ^a	69 (62–77)	78 (60–80)	78 (65–98)
Whole-body fasting <i>Ra</i> _{NEFA} (μmol/min)	116 (88–131)	128 (110–352)	111 (60–250)
HOMA-IR ^a	0.9 (0.4–1.3)*	1.1 (0.9–1.4)	1.7 (1.5–3.5)¶
Fasting plasma concentrations			
Insulin (mU/l) ^a	6.8 (0.5–10.0)*	8.6 (6.6–10.7)	13.5 (11.2–27.9)¶
Glucose (mmol/l)	4.7 (4.4–5.3)*	5.4 (4.5–6.1)	5.5 (4.5–6.7)
TGs (μmol/l) ^a	969 (423–1510)	916 (581–1327)	1,223 (832–2119)§
VLDL TG (μmol/l) ^a	314 (102–551)	257 (183–530)	508 (148–930)‡
NEFAs (μmol/l)	697 (553–850)	636 (405–860)	567 (298–935)
HDL cholesterol (mmol/l) ^a	1.09 (0.89–1.44)	1.11 (0.98–1.68)	1.19 (0.57–1.63)
ALT (units/l) ^a	18 (9–21)	16 (10–23)	22 (14–73)§
Blood 3-OHB (μmol/l) ^a	73 (60–207)	85 (23–437)	46 (13–245)
Time-averaged postprandial plasma concentrations			
Insulin (mU/l) ^a	17.9 (6.27–32.9)	17.7 (13.4–27.7)	30.8 (23.3–65.5)§
Glucose (mmol/l)	5.37 (4.56–6.02)*	6.07 (5.25–6.41)	5.65 (5.05–7.16)

Data are median (range). ^aData was log transformed for analysis. **P* < 0.05, [†]*P* < 0.001, women vs. insulin-sensitive men; [‡]*P* < 0.05, [§]*P* < 0.01, [¶]*P* < 0.001, insulin-sensitive men vs. insulin-resistant men.

compared with the insulin-sensitive men, and this was also reflected in a significantly (*P* < 0.001) higher value for homeostasis model assessment of insulin resistance (Table 1). When compared with the insulin-sensitive men, women had a significantly (*P* < 0.05) lower fasting plasma insulin concentration and homeostasis model assessment of insulin resistance (Table 1).

Fasting plasma ALT, TG, and VLDL TG concentrations were significantly higher in the insulin-resistant men compared with the insulin-sensitive women (Table 1). The postprandial insulin response was two times greater (*P* < 0.01) in the insulin-resistant than in the insulin-sensitive men (Table 1).

Unlabeled biochemical parameters. During the postprandial period, there was a significant increase (*P* < 0.001) in plasma TG concentrations in all three groups (Fig. 1A). The increase in plasma TGs over time was significantly greater in the insulin-resistant than the insulin-sensitive men (*P* < 0.01). There was no difference between women and insulin-sensitive men. After the consumption of the mixed meal, systemic plasma NEFA concentrations rapidly decreased, reaching nadir ~90 min, after which concentrations began to increase. There was no difference between the groups (Fig. 1B).

Consumption of a mixed meal caused an initial suppression of blood 3-OHB until ~120 min, when 3-OHB concentrations began to increase. There was a significant difference between insulin-sensitive and insulin-resistant men for blood 3-OHB over time (*P* < 0.05), with insulin-sensitive men having a more dynamic response of greater suppression and a greater increase in concentration over time (Fig. 1C). The postprandial response for chylomicron and chylomicron remnant TGs was similar in both the insulin-resistant and insulin-sensitive groups (Fig. 2A and B). However, insulin-resistant men had significantly greater (*P* < 0.01) postprandial VLDL TG concentrations (Fig. 2C).

The contribution of systemic plasma NEFAs to VLDL TGs and the concentration of [²H₂]palmitic acid in plasma NEFAs and VLDL TGs. The TTRs for [²H₂]palmitate in VLDL TGs were substantially lower than the TTRs for [²H₂]palmitate in systemic plasma NEFAs in all groups for the first 240 min of the postprandial period (Fig. 3A–C). However, between 240 and 360 min, the TTRs of systemic plasma NEFAs and VLDL TGs started converging in women and insulin-sensitive men. This did not occur to the same extent in the insulin-resistant men (Fig. 3A–C). No enrichment of [²H₂]palmitate was detected in the *S*_f >400 fraction.

