Original Article

Increased Dietary Substrate Delivery Alters Hepatic Fatty Acid Recycling in Healthy Men

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Sources of fatty acids flowing to the liver may be used for triacylglycerol (TAG) synthesis. Our objective was to quantify contributions of nonesterified fatty acids (NEFAs), de novo lipogenesis, and dietary fatty acids to VLDL-TAG in the fed state after meal feeding in healthy subjects ($n = 6$). The effect of substrate delivery rate was also determined by comparison with data obtained under a continuous-feeding regimen. A liquid diet was administered by mouth or via feeding tube. Contributions of NEFAs, de novo lipogenesis, and dietary fatty acids to VLDL-TAG were quantified using stable isotopes and gas chromatography–mass spectrometry. Contribution of NEFAs to VLDL-TAG was similar under meal feeding and continuous feeding, although insulin area under the curve (AUC) was greater under meal feeding (1,597 ± 455 vs. 471 ± 484 pmol · h · l^{-1}, $P < 0.004$). Lipogenesis achieved a higher AUC with meal feeding versus continuous feeding (88.7 ± 84.4 vs. 1.9 ± 19.3 μmol · h · l^{-1}, $P = 0.03$) supporting greater stimulation of de novo lipogenesis from increased glucose delivery rate. The contribution of dietary fatty acids to VLDL-TAG was also greater with meal feeding. These data demonstrate for the first time in humans the well-coordinated use of fatty acids by the liver during the transition from fasted to fed states and highlight the dominant role of NEFAs for VLDL-TAG synthesis in both states. *Diabetes* 54:2694–2701, 2005

Throughout the day, the human liver responds to changes in metabolism associated with fasting and feeding. The liver plays a primary role as a regulator of glucose metabolism, and at the onset of eating, the effect of increased blood glucose is immediately sensed by the organ, which orchestrates storage and use of this metabolite (1). By contrast, within the body's systems of control for fatty acid metabolism, the liver responds to and manages fatty acids that originated from the diet, from adipose stores, and from its own de novo production of fatty acids. The plasma NEFA pool contributes the majority of fatty acids that flow to the liver in the fasted state and thus provides the bulk of fatty acids secreted by the liver in VLDL (2). By contrast, an understanding of fatty acid regulation in the fed state should take into account the many sources of fatty acids that flow to the liver after food consumption. These sources can include nonesterified fatty acids (NEFAs), de novo lipogenesis, and dietary fatty acids. In healthy individuals, consumption of a meal induces an increase in plasma insulin concentration and subsequent suppression of adipose hormone-sensitive lipase, leading to a reduction in plasma NEFA concentration. Furthermore, a meal that elicits a higher insulin response is associated with a greater suppression of NEFAs (3,4). A second source of fatty acids that enter liver-triacylglycerol (TAG) pools, and may contribute to VLDL-TAG, is the pathway of de novo lipogenesis. Early research demonstrated that lipogenesis increased in the fed state, but the absolute contribution of this source to VLDL-TAG appeared to be minimal (5). More recent studies have shown that fed-state lipogenesis is elevated after the chronic ingestion of high-carbohydrate diets (6–8). These studies have expanded our knowledge of the rates of lipogenesis in the postprandial state, but only one study measured lipogenesis concurrently with other fatty acid sources (2). Moreover, recent reports have demonstrated an increase in lipogenesis after a single bolus of food (9) and higher lipogenic rates in insulin resistance (10). A final potential source of fatty acids flowing to the liver postprandially comes from dietary fatty acid influx, which is mediated by chylomicrons produced by the intestine. Animal studies demonstrated that dietary fatty acids were cleared to the liver in vivo (11,12) and that chronic consumption of higher-fat diets results in elevations in liver fat (13,14). However, this pathway has not been measured directly in humans and may provide a significant route of fatty acids used for hepatic TAG synthesis (15). For all fatty acid sources, the meal characteristic that would most likely impact liver fatty acid partitioning is the rate of substrate delivery. The greater the influx of carbohydrates, the greater the response of insulin, which could impact NEFA flux and lipogenesis. The rate of influx of dietary lipid could also potentially influence dietary fatty acid storage in adipose and liver. Thus, although the plasma NEFA pool can provide the majority of fatty acids for VLDL-TAG synthesis in the fasting state, changes in fed-state metabolism may result in the replacement of adipose NEFAs with dietary and

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AUC, area under the curve; NEFA, nonesterified fatty acid; TAG, triacylglycerol.

