Plasma free fatty acid (FFA) kinetics in humans are often measured with only one tracer. In study 1, healthy volunteers received infusions of [U-13C]linoleate, [U-13C]oleate, and [U-13C]palmitate during continuous feeding with liquid meals low (n = 12) and high (n = 5) in palmitate and containing three labeled fatty acids to measure FFA appearance and fractional spillover of lipoprotein lipase (LPL)-generated fatty acids. Study 2 used an intravenous lipid emulsion to increase FFA concentrations during infusion of linoleate and palmitate tracers. In study 1, there were no differences in spillover of the three fatty acids for the low-palmitate meal, but linoleate spillover was greater than oleate or palmitate for the high-palmitate meal. In studies 1 and 2, clearance was significantly greater for linoleate than for the other FFAs. There was a negative correlation between clearance and concentration for each fatty acid in the two studies. In study 1, concentration and spillover correlated positively for oleate and palmitate but negatively for linoleate. In conclusion, linoleate spillover is greater than that of other fatty acids under some circumstances. Linoleate clearance is greater than that of palmitate or oleate, indicating a need for caution when using a single FFA to infer the behavior of all fatty acids.

Elevation of plasma free fatty acid (FFA) concentrations and flux have been linked to adverse metabolic consequences such as increased VLDL triglyceride (TG) production (1) and reduced hepatic insulin sensitivity (2,3). Previous fatty acid kinetic studies in humans have demonstrated that spillover of fatty acids derived from the intravascular lipolysis of chylomicron TGs by LPL makes a substantial, direct contribution to the FFA pool (4-6). The regulation of spillover is poorly understood. Differences in albumin binding affinity (7) and membrane transport (8) based on degree of fatty acid saturation have been described that could influence both partitioning of LPL-generated fatty acids and the clearance of individual fatty acids from the circulating FFA pool. However, there is little in vivo evidence to support true differences between the various classes of FFA. The present studies provide evidence that there are class-dependent differences in fatty acid transport in humans.

RESEARCH DESIGN AND METHODS

Participants. Studies were conducted according to the principles expressed in the Declaration of Helsinki under two separate protocols approved by the Mayo Institutional Review Board. Study 1 included 17 lean, nondiabetic adults. Volunteers were weight-stable, did not engage in regular vigorous exercise, and had no history of diabetes or kidney, liver, thyroid, or heart disease. None of the participants had a history of diabetes in first-degree relatives. Study 2 was conducted independently and included 16 nondiabetic volunteers. Participant characteristics are provided in Table 1.

Study protocol. Volunteers for both studies received all meals from the metabolic kitchen for 5 days before the study. The meals were isonenergetic, with a macronutrient distribution of 20% protein, 35% fat, and 45% carbohydrate. Participants were admitted to the Clinical Research Unit the day before the study and were given an evening meal.

The experiments began the next morning after the volunteers had fasted overnight. The objective for study 1 was to determine if spillover was influenced by degree of fatty acid saturation. In study 1, participants were assigned to either protocol A (n = 12) or protocol B (n = 5). The only difference between the protocols was the composition of the test meal. The meal for protocol A consisted of chocolate-flavored Ensure Plus with additional canola oil to achieve the macronutrient distribution described. The meal for protocol B provided the same macronutrient distribution but consisted of skim milk, palm oil, and powdered lemonade sweetened with aspartame. The fatty acid distributions of meal A (linoleate 31%, oleate 48%, and palmitate 12%) and meal B (linoleate 19%, oleate 39%, and palmitate 39%) were confirmed by laboratory analysis. For both protocols, TG tracers consisting of 500 μCi of [9, 10-3H]trilinolein and 200 mg each of [1-13C]triolein and [1-13C]tripalmitin were added to the meal. The meal was sonicated with a Sonicator XL ultrasonic probe (Misonix, Farmingdale, NY) to ensure thorough mixing of the ingredients and tracers. For each individual, the complete meal provided one-third of the estimated daily basal energy expenditure (Harris-Benedict equation) and was administered as a priming dose (80 mL at 0 min), followed by ~20 mL (range, 14-28 mL) doses every 15 min beginning 120 min after the priming dose and continuing until 15 min before study conclusion at 360 min. This protocol produces nearly steady-state chylomicron TG and FFA concentrations (9,10). At 240 min, infusions of [U-13C]oleate and [U-13C]linoleate (~0.5 nmol kg⁻¹ min⁻¹ each) together with [1-13C]palmitate (~0.3 μCi·min⁻¹) were initiated and continued to the end of the study. Blood samples were taken every 15 min from 300 to 360 min for measurement of FFA, TG, chylomicron TG, and FFA concentrations, as well as FFA tracer-to-trace ratio (specific activity for radioisotopes and isotopic enrichment for stable isotopes). The averages of the data from these five time points were used for calculations.