There were no differences in plasma NEFA [²H₂]palmitate concentrations during the postprandial period between the groups (Fig. 3D–F). The concentration of [²H₂]palmitate in VLDL TGs increased during the postprandial period and appeared to be reaching equilibrium in all groups at 360 min (Fig. 3D–F). There were no significant differences in the concentration of [²H₂]palmitate over time in VLDL TGs between the groups (Fig. 3D–F). Insulin-resistant men had a significantly lower contribution of systemic plasma NEFAs to VLDL TG production than insulin-sensitive men (39 ± 4% vs. 69 ± 6%; *P* < 0.001).

The contribution of different fatty acid sources to VLDL TGs. The proportion of dietary fatty acids contributing to VLDL TGs did not differ between the groups 360 min after the meal (Fig. 4A). The proportion from endogenous systemic fatty acids was over twofold higher in insulin-sensitive men than insulin-resistant men (59 ± 7% vs. 31 ± 4%; *P* < 0.01) (Fig. 4A). Conversely, the proportion from splanchnic sources was double the value (*P* < 0.01) in insulin-resistant men compared with insulin-sensitive men (Fig. 4A). When expressed as concentrations, the contributions from dietary remnants, dietary spillover NEFAs, and endogenous systemic NEFAs were not different between the groups (Fig. 4B). The contribution from

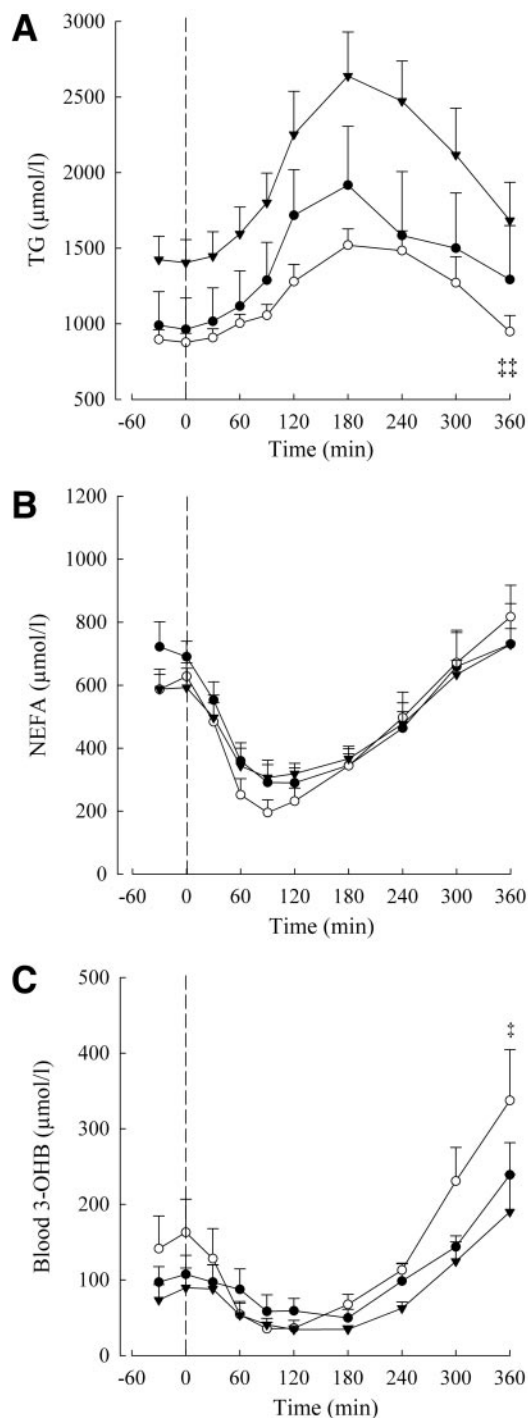


FIG. 1. Plasma concentrations of TGs (A), NEFAs (B), and the blood 3-OHB concentration (C) after a mixed meal in female subjects (●), insulin-sensitive men (○), and insulin-resistant men (▼). There were significant differences between the groups. A: ‡‡*P* < 0.01 insulin-sensitive men vs. insulin-resistant men. C: ‡*P* < 0.05 insulin-sensitive men vs. insulin-resistant men.