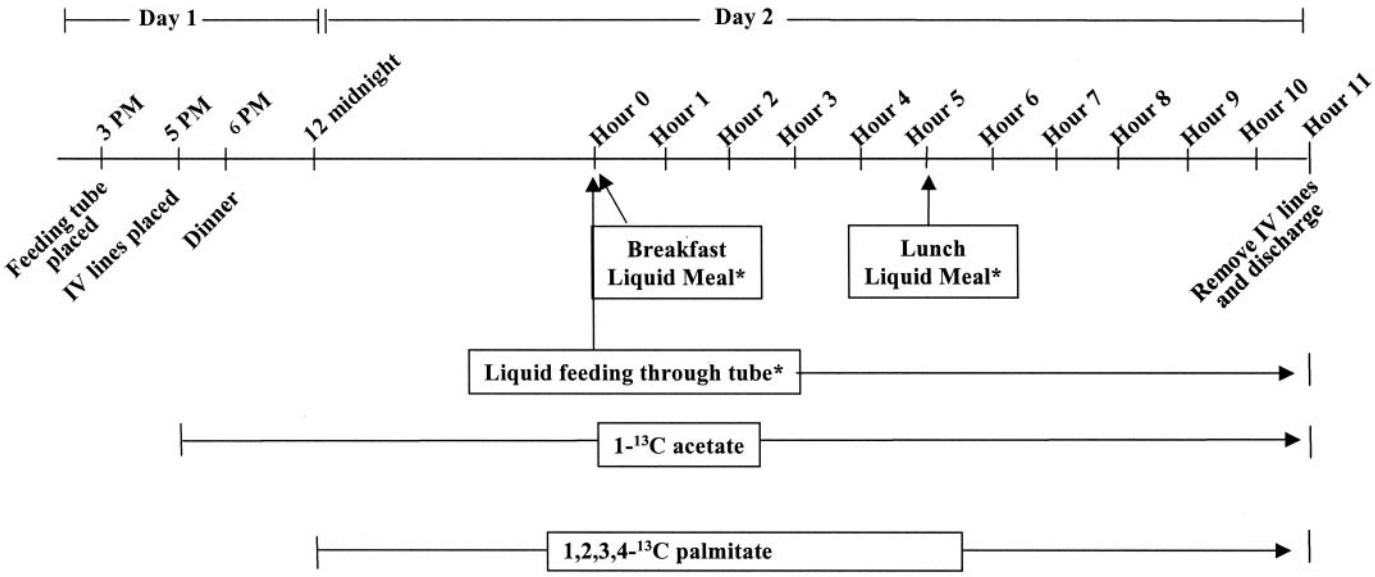

FIG. 1. Timeline for the meal-feeding and continuous-feeding protocols. *The liquid meal contained the stable isotope glyceryl tri(hexadecanoate-d_{31}) and was served at breakfast and lunch for the meal-feeding regimen and infused duodenally for the continuous-feeding regimen.

newly made fatty acids. We believe that disease risk stems from the dysregulation of metabolic substrate flux that occurs between fasting and intermittent food intake. A better understanding of changes that occur in fatty acid flux with feeding will provide the basis for the study of how changes in liver-fatty acid and TAG flux contribute to insulin resistance. Accordingly, the goal of the present study was to quantify the contributions of NEFAs, lipogenesis, and dietary fatty acids to VLDL-TAG in the fed state after two successive liquid meals fed to healthy subjects. We also sought to test the effect of the rate of substrate delivery by comparing these data with those obtained under a slow, continuous-feeding regimen. Our hypotheses were that: *1*) compared with a continuous-feeding regimen, a meal-feeding regimen would result in a greater percentage of VLDL-TAG derived from lipogenesis; and *2*) bolus consumption of food (through meal-feeding) would be associated with a greater reduction in the amount of plasma NEFAs flowing to the liver and subsequently used for VLDL-TAG synthesis.

RESEARCH DESIGN AND METHODS

Six men were recruited by advertisement and gave written informed consent after all procedures and potential risks were explained. The protocols were approved by the University of Minnesota Human Subjects Committee (UMN IRB 9908M15861 and 0106M01641). Recruitment criteria were nonsmoking status, age of 20–55 years, stable body weight, and maintenance of normal exercise and activity patterns. Subjects were excluded if they had diabetes or any other metabolic disease or were taking medication known to affect lipid metabolism. Screening blood draws were performed after subjects fasted for at least 12 h and abstained from alcohol for at least 48 h. Body composition was determined by dual-energy X-ray absorptiometry (Lunar, Madison, WI). The present research was conducted over a 2-year period in which each subject participated in two metabolic studies. On one occasion, a subject was fed an enteral formula through a feeding tube placed in the duodenum. Hereafter, this protocol will be referred to as the continuous-feeding regimen. On a second occasion, the subject consumed the same amount of liquid formula by mouth: one-half at breakfast and one-half 5 h later for lunch. This protocol will be referred to as the meal-feeding regimen. Subject activities for the 3 days before both studies were standardized for diet, exercise, and alcohol consumption. During this time, the subject was placed on a weight-maintaining diet of constant energy (55% of energy from carbohydrate, 30% from fat, and 15% from protein) based on the Harris-Benedict equation (16) with comparison to 3-day food records of usual intake. Following direction from a registered dietitian, diets were prepared by the subjects themselves and consisted of whole foods. Alcohol consumption was prohibited during this time. The metabolic study protocol is shown in Fig. 1. On day 1 of the metabolic study, the subject reported to the General Clinical Research Center between 1100 and 1200 for the continuous-feeding regimen and between 1630 and 1700 for the meal-feeding regimen. For the continuous-feeding protocol, between 1430 and 1600 on day 1, a size 8 French feeding tube was placed in the duodenum via the nasal cavity. Fluoroscopy was used to confirm accurate placement of the tube, 15 cm distal to the pyloric sphincter. The feeding tube was put in the day before the study to increase the amount of time between the short-term stress that occurred during placement and the time of data collection. We also wanted to allow the subject some time to get accustomed to the feeding tube. For both studies, at 1730, an intravenous line was placed in the antecubital vein of each arm: one for the administration of stable isotope and the other for drawing blood. Between 1730 and 1800, the subject consumed a solid-food dinner by mouth that met 40% of his total daily energy needs. The subject slept overnight at the General Clinical Research Center and remained fasted until feeding was initiated (denoted time 0 h on the figures). At this time, the tube feeding was started in the continuous-feeding regimen and the first meal was served in the meal-feeding regimen followed by meal two at noon. The liquid formulas fed for both the continuous-feeding and meal-feeding regimens were of the same composition, were designed to mirror the same macronutrient composition as the 3-day prestudy diet, and were prepared as follows: an enteral nutritional supplement (Mead Johnson & Company, Evansville, IN), pasteurized egg yolk, heavy whipping cream, vegetable oil, and the dietary stable isotope glyceryl tri(hexadecanoate-d_{31}) were combined immediately after heating and mixed thoroughly for at least 1 min. To achieve homogeneity, the liquid formula was processed twice via a microfluidizer (110Y; Microfluidics, Newton, MA). The total energy provided by the formula was 7,260 ± 1,346 kJ, with a macronutrient profile of 54 ± 1% of energy from carbohydrate, 32 ± 1% from fat, and 14 ± 1% from protein. The amount of dietary label incorporated into the formula was dependent on the amount of energy needed by the subject. On average, 1.59 g of label was added to the formula, which resulted in a final TAG-palmitate enrichment of ~20%. The carbohydrate source of the formula was maltodextrins, and of the total percentage of energy coming from carbohydrate (54%), 31% was derived from mono- and disaccharides and 23% from polysaccharides. The most prominent fatty acids provided by the formula were oleic acid (45.7%) and linoleic acid (23.3%), with palmitic acid contributing 18.7% by weight. The liquid formula consumed on day 2 met two-thirds of the total daily energy requirement for the subject. Subjects rested, watched TV, or read during the infusion study. Non–energy-containing, noncaffeinated drinks were available upon request.