Participants in study 2 included nonobese and obese men (n = 8 each). The participants were part of an unpublished study that was conducted to compare nonoxidative FFA disposal rates in nonobese and obese men. After an overnight fast, the participants received a 3-h intravenous infusion of a commercial 20% lipid emulsion (Liposyn) administered as a primed, continuous infusion (1.0 mL/min for 20 min, then 0.33 mL/min for the remainder of the study). The abundance of linoleate, oleate, and palmitate in the lipid emulsion is approximately 66%, 18%, and 9%, respectively. Each volunteer received continuous intravenous infusions of [U-13C]linoleate and [U-13C]oleate at rates of ~7 nmol kg⁻¹ min⁻¹ beginning at 60 min. A series of six blood samples were obtained and the results were averaged to calculate steady-state rate of disappearance and clearance rate as noted.

Analyses. For study 1, blood samples were collected in chilled 10 mL EDTA tubes containing 0.5 mg of paraoxon to inhibit LPL (11) and kept on ice until centrifugation at 4°C. Chylomicrons were isolated using a triple-spin ultracentrifugation method previously published (10). Plasma FFA and chylomicron concentrations and FFA specific activity were determined using a modification (12) of a high-performance liquid chromatography method (13). Plasma TG concentrations were measured using a COBAS Integra autoanalyzer. For study 2, plasma FFA concentrations were determined by an ultra performance liquid chromatography, modification of the high-performance liquid chromatography method previously cited (13). Liquid chromatography–mass spectrometry (14) was used to measure isotopic enrichments for both studies.
TABLE 1
Participant characteristics

| Study | Protocol A (n = 12) | Protocol B (n = 5) | Nonobese (n = 8) | Obese (n = 8) |
|-------|---------------------|-------------------|-----------------|--------------|
| Age   | 26 ± 1              | 33 ± 2*           | 27 ± 1          | 30 ± 3       |
| Sex   | M/F                 | M/F               | M/F             | M/F          |
| Weight (kg) | 62 ± 3       | 60 ± 3            | 89 ± 5          | 100 ± 7      |
| BMI (kg/m²) | 23 ± 1       | 22 ± 1            | 26 ± 1†         | 30 ± 1       |
| Total cholesterol (mg/dL) | 172 ± 13 | 142 ± 13          | 141 ± 7†        | 173 ± 6      |
| HDL cholesterol (mg/dL) | 58 ± 3      | 58 ± 5            | 47 ± 3          | 40 ± 2       |
| LDL cholesterol (mg/dL) | 96 ± 12     | 74 ± 9            | 83 ± 8          | 104 ± 5      |
| TG (mg/dL) | 83 ± 11   | 51 ± 7*           | 53 ± 7†         | 148 ± 30     |
| Fasting glucose (mg/dL) | 85 ± 2      | 85 ± 3            | 81 ± 2†         | 88 ± 2       |
*F, female; M, male. *P ≤ 0.03; vs. A; †P ≤ 0.03; ††P < 0.01 vs. obese.

