Fractional oxidation of chylomicron-derived oleate is greater than that of palmitate in healthy adults fed frequent small meals[1]

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Abstract Differences in oxidation of individual dietary fatty acids could contribute to the effect of dietary fat composition on risk factors for non-insulin-dependent diabetes mellitus and cardiovascular disease. Using a novel stable isotope technique, we compared fractional oxidation of chylomicron-derived oleate and palmitate in 10 healthy adults in a crossover study. 1-^{13}C-labeled oleate or palmitate was emulsified into a eucaloric formula diet administered each 20 min for 7 h to produce a plateau in excretion of ^{13}C label in breath CO_2. Unlabeled oleate and palmitate each provided 16% of dietary energy, and other fatty acids provided 8% of energy. Total dietary fat was 40% of energy, carbohydrate was 46%, and protein was 14%. Diet without tracer was fed for 2 h before beginning tracer administration to establish a baseline fed state. Relative oxidation of oleate versus palmitate was defined as fractional oxidation of oleate divided by fractional oxidation of palmitate. Relative oxidation averaged 1.21 (99.5% confidence interval = 1.03–1.39), indicating that fractional oxidation of oleate was significantly greater than that of palmitate.—Schmidt, D. E., J. B. Allred, and C. L. Kien. **Fractional oxidation of chylomicron-derived oleate is greater than that of palmitate in healthy adults fed frequent small meals.** ***J. Lipid Res.*** **1999.** 40: **2322–2332.**

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It is well established that fatty acid composition of the diet markedly affects various aspects of human health. The saturated fatty acid palmitate consistently raises plasma low density lipoproteins when it replaces dietary carbohydrate, whereas monounsaturated oleate is "neutral" in this respect (1). Oleate, moreover, improves indices of glycemic control in persons with non-insulin-dependent diabetes mellitus when substituted for carbohydrate at total fat intakes of 40 to 50% of dietary energy (2–4).

Understanding the beneficial effects of dietary oleate as compared with palmitate may be helpful in formulating dietary fat recommendations, particularly as they are the two most common fatty acids in the food supply (5). However, the mechanisms responsible for these differences have remained elusive. One factor could relate to the inhibitory effect of unsaturated fatty acids on hepatic fatty acid biosynthesis (6–8). Linoleate compared to palmitate is a more effective inhibitor of hepatic acetyl-CoA carboxylase and fatty acid synthetase (6–8). Another factor may be differences in oxidation in the fed state. Several studies have compared oxidation of various fatty acids after their ingestion. This is achieved by feeding tracers labeled with carbon isotopes and measuring appearance of the label in breath CO_2. Some marked differences have been shown, particularly between saturated and unsaturated fatty acids, in humans (9), rats (10, 11), and mice (12). In other studies different fatty acids were equally oxidized in rats (13–15). However, oxidation of oleate and palmitate has never been directly compared in humans, and in the two comparisons that used rats, one found that more oleate was oxidized (11) while the other found equal oxidation of the two substrates (13).

The present study compares oxidation of dietary oleate and palmitate in healthy adults while controlling for potential differences in digestive and absorptive processes. This was done to narrow the experimental focus such that our results would be complementary to those that include such differences. To that end, we used a novel approach. Using a crossover design, 1-^{13}C-labeled oleate and palmitate tracers were fed to healthy adults in frequent small meals to produce a steady state in appearance of labeled

Abbreviations: APE, atoms percent excess; CM, chylomicrons; F%, fraction of energy from fat oxidation; FABP, fatty-acid binding protein; FAMES, fatty acid methyl esters; GC–FID, gas chromatography–flame ionization detection; MPE, moles percent excess; NEFA, non-esterified fatty acids; Rel_{OX}, relative oxidation of oleate versus palmitate; RQ, respiratory quotient; TAG, triacylglycerol; VCO_2, rate of carbon dioxide excretion; VO_2, rate of oxygen consumption.