Isotopes and samples analyses. Sodium acetate-1-^{13}C, potassium hexadecanoate-1,2,3,4-$^{13}C_4$, and glyceryl tri(hexadecanoate-d_{31}) were purchased from Cambridge Isotope Laboratories (Andover, MA) and from Isotec (Miamisburg,

OH). Isotopic purity was >98% for all isotopes, and intravenous isotopes were administered as previously described (2). For all subjects, sodium acetate-1-^{13}C was given at a dose of 10 g over a 24-h period starting with dinner the night before the study (Fig. 1). Potassium hexadecanoate-1,2,3,4-$^{13}C_4$ was administered at a dose of 7 $\mu g \cdot kg^{-1} \cdot min^{-1}$ complexed to albumin in a ratio of 2:1. Blood samples were taken starting at the onset of feeding and continued throughout the 11-h feeding period. Serum was separated immediately by centrifugation at 1,500*g* for 20 min at 4°C and kept on ice as a preservative cocktail was added (17). Concentrations of glucose in serum samples were measured via a Vitros Analyzer 950 (Ortho-Clinical Diagnostics, Rochester, NY) and insulin via chemiluminescent immunoassay (Diagnostic Products, Los Angeles, CA). Serum samples for NEFAs were extracted immediately, and within 24 h of each infusion study, serum lipoproteins were isolated (2). For lipids extracted from lipoprotein particles, trinonadecanoate (19:0 TAG) internal standard was added to the sample; for serum NEFAs, 15:0 fatty acid internal standard was added. Concentrations were measured both by gas chromatography and by enzymatic means using assays for serum and lipoprotein-TAG (kit 336; Sigma Diagnostics, St. Louis, MO) and for serum NEFAs (994-75409W; Wako Chemicals USA, Richmond, VA). Stable isotope enrichments were measured as previously described (2). Newly made fatty acids from the de novo lipogenesis pathway were calculated using Mass Isotopomer Distribution Analysis (18).

Calculations and statistical analysis. The infusate composition and enrichments were analyzed by gas chromatography and gas chromatography–mass spectrometry, and the calculations were adjusted for the amount of unlabeled fatty acids present in the NEFA pool that were derived from <100% purity of the isotope and from fatty acids present on the albumin used in the infusion. The rate of appearance of fatty acids from adipose-TAG lipolysis (R_a NEFA) discussed in the present paper takes the inputs of these fatty acids into account, along with the amount of unlabeled (cold) dietary fatty acids that spilled over into the plasma NEFA pool. Blood sampling was performed via a peripheral catheter placed in an antecubital vein, which would underestimate NEFA tracer enrichments in arteries because of release of fatty acids from local stores (19). It is acknowledged that this effect could result in a systematic underestimation (by as much as ~27%) of the NEFA enrichment used to calculate the percentage of VLDL-TAG derived from NEFA sources (19). By contrast, peripheral venous sampling could also result in an overestimation of NEFA enrichment if the fatty acid tracer was diluted by visceral fatty acid release before entry into liver. Given that the subjects were all relatively lean (e.g., mean ± SD of body fat percentage 18.2 ± 5.2, range 10.3–24.2%), this effect is expected to result in an overestimation of NEFA enrichment of only 5–10% (20). The calculation of R_a NEFA from adipose was as follows:

Equation 1. % NEFAs from adipose-TAG lipolysis:

$$\left(1 - \frac{\text{fraction of } d_{31}\text{hexadecanoate in NEFA}}{\text{fraction of } d_{31}\text{hexadecanoate in liquid formula TAG}}\right) \times 100$$

Given equation 1, the percentage of VLDL-TAG palmitate derived only from adipose tissue release was calculated by incorporating equations from Steele and colleagues (21,22) for non–steady-state kinetics, to give equation 2.