Calculations. Rates of appearance (Ra) of the individual FFA were calculated using steady-state equations in which rate of disappearance (Rd) equals Ra (15). FFA clearance was calculated as FFA tracer infusion rate divided by plasma tracer concentration (16); this calculation assumes that at steady-state, the rate of disappearance of the tracer equals the infusion rate.

Spillover of each fatty acid was calculated using each of the three FFA tracers and was expressed as a percent of total chylomicron fatty acids generated by LPL. The calculation of the Ra of the meal fatty acid tracer in plasma FFA is described by the equation:

\[
\text{Ra} = \frac{\text{FFA} \times \text{IV tracer}}{\text{meal FFA tracer (TG tracer)}},
\]

where *FA is the labeled fatty acid from the TG tracer in the meal (5).

Fractional spillover (%) can then be calculated using the following equation:

\[
\text{Spillover} = \frac{\text{Ra} \times \text{FAD}}{\text{Rd} \times \text{TG tracer}} \times 100
\]

where Rd (rate of disappearance) of the chylomicron TG tracer from plasma is equal to the ingestion rate of the TG tracer in the meal at steady state (5).

Statistical methods. For study 1, results from protocol A were compared with protocol B using t tests for means with unequal variance. The three classes of fatty acids were compared with each other using a Tukey mean difference test for means with unequal variance (α < 0.05). Statistical analyses were performed using JMP version 9.1.0 software (SAS Institute, Cary, NC). P values for regression analyses were based on Spearman r. All data are presented as mean ± SEM.

RESULTS
Baseline characteristics of the volunteers for both studies are shown in Table 1.

Study 1. Tracer data were not available for all volunteers because of technical difficulties. Data for all 17 individuals are presented except when noted. Plasma total TG concentrations increased in protocol A from a baseline of 76 ± 7 to 95 ± 9 mg/dL (P < 0.005) during the final hour, whereas values for protocol B increased from 50 ± 6 to 68 ± 11 mg/dL (P = 0.13). Chylomicron TG concentrations during the last hour averaged 10 ± 1 mg/dL in both groups. Total FFA concentrations in groups A and B during the meal ingestion period were not significantly different (107 ± 10 vs. 79 ± 7 μmol/L, respectively; P = 0.39). Individual plasma FFA concentrations differed significantly from each other during meal absorption for group A (Table 2). In group B, linoleate concentration was less than concentrations of oleate (P < 0.001) and palmitate (P = 0.046), with only slight differences between palmitate and oleate concentrations (P = 0.064). Oleate Ra was significantly less in group B than in group A, but there was no difference in the Ra of linoleate (P = 0.27) and palmitate (P = 0.28) between groups A and B. Palmitate Ra was significantly lower than the Ra of the other two FFAs in group A, whereas in group B the Ra of linoleate was significantly greater than the Ra of the other two fatty acids.

The ingestion rates of meal tracers were ~0.45 μg/min, ~2.2 μmol/min, and ~2.5 μmol/min for linoleate, oleate, and palmitate, respectively. In group A, there were no significant differences in fractional spillover among the three fatty acids when the corresponding primary tracers were used, whereas in group B linoleate spillover was greater than that of the other two fatty acids with the primary tracer (Table 3). In both groups, the use of an alternate (secondary) FFA tracer (e.g., a palmitate tracer for linoleate) produced both higher and lower spillover values than those from the primary tracer calculation.

There was a positive correlation between fractional spillover and plasma concentration that was significant for palmitate (r = 0.71; P = 0.006) and that of borderline significance for oleate (r = 0.46; P = 0.07), as can be seen in Fig. 1. In contrast, fractional linoleate spillover correlated negatively with concentration (r = −0.49; P = 0.048).

Study 2. Total FFA concentrations did not differ between nonobese and obese participants during lipid infusion (509 ± 57 vs. 523 ± 47 μmol/L, respectively; P = 0.84). Linoleate concentrations were greater (P < 0.02) than oleate concentrations for both groups (Table 2). Linoleate Rd also was significantly greater in both groups than oleate Rd (P < 0.001). For both oleate and linoleate, there was no difference in concentration or Rd between the two groups.