splanchnic sources in insulin-resistant men was markedly greater (*P* < 0.01) than in insulin-sensitive men (Fig. 4B). **Correlations between postprandial biochemical parameters and the contribution of fatty acids from different sources.** The contribution of different fatty acid sources, was expressed as a percentage or concentration, was significantly correlated with a number of biochemical parameters measured at 360 min (Table 2). A high VLDL

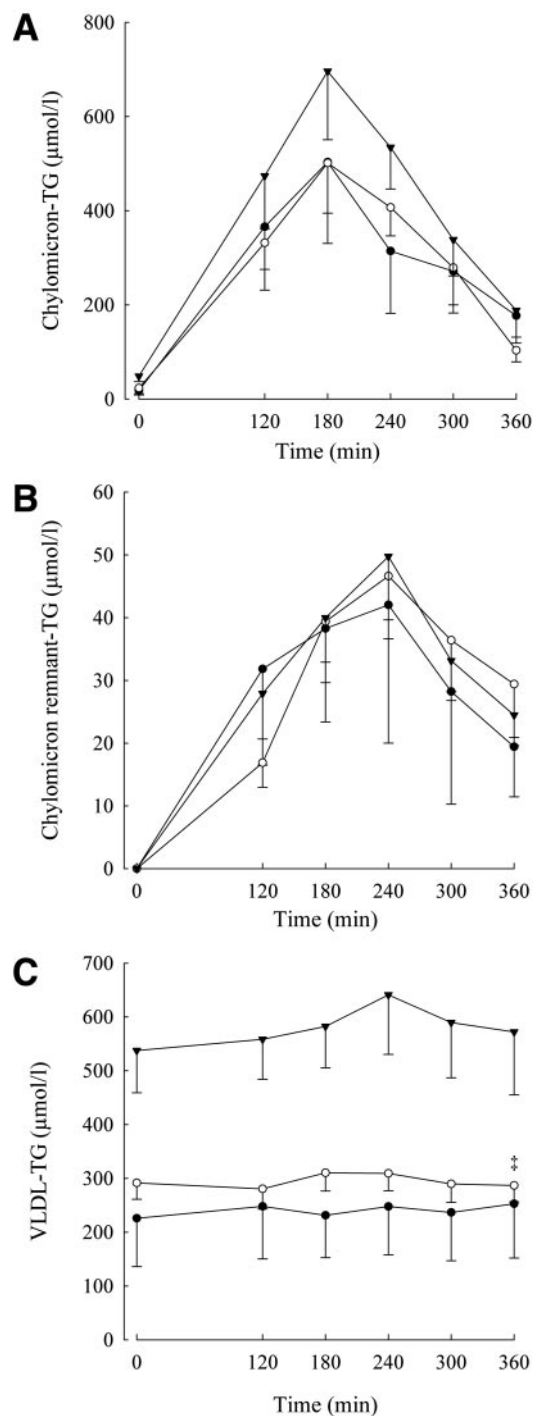


FIG. 2. Chylomicron TG (A), chylomicron remnant-rich fraction TG (B), and VLDL TG (C) concentrations after a mixed meal in female subjects (●), insulin-sensitive men (○), and insulin-resistant men (▼). There were significant differences between the groups. C: ‡*P* < 0.05 insulin-sensitive men vs. insulin-resistant men.

TG concentration was significantly correlated to a high contribution from endogenous systemic NEFAs (μmol/l) (*r* = 0.75, *P* < 0.001), dietary spillover NEFAs (μmol/l) (*r* = 0.72, *P* < 0.001) (Table 2), and splanchnic sources when expressed as a percentage (*r* = 0.48, *P* < 0.01) (Table 2; Fig. 5A) and concentration (*r* = 0.77, *P* < 0.001) (Table 2; Fig. 5B). Subjects who had a high blood 3-OHB concentration at 360 min tended to have a lower contri-