Equation 2. % VLDL-TAG from adipose NEFAs:

$$\frac{\%^{13}C_4\text{hexadecanoate in VLDL-TAG}}{\%^{13}C_4\text{hexadecanoate in NEFA}} \times \%\text{NEFA from adipose-TAG lipolysis}$$

In the present article, the amount of VLDL-TAG derived from dietary fatty acids reflects all combined inputs of dietary label including spillover of dietary fatty acids into the serum NEFA pool and any additional entry of dietary fatty acids through either chylomicron remnant uptake by the liver or dietary fatty acids liberated at the liver, which may have been immediately taken up into the hepatocyte (these latter two sources cannot be differentiated by the present methodology). In other words, for the present analysis, these different sources of dietary fatty acids used for VLDL-TAG synthesis are not delineated but treated as a single source, which allows for a clearer comparison of dietary input with adipose and de novo lipogenesis. A comparison of the entry of the different dietary-TAG fatty acid sources (e.g., spillover and remnant uptake) is presented in the companion article by Barrows et al. (23) in this issue of *Diabetes*, along with the differences in these dietary sources between the continuous-feeding and meal-feeding regimens.

By tracking the variously labeled methyl-palmitate isotopomers in VLDL-TAG (M_0, M_1, M_2, M_4, M_{30}, and M_{31}) using gas chromatography–mass spectrometry, information regarding the sources of fatty acids used for liver-TAG synthesis was obtained. These data are presented as the proportion of fatty acids derived from the three sources (i.e., data presented as percentage of fatty acids in VLDL-TAG from NEFAs, lipogenesis, and diet). As will be demonstrated, data considered in this way provide a perspective that is focused on the physiology of intrahepatic fatty acid flux of the various sources into VLDL-TAG. These numbers were then multiplied by the absolute pool size of VLDL-TAG 16:0 (for each subject at each time point) to generate the absolute contribution of the various sources to VLDL-TAG in units of millimoles per liter. The absolute concentrations were used to calculate the area under the curve (AUC) for the two feeding regimens. Calculations were performed using Microsoft Excel (version 2000; Microsoft, Seattle, WA) and statistical analyses using Statview for Windows (version 5.0.1; SAS Institute, Berkeley, CA). Differences between feeding regimens were analyzed using paired Student's *t* test. Correlations were analyzed using simple regression, and effects of time were analyzed using multiple regression. A P value of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics (mean ± SD) of subjects were: age, 35 ± 9 years (range 21–50); BMI, 25.2 ± 3.0 kg/m^2 (20.9–28.6). Fasting serum concentrations were: insulin, 39 ± 25 pmol/l (18–84); glucose, 4.7 ± 0.2 mmol/l (4.4–5.0); and TAG, 1.21 ± 0.72 mmol/l (0.58–2.59). Baseline concentrations of glucose and insulin were not different between the two feeding regimens, and Fig. 2*A* demonstrates the changes in glucose and insulin concentrations that occurred with feeding. At the end of the studies (11 h), values were significantly lower in the meal-feeding regimen for glucose (4.8 ± 0.6 vs. 5.7 ± 0.4 mmol/l, for meal-feeding and continuous-feeding, respectively, $P = 0.01$) and insulin (39 ± 26 vs. 95 ± 42 pmol/l, $P = 0.043$). When baseline concentrations were compared with end point concentrations within each feeding regimen, no significant difference was identified for glucose or insulin. The AUC for glucose was not significantly different between the feeding regimens, although the AUC for insulin was significantly greater under the meal-feeding regimen (1,597 ± 455 vs. 471 ± 484 pmol · h · l^{-1}, $P < 0.004$). Changes in VLDL-TAG concentration are depicted in Fig. 2*B*. Concentrations of VLDL-TAG at baseline (0 h) were not different between regimens; the same was true for the 11-h time point. Because VLDL-TAG concentration increased after consumption of meals and returned toward baseline 3–4 h later, the AUC was greater in the meal-feeding regimen (0.93 ± 1.24 vs. −0.06 ± 0.65 mmol · h · l^{-1}, $P < 0.050$). Changes in NEFA concentration are shown in Fig. 2*C*. The average NEFA AUCs were similar between feeding regimens, and no significant differences in NEFA concentration from baseline to end point within each feeding regimen were found. Qualitatively, an increase in NEFA concentration was noted at the end of the study (9–11 h) in the meal-feeding regimen; a higher peak and lower final value of insulin may have contributed to this rebound in NEFAs. Lastly, the AUC for R_a NEFA (Fig. 2*D*) indicated similar suppression of adipose NEFAs under both feeding regimens (see also Table 1), but a significant reduction in R_a NEFA from baseline to end point was observed within the meal-feeding regimen ($P = 0.013$).