Clearance in study 1 and study 2. In study 1, linoleate clearance was markedly greater than oleate or palmitate.

TABLE 2
Unlabeled fatty acid concentrations and rate of appearance for both studies

| Study 1 | Study 2 |
|---------|---------|
| Protocol A | Protocol B | Nonobese | Obese |
| Concentration (μmol/L) | | | |
| Linoleate | 30 ± 3* | 18 ± 2† | 174 ± 14* | 185 ± 11* |
| Oleate | 38 ± 3 | 26 ± 2 | 161 ± 25 | 156 ± 15 |
| Palmitate | 21 ± 2* | 22 ± 2 | | |
| Rate of disappearance (μmol/min) | | | |
| Linoleate | 57 ± 4 | 51 ± 4* | 261 ± 27* | 237 ± 17* |
| Oleate | 49 ± 4 | 24 ± 2‡ | 128 ± 19 | 122 ± 12 |
| Palmitate | 29 ± 2* | 26 ± 2 | | |
| Clearance (L/min) | | | |
| Linoleate | 2.13 ± 0.32 | 2.80 ± 0.15 | 1.54 ± 0.16 | 1.33 ± 0.14 |
| Oleate | 1.38 ± 0.12 | 0.93 ± 0.05 | 0.94 ± 0.20 | 0.86 ± 0.15 |
| Palmitate | 1.53 ± 0.14 | 1.19 ± 0.16 | | |

Ra and clearance comparisons in study 1 group A were made for 11 subjects. For within group comparisons, *P < 0.017 vs. other fatty acids in group; †P < 0.0001 vs. other fatty acids in group; ‡P < 0.017 vs. oleate only. For between group comparisons, ††P < 0.017 vs. A.
clearance ($P < 0.001$ for both), whereas palmitate clearance was significantly greater than oleate clearance (Table 2). In study 2, linoleate clearance was greater than oleate clearance ($P < 0.001$). Figure 2 depicts the relationship between FFA concentration and clearance in study 1. There was a negative correlation between linoleate clearance and concentration ($r = -0.79, P < 0.001$) and a borderline significant correlation for palmitate ($r = -0.495; P = 0.05$), but no significant correlation between clearance and concentration for oleate ($r = -0.29; P = \text{not significant}$). In addition, there were significant negative correlations between clearance and Ra for linoleate and oleate, but not palmitate (not shown). There was a significant negative correlation between clearance and concentration for both linoleate ($r = -0.57; P = 0.02$) and oleate ($r = -0.70; P < 0.005$) in study 2 (Fig. 3).

**DISCUSSION**

In study 1, we found that fractional spillover (calculated using primary FFA tracers) was similar for palmitate, oleate, and linoleate when a meal low in palmitate was administered (group A). However, when a meal high in palmitate was given (group B), linoleate spillover was higher than that of oleate or palmitate. When spillover was calculated using secondary tracers (palmitate for linoleate, linoleate for oleate, and so on), there were marked differences compared with the primary tracer estimates. The clearance of plasma linoleate as an FFA during meal ingestion was $\sim 75\%$ greater than clearance of oleate or palmitate at both the low plasma FFA concentrations that prevail during meal absorption (study 1) and at the higher concentrations observed during infusion of a lipid emulsion (study 2). Our data also show a positive correlation between spillover and fatty acid concentration for oleate and palmitate, and a negative correlation between linoleate spillover and concentration. Finally, there was a significant negative correlation between clearance and plasma concentration of the three fatty acids.