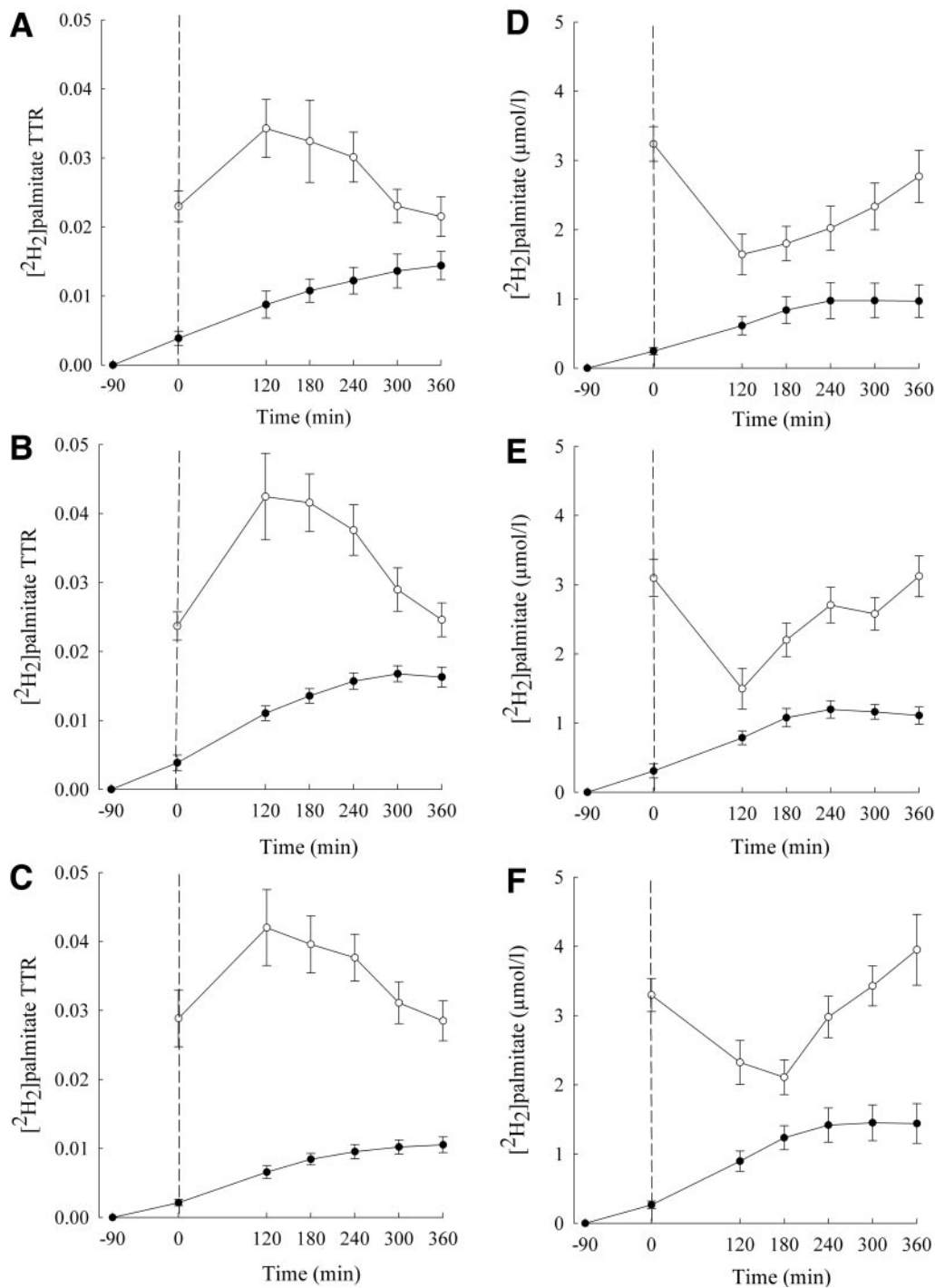


FIG. 3. The TTRs (A–C) and concentration ($\mu\text{mol/l}$) (D–F) of $[^2\text{H}_2]$ palmitate in VLDL TGs (●) and plasma NEFAs (○) after a mixed meal in female subjects (A and D), insulin-sensitive men (B and E), and insulin-resistant men (C and F). The contribution of systemic NEFA-to-VLDL TG production was calculated at 360 min using the product to precursor relationship and was $68 \pm 6\%$ for female subjects (A), $69 \pm 6\%$ for insulin-sensitive men (B), and $39 \pm 4\%$ for insulin-resistant men (C). This was significantly lower ($P < 0.001$) than the insulin-sensitive men.

bution of fatty acids from splanchnic sources when expressed as a percentage ($r = -0.51$, $P < 0.01$) (Table 2; Fig. 5C) or as a concentration ($r = -0.47$; $P < 0.05$) (Table 2). The postprandial whole-body Ra_{NEFA} was significantly correlated with the contribution of endogenous systemic NEFAs when expressed as a percentage ($r = 0.56$, $P < 0.01$) but negatively correlated with the percentage contribution from splanchnic sources ($r = -0.50$, $P < 0.01$) (Table 2).