Sources contributing to VLDL-TAG. With regard to the contribution of the serum NEFA pool to VLDL-TAG, between baseline and 1 h, a significant reduction in the percentage contribution of NEFAs was observed, a pattern that was similar between the two feeding regimens (Fig. 3*A*, $P < 0.004$ for both). Figure 3*B* demonstrates the variability in the absolute contribution of the serum NEFA pool to VLDL-TAG; no significant difference was found between feeding regimens for the AUC (Table 1). Comparison of baseline and end point concentrations of VLDL-TAG from plasma NEFAs between regimens were also not

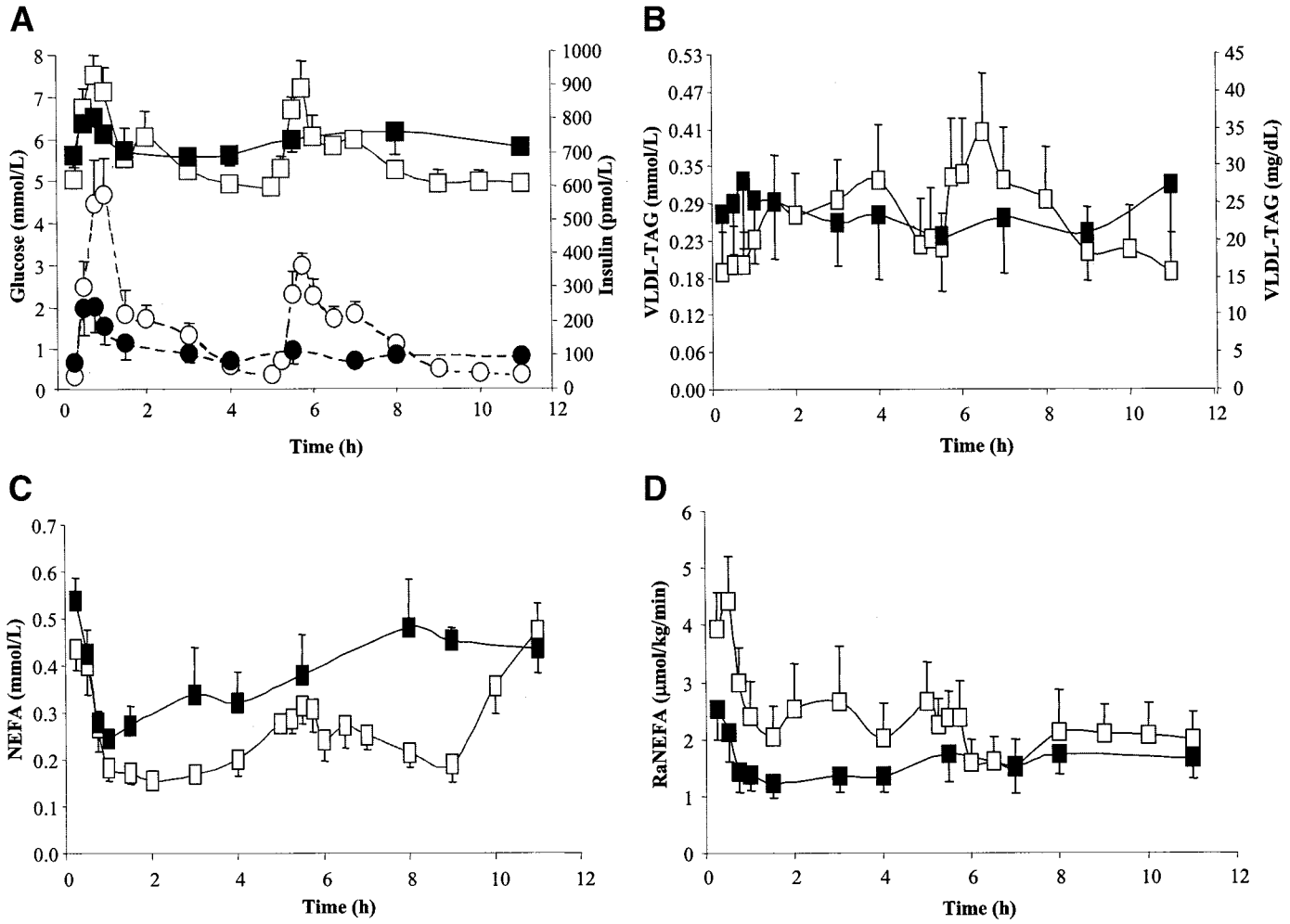

FIG. 2. Concentrations of insulin, glucose, VLDL-TAG, NEFAs, and the R_a NEFAs under two feeding regimens. *A*: Concentrations of glucose (squares) and insulin (circles); filled symbols, continuous feeding; unfilled symbols, meal feeding. *B–D*: Concentrations of VLDL-TAG (*B*), NEFAs (*C*), and R_a NEFAs (*C*); ■, continuous-feeding; □, meal feeding. Values are mean ± SE, $n = 6$.