Most investigators, including our group, have used a single tracer to study FFA kinetics and the spillover process, with the implicit assumption that the metabolic characteristics of all fatty acids can be inferred from the results. Hodson et al. (17) used a palmitate FFA tracer to measure spillover rates for oleate, palmitate, and linoleate in healthy adults during absorption of a bolus meal and found no differences in spillover among the three fatty acids. We measured spillover with three FFA tracers corresponding to the labeled fatty acid in meal TG. In addition, we administered frequent aliquots of a liquid mixed meal to achieve the steady-state conditions, rather than giving a bolus meal. This allowed the use of the Steele equation for steady-state conditions, a requirement for measurement of precursor–product relationships as previously discussed by Hellerstein et al. (18). It should be

| Spillover | Plasma FFA Tracer | Linoleate | Oleate | Palmitate |
|----------|-------------------|-----------|--------|-----------|
| Protocol A | Linoleate ($n=11$) | 15.4 ± 1.1 | 12.9 ± 0.9 | 8.1 ± 0.8† |
|          | Oleate ($n=10$)   | 17.9 ± 1.4 | 18.2 ± 1.9 | 10.8 ± 6.7† |
|          | Palmitate ($n=8$) | 41.5 ± 5.1† | 35.7 ± 5.9* | 22.6 ± 4.3* |
| Protocol B ($n=5$) | Linoleate | 25.0 ± 3.8‡ | 11.9 ± 1.8* | 12.5 ± 1.6* |
|          | Oleate | 15.8 ± 3.7 | 12.8 ± 1.7 | 13.4 ± 1.2 |
|          | Palmitate | 32.3 ± 6.3* | 15.3 ± 2.9 | 15.8 ± 2.4 |

Data presented are percentages. Results using primary tracer are boxed. ‡$P < 0.017$; †$P < 0.002$ vs. primary tracer (horizontal comparison); †$P < 0.01$ vs oleate and palmitate using a linoleate FFA tracer (vertical comparison within group).
acknowledged that the continuous feeding model used in our studies may generate spillover rates lower than what occurs with bolus feeding (19). Despite these differences in experimental design, the results in group A are concordant with those of Hodson et al. This suggests that differences in albumin binding affinity (7) and in rate of cellular transport among the fatty acids (8), if present, exert little if any influence on the partitioning of LPL-generated fatty acids between plasma FFA and local cellular uptake. It seems likely that fractional spillover is regulated, at least in part, by local release of FFA from intracellular lipolysis. We previously reported a strong correlation between fractional spillover and FFA release in extrahepatic splanchnic tissues, presumably visceral fat (20). Moreover, intravenous infusion of niacin, a potent inhibitor of intracellular lipolysis, produces significant decreases in spillover in healthy volunteers (21).

The results in group B of study 1, however, show significantly higher spillover of linoleate compared with oleate and palmitate. Although the meal in this protocol contained more saturated fat and less unsaturated fat, the explanation for this finding is not apparent. Additional studies in a larger number of subjects would be required to confirm the results.

The greater clearance of plasma linoleate compared with plasma palmitate or oleate may be a result of lower albumin binding affinity and/or more rapid membrane transport. Ashbrook and Spector (7) found a hierarchy of binding affinities of albumin for different fatty acids, with oleate binding being the most avidly binding, linoleate being the least binding, and palmitate being intermediate. Kleinfeld (17) reported that linoleate has a more rapid rate of transport across red cell membranes than oleate or palmitate (8), which may explain the more rapid incorporation of dietary linoleate into erythrocyte membrane phospholipids, VLDL, TG, and cholesterol esters relative to oleate or palmitate.

Other investigators have compared the clearance of different FFAs in humans. Our findings differ from those of Mittendorfer (22), who did not find a significant difference in the clearance of these three fatty acids, but the findings are consistent with the observation that the net splanchnic fractional uptake of plasma linoleate is greater than that of palmitate or oleate (23). Carpentier et al. (24) measured palmitate and linoleate clearance during infusions of insulin at two different doses with and without intralipid heparin. Although there was not a formal statistical comparison between linoleate clearance and palmitate clearance within each of four groups, linoleate clearance in that study was similar to or lower than that of palmitate. The differences among these studies may be attributable to the use of different analytical methods (gas chromatography and mass spectrometry in Mittendorfer, liquid chromatography and mass spectrometry in Carpentier, and high-performance liquid chromatography/ultra performance liquid chromatography in our study). Our results also could be interpreted as contradictory to those of Summers et al. (25), who found no differences in the net uptake of linoleate, oleate, and palmitate across abdominal subcutaneous fat. However, it is difficult to compare our results with that study because it did not use tracers and therefore only provided net balance data.