DISCUSSION

Insulin-resistant men had significantly higher postprandial lipemia than insulin-sensitive men, which was attributable to higher VLDL TG, but not chylomicron TG ($S_f > 400$), concentrations. The use of stable isotope techniques combined with immunoaffinity chromatography allowed us to make a novel calculation of chylomicron remnant TG concentrations, which we also found not to be different between groups. This indicates that VLDL TGs is the main

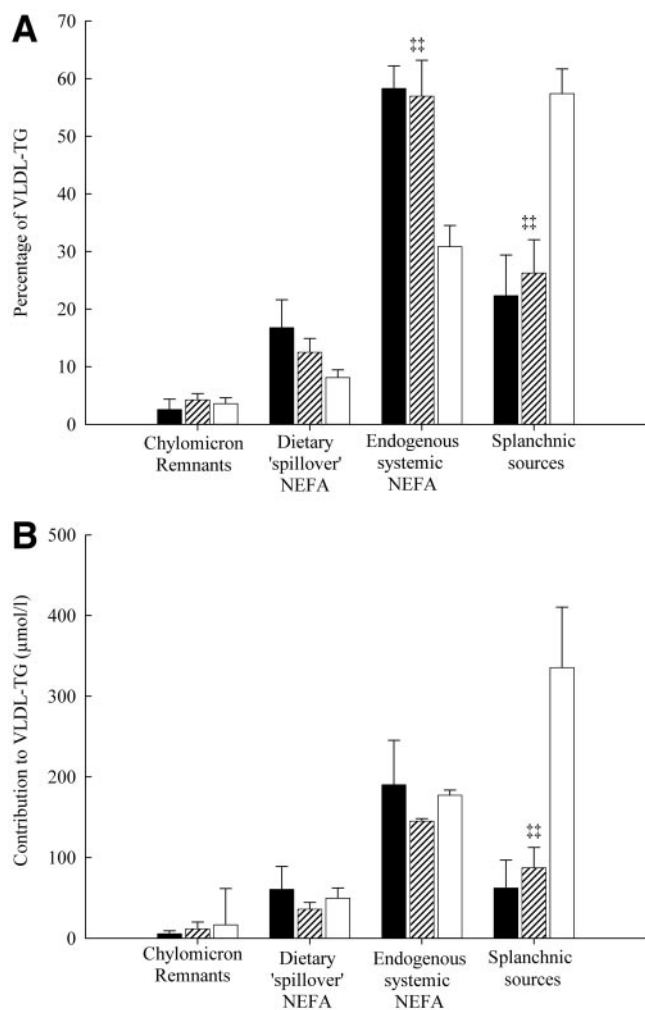


FIG. 4. The relative (A) and absolute (B) contributions of different sources of fatty acids to VLDL TGs in female subjects (■), insulin-sensitive men (▨), and insulin-resistant men (□). ‡‡P < 0.01 insulin-sensitive men vs. insulin-resistant men.

determinant of the difference in postprandial lipemia between insulin-sensitive and insulin-resistant men. Furthermore, our results show that splanchnic fatty acids provide the largest source of substrate for VLDL TG production in insulin-resistant men. Notably, these differences were found despite any significant difference in waist circumference between the male groups. The contribution from splanchnic sources was >3.5 times greater in insulin-resistant compared with insulin-sensitive men. We did not distinguish the different sources of splanchnic fat, and these would include fatty acids from visceral fat lipolysis, DNL, or the liver cytosolic TG pool. The contribution of dietary fatty acids to VLDL TGs was not different between insulin-resistant and insulin-sensitive groups, which is supported by our finding of no difference in chylomicron remnant TG concentrations. Endogenous systemic NEFAs provided a similar contribution in insulin-resistant and insulin-sensitive groups. Furthermore, the difference in blood 3-OHB concentrations suggests that insulin-resistant men have lower hepatic fatty acid β-oxidation postprandially compared with insulin-sensitive men. To our knowledge, we are the first to report on the contribution of different fatty acid sources to VLDL TGs after a standard mixed meal. Furthermore, we have demonstrated that there are no differences in the contributions of different fatty acid sources for VLDL TG production between insulin-sensitive women and men.