significant. For lipogenesis, the percentage of VLDL-TAG derived from newly made fatty acids supported a physiologic stimulation of fatty acid synthesis after meals, an effect more pronounced with meal feeding (Fig. 3*C*). During the final 3 h of the studies (9–11 h), lipogenesis was steady under the continuous-feeding regimen but falling after meal feeding. When VLDL-TAG pool size was taken into account, a significantly greater amount of de novo fatty acids were found under the meal-feeding regimen (Fig. 3*D* and Table 1, $P = 0.033$). Lastly, both feeding regimens exhibited significant increases in the percentage of VLDL-TAG derived from dietary TAG from hours 1 to 5 ($P < 0.004$, slopes significantly different from zero, Fig. 3*E*) and 7 to 11 ($P < 0.0001$). When these increases were compared between regimens, the dietary contribution to VLDL-TAG under the meal-feeding regimen was significantly greater than under the continuous-feeding regimen from 1 to 5 h ($P = 0.028$) and 7 to 11 h ($P = 0.020$), indicating both faster and greater incorporation of dietary fatty acids into VLDL-TAG. A peak value of 21.2 ± 9.0% was observed in the continuous-feeding regimen at the end of the study (11 h), whereas a peak of 33.4 ± 7.7% occurred on the meal-feeding regimen 5 h earlier. When the values as a percentage were averaged across all time points, the percentage of TAG derived from diet under the continuous-feeding regimen (9.1 ± 3.2%) was significantly lower

TABLE 1
Change in R_a NEFA and sources of fatty acids contributing to VLDL-TAG

	Continuous-feeding AUC	Meal-feeding AUC	*P* value
R_a NEFA (μmol/kg total body wt)	−794.2 ± 318.9	−1,045.8 ± 118.2	0.24
Sources of fatty acids in VLDL-TAG			
NEFA (μmol · h · l^{-1})	−98.8 ± 106.4	−63.3 ± 74.1	0.37
Lipogenesis (μmol · h · l^{-1})	1.9 ± 7.9	88.7 ± 34.4	0.03
Diet (μmol · h · l^{-1})	64.9 ± 24.4	137.0 ± 41.6	0.05

Data are means ± SE.