The lower oleate Rd in group B versus group A of study 1 cannot be explained by differences in meal composition. It is possible that there were differences in insulin sensitivity between the groups to account for this difference. We do not have an explanation for the lower oleate clearance observed in this group of subjects.

It is clear from our results and previous work (4,5,20,26) that a major fraction of the fatty acids generated by the action of LPL on circulating chylomicron TGs do not mix with plasma FFA. Presumably, these fatty acids are very rapidly transported across the capillary endothelium from a subcompartment of plasma; it previously has been suggested that LPL-generated fatty acids are preferentially extracted by tissues compared with arterial-borne FFAs (26).

We found a significant negative correlation between the clearance of FFA and their plasma concentration, a relationship that prevailed at both low and elevated plasma FFA concentrations. This nonlinear relationship between

![FIG. 2. Relationship between fatty acid concentration and clearance for linoleate (top panel), oleate (middle panel), and palmitate (lower panel) in study 1.](diabetes.diabetesjournals.org)
transport and concentration has been previously reported for FFA (13) and also applies to glucose (27). We found that oleate clearance is higher during intravenous niacin infusion compared with saline infusion in healthy volunteers; this occurs in the context of a marked decrease in oleate concentration and no change in insulin concentrations (R. Nelson et al., unpublished data). The inverse relationship between pool size and clearance also may apply to amino acids; we calculated clearance of leucine before and during a leucine infusion in healthy subjects from a previous study by Nair et al. (28) and found that clearance was significantly lower during leucine infusion ($P < 0.002$). A greater relative affinity of tissues for circulating fatty acids at lower concentrations may explain why there was a positive correlation between FFA concentration and fatty acid spillover for palmitate and oleate, but it does not explain the negative correlation between linoleate concentration and spillover. However, intracellular lipolysis itself (20), rather than tissue affinity for FFA, may be the true regulator of spillover in adipose tissue. It should be acknowledged that the relationship between plasma FFA and spillover is potentially bidirectional because spillover contributes to plasma FFA concentration.

Figures have implications for studies in which total FFA flux is measured with a single FFA tracer. The calculation of total FFA flux with a single fatty acid tracer is made by dividing the Ra of the individual fatty acid by the proportional contribution of that fatty acid to the total plasma FFA concentration. If the clearance of the FFA that is traced is different than the clearance of other FFAs, then quantitative errors in estimates of total FFA flux will be introduced (22). Specifically, if a fatty acid such as stearate (which has lower clearance than other FFAs) is used (22), then total FFA flux will be underestimated, whereas the use of a fatty acid tracer that has a high clearance (linoleate in the current study) will result in an overestimate of total FFA flux.

In summary, the spillover of LPL-generated fatty acids into plasma FFA is similar for saturated, monounsaturated, and polyunsaturated fatty acids. FFA clearance decreases as plasma concentration increases for all three classes of fatty acids. There was a positive correlation between fractional spillover and plasma concentration for palmitate and oleate, suggesting that dietary fat storage is less efficient when suppression of adipose tissue lipolysis is impaired, such as is the case in states of insulin resistance. The negative correlation between linoleate spillover and plasma concentration is surprising and will require further elucidation.

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R.H.N., as primary investigator of study 1, was involved in every aspect of the project and wrote the manuscript. D.T.V., A.S., K.M., J.P.A., and E.S. reviewed the manuscript. K.M., J.P.A., and E.S. contributed to the discussion. D.T.V. and A.S. assisted with study planning, execution, and data analysis. M.S.M. and M.D.J. conducted study 2 and contributed to discussion and to the manuscript. J.M.M. assisted with all aspects of study 1 and manuscript preparation. R.H.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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