The relative contribution of dietary spillover NEFAs to VLDL TGs in the present study is comparable with that previously reported (13). The relative contribution from dietary remnants to VLDL TGs was similar (4%) across the groups in the present study. This is in agreement with the findings of no difference between the groups for chylomicron remnant TGs. The lower contribution than previously reported (15%) (13) could be due to the more specific measure of VLDL TGs in the present study, the difference in feeding protocol, or the length of the postprandial period. Furthermore, when calculating the contribution of dietary fatty acid sources we used the highest isotopic enrichment in the $S_f > 400$ fraction rather than the isotopic

TABLE 2

Correlations of the contributions from different sources of fatty acids with plasma metabolites at the end of the postprandial period (360 min)

	Postprandial plasma insulin (mU/l)	Postprandial VLDL TGs (μmol/l)	Postprandial plasma NEFAs (μmol/l)	Postprandial 3-OHB (μmol/l)	Postprandial whole-body Ra_{NEFA} (μmol/min)
Postprandial VLDL TGs (μmol/l)	0.51†				
Postprandial plasma NEFAs (μmol/l)	-0.02	-0.08			
Postprandial 3-OHB (μmol/l)	-0.07	-0.17	0.57†		
Postprandial whole-body Ra_{NEFA} (μmol/min)	0.02	-0.02	0.26	0.38*	
Contributions from different fatty acid sources as a percentage					
Endogenous systemic NEFAs	-0.50†	-0.45*	0.44*	0.50†	0.56†
Dietary spillover NEFAs	-0.34	0.06	0.04	0.43*	0.08
Dietary remnants	-0.01	-0.30	-0.30	-0.56†	-0.23
Splanchnic sources	0.52†	0.48†	-0.43†	-0.51†	-0.50†
Contributions from different fatty acid sources as a concentration (μmol/l)					
Endogenous systemic NEFAa	0.16	0.75‡	0.24	0.08	0.22
Dietary spillover NEFAa	0.13	0.72‡	-0.02	0.16	-0.02
Dietary remnants	0.12	0.08	-0.50†	-0.69‡	-0.38*
Splanchnic sources	0.46*	0.77‡	-0.37	-0.47*	-0.37

*P < 0.05; †P < 0.01; ‡P < 0.001.