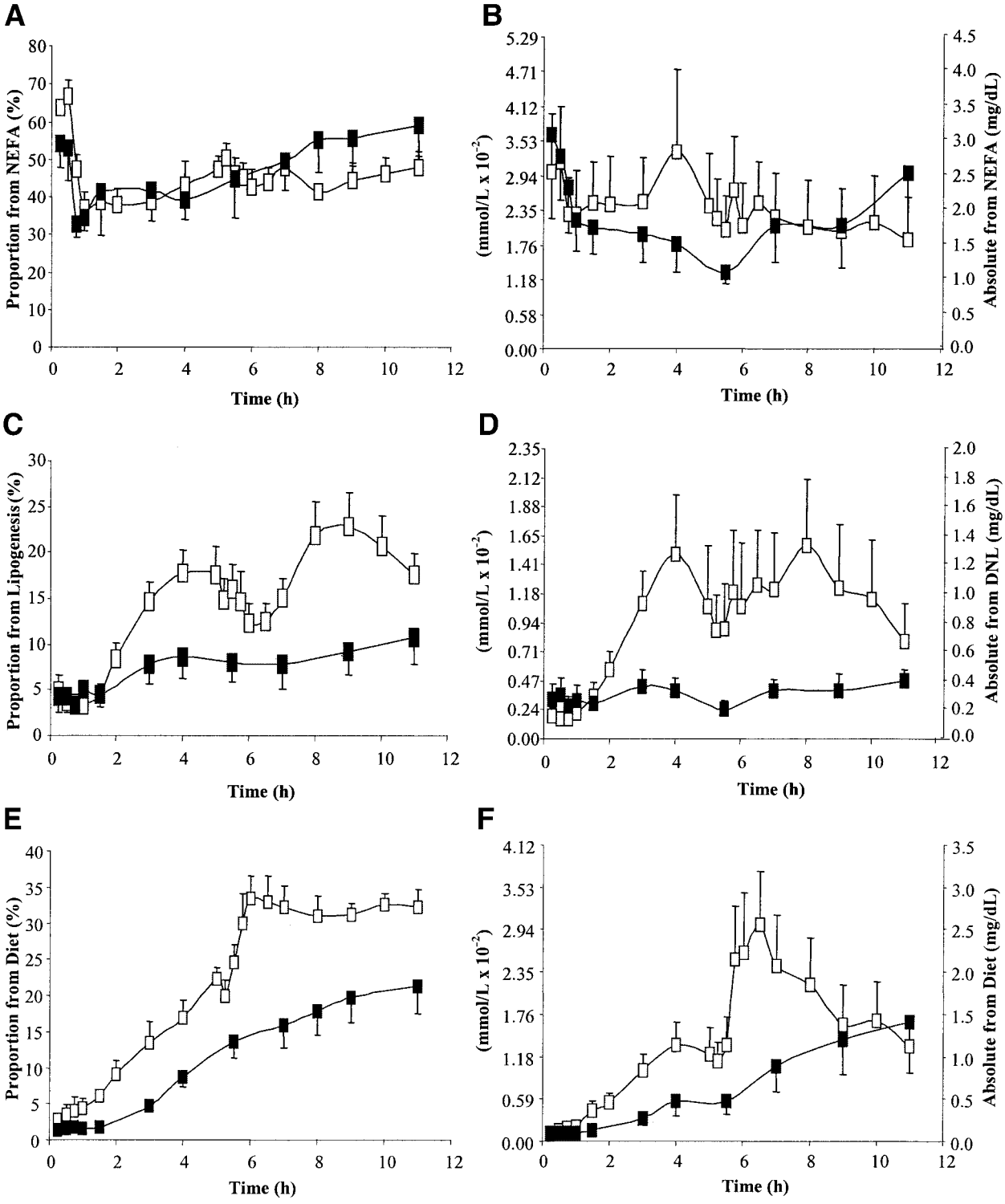

FIG. 3. Percentage and absolute contributions of VLDL-TAG fatty acid sources. Percentage contributions from NEFAs (*A*), de novo lipogenesis (*C*), and dietary fatty acids (*E*). Absolute contribution of sources to VLDL-TAG from NEFAs (*B*), de novo lipogenesis (*D*), and diet (*F*). ■, continuous-feeding; □, meal feeding. The absolute concentrations are presented as units of mmol/l (left axis) and mg/dl (right axis). Values are mean ± SE, $n = 6$.

than during the meal-feeding regimen (20.2 ± 4.0%, $P = 0.003$). Under both regimens, the absolute presence of dietary fatty acids in VLDL-TAG (Fig. 3*F*) could be explained by a progressive increase in the percentage of TAG derived from diet as opposed to change in the VLDL-TAG pool size (Fig. 2*B*). The absolute amount of VLDL-TAG derived from dietary fatty acids was significantly greater with meal feeding when analyzed as the average over the postprandial period (meal feeding, 12.9 ± 3.4 vs. continuous-feeding, 5.5 ± 1.9 μmol/l, $P = 0.026$) or by AUC (meal feeding, 137 ± 42 vs. continuous-feeding, 65 ± 24 μmol · h · 1^{-1}, $P = 0.051$). A greater variability was observed for the

absolute contribution of all three sources under meal feeding compared with continuous feeding (Fig. 3*B*, *D*, and *F*).

DISCUSSION

The present study was conducted to quantitate the contributions of various fatty acid sources (NEFAs, lipogenesis, and diet) to VLDL-TAG in the fed state after two liquid meals were consumed in healthy subjects. Comparison of these data with those obtained under a continuous-feeding regimen was performed to determine whether differences in the rate of entry of dietary substrate would alter fatty acid usage by the liver. We have recently documented the contributions of multiple fatty acid sources to liver-TAG in patients with fatty liver (24). To our knowledge, the present data are the first in healthy subjects to investigate the immediate changes in fatty acid sources after the consumption of a meal. We hypothesized that the feeding regimen eliciting the greatest reduction in NEFA concentration (i.e., meal feeding) would result in the lowest amount of these fatty acids being used for VLDL-TAG synthesis. This hypothesis was based on studies demonstrating a greater insulin response and R_a NEFA suppression after consumption of increased carbohydrate loads (25–27). In the present study, no significant difference in concentration of NEFAs, absolute NEFA 16:0 (AUC), or percent VLDL-TAG from NEFAs was found between the two feeding regimens, even though the meal-feeding insulin concentration was significantly greater. One explanation for this could be that the minimum insulin response needed to reduce NEFA concentration, 100–120 pmol/l (27), was attained under both feeding regimens. Although NEFA concentration and R_a NEFA were reduced significantly in both studies, a steady increase in NEFA concentration was observed under the continuous-feeding regimen throughout the day when R_a NEFA exhibited steady-state metabolism. Likewise, an acute rise in NEFA concentration was observed in the meal-feeding regimen at the end of the study (9–11 h) when R_a NEFA reflected steady-state metabolism, suggesting other sources besides adipose fatty acid flux could have contributed to the NEFA pool. One potential source that may account for these observations is spillover of dietary fatty acids into the plasma via the action of lipoprotein lipase (15,28,29). The appearance of dietary-derived NEFA and its use for VLDL-TAG synthesis is the subject of a companion article (23).

Compared with the fasting-state, higher fed-state lipogenesis has been documented in previous research, but the intervals between blood draws were longer (from 1 to 6 h) than the 15 min used in the present study, and as a result, the immediate stimulation of lipogenesis was not apparent (6,8,9). We expected a greater percentage of VLDL-TAG to come from lipogenesis under the meal-feeding regimen as a result of a higher insulin AUC. The data support this hypothesis, with lipogenesis rates two-fold higher in the meal-feeding versus the continuous-feeding regimen. In earlier work of Hellerstein et al. (5), the effect of multiple meals was examined when healthy subjects received either a liquid formula by mouth on an hourly basis or an intravenous glucose infusion. The percentage of newly made fatty acids observed (1–2% of VLDL-TAG) was similar under both oral feeding and intravenous feeding in that study but significantly lower than found here (between 9 and 20%). The higher lipogenesis rates observed in the present study are most likely due to the lipoprotein fractionation procedure used and a longer time of isotope infusion. Moreover, compared with continuous feeding, the higher lipogenesis that occurred with the meal-feeding regimen could have been due to differences in rate of digestion or rate of delivery of substrate. Given that the food consumed in both feeding regimens was liquid in nature, differences in rates of digestion are unlikely. The overriding question is why lipogenesis was higher with meal feeding. Was this because of an increased rate of glucose entry (i.e., a substrate effect) or due to the higher insulin concentrations associated with faster glucose flux rate (a hormonal effect)? Research has suggested indirectly that the rate of carbohydrate delivery can affect this process (6,7). Hudgins et al. demonstrated that meals high in mono- and disaccharides elicited higher rates of lipogenesis (6,7). When these meals were formulated to be high in starch (complex carbohydrate), stimulation of lipogenesis was mitigated. A similar low lipogenic rate has also been observed when dietary carbohydrate was primarily complex in nature (2). However, the present in vivo data also highlight the relatively fast stimulation of a synthetic pathway in humans (29). The "on-off " nature of lipogenesis, shown over 5 h after the peak in insulin concentration, raises the question of whether greater insulin responses are associated with increases in lipogenesis. If so, a lesser lipogenic rate may occur if meals are consumed more frequently and insulin peaks are not as high. It is currently unknown whether allowing a definite period of fasting to occur between three meals per day will restrict lipogenesis, or whether consuming more frequent small meals each day will more likely reduce it. What is clear is that overconsumption of carbohydrates beyond energy needs is associated with a significant stimulation in fatty acid synthesis (8). Furthermore, although the meals were composed of liquid formula, they provide a good model to predict the stimulation of lipogenesis when energy is provided in liquid form.