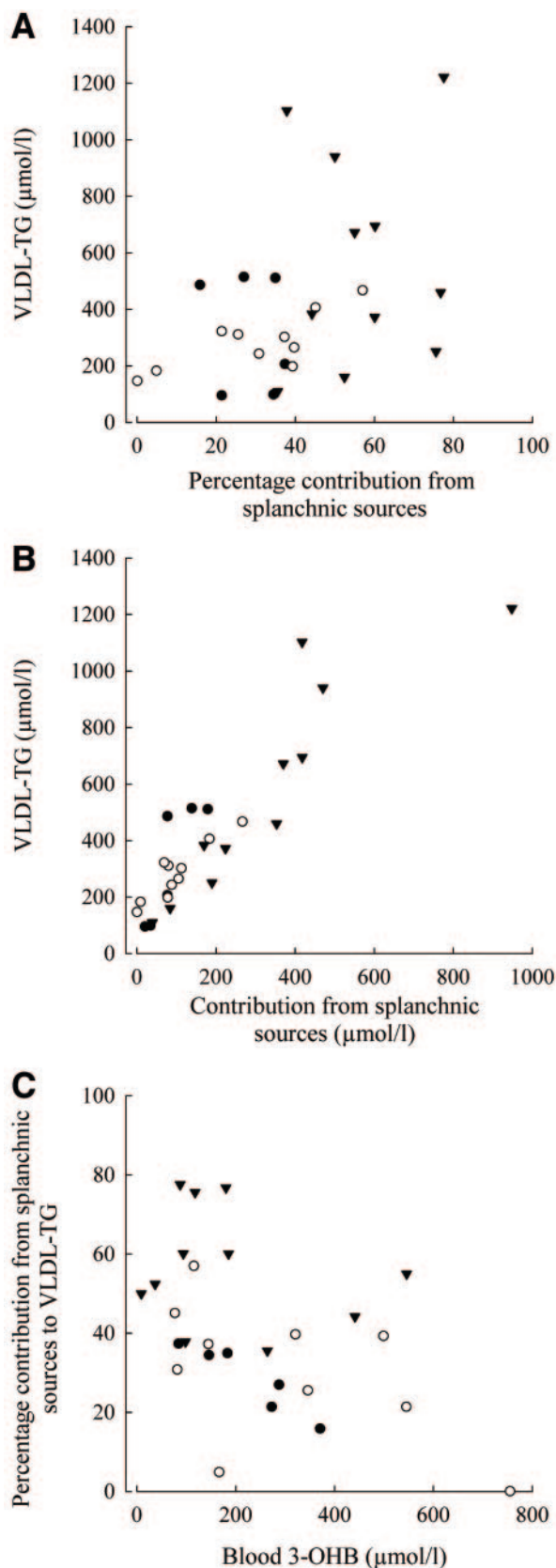


FIG. 5. The relationship between the contribution of fatty acids from splanchnic sources as a percentage and concentration and VLDL TGs (A and B) and blood 3-OHB concentrations (C) for female (●), insulin-sensitive men (○), and insulin-resistant men (▼). A: The correlation for female subjects was $r = 0.09$; insulin-sensitive men, $r = 0.78$, $P < 0.01$; insulin-resistant men 0.26 . B: The correlation for female subjects was $r = 0.60$; insulin-sensitive men, $r = 0.80$, $P < 0.01$; and

enrichment of the test meal because this allows for individual variability in the metabolic handling of the test meal in the intestine.

In the present study, fasting and postprandial systemic plasma NEFA concentrations and Ra_{NEFA} were similar between the insulin-resistant and insulin-sensitive groups, indicating no difference in the suppression of insulin-induced lipolysis of subcutaneous adipose tissue. We determined the contribution of endogenous systemic NEFAs to VLDL TGs by labeling the endogenous NEFA pool; this would contain fatty acids that are predominantly derived from subcutaneous adipose tissue lipolysis. At 360 min, we noted equilibrium in VLDL TG [$^2\text{H}_2$]palmitate concentration, indicating that the rate of endogenous systemic NEFA incorporation into VLDL TGs had become fairly constant. The relative contribution of endogenous systemic NEFAs to VLDL TGs was lower in the insulin-resistant than the insulin-sensitive subjects. The relative postprandial contributions for the insulin-sensitive men (58%) are slightly higher than previously reported for healthy men (44%) (13), with the contribution for insulin-resistant men (31%) being comparable with that of subjects with nonalcoholic fatty liver disease (28%) (15). When expressed as a concentration, the contribution from endogenous systemic NEFAs was similar in the groups.

A lower isotopic enrichment of fatty acids in VLDL TGs than in the fatty acids entering the liver (endogenous systemic NEFAs) implies a dilution from other fatty acid sources. The degree of dilution was greater in insulin-resistant subjects, who had significantly greater VLDL TG concentrations than the insulin-sensitive men. Increasing amounts of visceral fat adds to the fatty acid supply being released into the portal vein (32). It is therefore possible that there was a higher contribution of fatty acids from visceral lipolysis to the hepatic VLDL TG production in the insulin-resistant men. Meek et al. (33) elegantly demonstrated that moderate hyperinsulinemia appeared to increase the proportions of fatty acids reaching the liver from visceral fat compared with systemic sources. Thus, it is plausible that insulin-resistant men, despite not having a significant difference in waist circumference (implying no difference in visceral fat) when compared with insulin-sensitive men, had a higher flux of visceral fatty acids to the liver. If this were the explanation, visceral fat stores must, of course, also be replenished, for instance by uptake of fatty acids from circulating TG-rich particles. Our studies do not clarify that process.

Additionally, the higher contribution of splanchnic fatty acids could be derived from hepatic DNL or from the stored cytosolic TG pool. Exposure of hepatic lipogenic enzymes to insulin has been shown to stimulate activity by increasing the transcription of the genes for fatty acid synthase and acetyl-coenzyme A carboxylase (34). In the postprandial state, the contribution of DNL fatty acids has been reported to be between 8 and 13% to S_r 60–400 TGs in healthy individuals (13,15,17,35), and up to 26% of DNL fatty acids to TRLs has been reported for individuals with nonalcoholic fatty liver disease (15) and hypertriglyceridemia (17). In the present study, DNL might therefore explain part of the higher contribution of splanchnic sources to VLDL TGs in insulin-resistant compared with insulin-sensitive subjects.

insulin-resistant men, $r = 0.95$, $P < 0.001$. C: The correlation for female subjects was $r = -0.89$, $P < 0.05$; insulin-sensitive men, $r = -0.65$, $P < 0.05$; and insulin-resistant men, $r = -0.20$.

It has been proposed that the liver stores TGs to accommodate fatty acids that have accumulated in excess of requirement for oxidation and/or secretion as VLDL (36,37). In fact, after a 24-h fast, there is a fourfold increase in the neutral fat content of the mouse liver (38), although this is yet to be demonstrated in humans. Increased lipid accumulation in the liver has been associated with insulin resistance (20), and with increasing concentrations of liver fat there is increased secretion of TG-rich VLDL (9). The insulin-resistant subjects in the present study had a significantly higher VLDL TG concentration compared with the insulin-sensitive subjects. Additionally, they had higher plasma ALT concentrations, suggesting higher liver fat (39) compared with the insulin-sensitive men.

The degree of β -oxidation of hepatic fatty acids would influence partitioning to VLDL TG synthesis. Evidence for differences in postprandial hepatic fatty acid β -oxidation in humans is sparse. We report here a significant difference over time in the postprandial response of blood 3-OHB between the insulin-resistant and insulin-sensitive individuals, the latter showing a more dynamic response, suggesting greater postprandial hepatic fatty acid β -oxidation. In contrast to the work of Halkes et al. (40), we did not note a difference in postprandial blood 3-OHB between women and insulin-sensitive men. One explanation for the lower rates of hepatic fatty acid β -oxidation in the insulin-resistant men could be a higher rate of DNL due to hyperinsulinemia. Elevation of malonyl-CoA would shift cellular metabolism away from fatty acid oxidation toward esterification (34).

Typically, women are reported to have lower fasting VLDL TG concentrations than men (41,42). Recently, it was suggested that women secrete fewer, but more TG-rich, VLDL particles than men and that they have increased plasma VLDL clearance (21). We report that the postprandial contributions of different fatty acid sources to VLDL TGs were the same in women and insulin-sensitive men. This is in agreement with Nielsen et al. (32), who reported that splanchnic release of fatty acids does not differ between lean men and women.

Our data illustrate the significance of VLDL TGs to postprandial lipemia and demonstrate the ability of the liver to utilize different fatty acid sources for VLDL TG production during this period. Our results show that splanchnic fatty acids provide >3.5 times the substrate for VLDL TG production in insulin-resistant men compared with insulin-sensitive men. The finding that a lower blood 3-OHB concentration was associated with a greater contribution from splanchnic sources suggests altered hepatic partitioning of fatty acids away from oxidation in insulin resistance. The contribution of systemic NEFAs was not greater in insulin-resistant men, reflecting no apparent dysregulation of subcutaneous adipose fatty acid release (43). At the end of one postprandial period, the relative contribution from dietary remnants was surprisingly low. However, this is in line with our findings of a low concentration of chylomicron remnant TG in all groups. It is likely that the contribution may increase with time, and to extend the postprandial phase longer than the 360 min reported here would be interesting. As most individuals consume multiple meals in a day, it would be of greater interest to investigate hepatic partitioning of fatty acids after the consumption of sequential meals. We did not directly assess VLDL production and clearance. However, it has been reported that hypertriglyceridemia is largely attributable to overproduction and secretion of VLDL by

the liver rather than impaired VLDL removal (34). Therefore, our data suggest that the higher concentration of VLDL TGs in the insulin-resistant men was due to differences in splanchnic fatty acid metabolism.

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