In this study, the percentage of VLDL-TAG derived from dietary TAG was significantly elevated in the meal-feeding regimen because both the rate of incorporation and peak incorporation of dietary fatty acids into VLDL-TAG were significantly greater with meal feeding. When differences in TAG pool size were taken into account, a greater absolute use of dietary fatty acid with meal feeding was observed. In the postprandial state, chylomicrons lose fatty acids through the action of lipoprotein lipase, and the rate of substrate delivery may help explain the differences observed. Research has suggested that competition due to influx of chylomicrons reduces VLDL-TAG clearance through lipoprotein lipase (30). In the continuous-feeding regimen, subjects were tube-fed such that two-thirds of their total daily energy requirements were met. By contrast, when they participated in the meal-feeding regimen, subjects consumed a liquid bolus containing one-third of their daily energy needs at breakfast and the other one-third at lunch, setting the stage for a large influx of chylomicrons to the bloodstream (see the companion

paper [23] for the analysis of the routes by which dietary lipid was used for VLDL-TAG synthesis).

Because the design of the present study (i.e., liquid meals and the use of a feeding-tube) did not represent the normal pattern of food consumption, a number of limitations must be considered. First, when food is consumed by mouth, cephalic and gastric responses occur that affect digestion and lipid metabolism. These physiologic responses would have been present in the meal-feeding regimen, when food was consumed by mouth, but not in the continuous-feeding (tube-feeding) regimen. Second, the continuous-feeding test was completed 1–3 months before the meal-feeding test in all but one subject, and this lack of randomization may have affected the results. Third, as described in RESEARCH DESIGN AND METHODS, blood sampling was performed via a peripheral catheter placed in an antecubital vein, which could underestimate (19) or overestimate (20) tracer enrichments in arteries because of release of fatty acids from local stores and visceral depots, respectively. The magnitude of these effects on the calculations used are unknown, although it is likely that the effects of arterial-venous differences in enrichments are greater than those caused by visceral dilution in these relatively lean subjects. Lastly, we acknowledge that stress was involved with the placement and use of a feeding tube in healthy subjects. The effect of stress during continuous feeding would have likely resulted in a greater serum NEFA concentration than exhibited here, an effect that would have amplified the regimens' differences in hepatic recycling of adipose fatty acids. However, of the three sources of fatty acids, the pattern of serum NEFA use by the liver was similar between meal feeding and continuous feeding.

In summary, compared with continuous feeding, an increased rate of glucose delivery during meal feeding resulted in higher insulin concentrations, significantly elevated rates of de novo lipogenesis, and similar suppression of adipose fatty acid release. We had hypothesized that greater NEFA suppression with meal feeding would lead to a reduction in the use of serum NEFAs for TAG synthesis in the liver. However, the concentration of NEFAs was not significantly different between feeding regimens, which may have been due to spillover of dietary fatty acids into the serum NEFA pool. Under both feeding regimens, serum NEFAs contributed the majority of fatty acids used for VLDL-TAG synthesis. The continued dominance of adipose NEFAs as the primary contributor to VLDL-TAG postprandially in healthy individuals provides the metabolic mechanism by which adipose insulin resistance could further elevate postprandial lipemia in obesity and type 2 diabetes. After the serum NEFA pool, dietary fatty acids provided the second most abundant source of lipid for VLDL-TAG. The use of dietary fatty acids was higher under the meal-feeding regimen, suggesting that a greater rate of dietary lipid delivery is associated with more of these fatty acids reaching the liver. If true, this concept suggests that meals high in fat may lead to a transient accumulation of TAG in the liver. The present data highlight a unique characteristic of the liver to coordinate the use of different sources of fatty acids during times of transition from fasting to feeding. The data suggest that in healthy individuals, this flexibility allows VLDL-TAG synthesis to be well regulated and optimizes the use of dietary substrates after meals. In the future, it will be critical to understand how dysregulation of this process may lead to elevations in postprandial lipemia contributing to chronic disease risk.

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