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chylomicrons (CM) in plasma. The triacylglycerol (TAG) content of the diet was identical in the two protocols, and the unlabeled oleate and palmitate were present in the diet in equal amounts. A comparison of the fractional oxidation of oleate versus palmitate was made by calculating differences in appearance of ^{13}C label in breath CO_2 corrected for the appearance of the 1-^{13}C-labeled fatty acids in CM.

METHODS

Experimental model

The experimental protocol was designed to produce data that would be applicable to fat oxidation during digestion and absorption of mixed meals, as this is typically the case during a large proportion of each day. Briefly, the fat content of the test diet, 40% of energy, is approximately equal to the upper quartile of the range of usual intakes in the American diet reported in NHANES II (16). Feeding 150% of fasting resting energy expenditure was intended to deliver food energy at a rate moderately above fed energy expenditure. The continuous delivery of carbohydrate and protein throughout the study was intended to maintain a consistent level of insulin secretion. Insulin inhibits hormone-sensitive lipase, thereby suppressing release of fatty acids from adipose stores (17).

Thus, it was anticipated that the primary source of fat oxidation would be dietary TAG. Note that this approach does not preclude oxidation of endogenous fatty acids. However, to the extent that whole-body fat oxidation was due to endogenous sources, this also represents normal physiological processes in a fed state. In effect, by analyzing the enrichment and proportion of oleate and palmitate in CM, we compared the oxidation of these two substrates both from the diet and from potential endogenous sources incorporated into CM within the enterocyte (18–20). Simultaneously, the method excludes assessment of other sources of endogenous fat oxidation, such as adipose stores, VLDL, or intracellular hydrolysis in metabolically active tissues. Although variability in these other fatty acid sources might influence oxidation of CM-derived fatty acids, it represents a source of random error in the present protocol.

Because CM are partially hydrolyzed to non-esterified fatty acids (NEFA) in the periphery, it might seem logical to use NEFA rather than CM, as representing the form of plasma fatty acid most proximal to oxidation. They are, in fact, the usual precursor analyzed in fasting fat oxidation studies. However, in a fed state, NEFA may not be entirely indicative of diet-derived fatty acid metabolism (21–23). This is because some of the fatty acids, which are released from CM in capillaries, are taken up immediately by muscle and could be oxidized directly in this tissue (21, 22), and some TAG is taken up directly as CM remnants and undergoes intracellular hydrolysis (23). Thus, there are two routes by which dietary fat bypasses the arterial NEFA pool.

Subjects

Ten healthy young adult volunteers were studied. Subjects were recruited via fliers posted at The Ohio State University General Clinical Research Center and College of Human Ecology. Informed written consent was obtained from each subject, and the experimental protocol was approved by the Biomedical Sciences Institutional Review Board for Research Involving Human Subjects, The Ohio State University. Characteristics of subjects are given in **Table 1**. Exclusion criteria included: obesity, defined as body mass index greater than the 85th percentile for height, age, sex, and race (24, 25); fasting blood lipid abnormalities, including TAG or low density lipoproteins above the 85th percentile or high density lipoproteins less than 15th percentile for age and sex (26); and chronic health problems or use of drugs that may affect lipid metabolism, including oral contraceptives and nicotine. Subjects were also verified to be afebrile on the morning of each study. In order to minimize variation in estrogen levels, female subjects were studied in early to mid-follicular phase of the menstrual cycle (in the first 10 days, by self-report).

TABLE 1. Characteristics of participants

Subject	Age	Body Weight	BMI	Fasting EE	Sex	Race
	yr	*kg*	*kg/m²*	*kJ/d*		
3020	34	67	21	6443	m	white
3022	29	79	27	6485	m	black
3024	22	71	26	5540	f	white
3025	21	51	19	4569	f	white
3026	20	78	23	7770	m	white
3027	20	50	21	5176	f	white
3028	34	56	22	5519	f	white
3029	23	73	27	7531	m	white
3030	24	51	20	5234	f	black
3031	20	57	23	4883	f	black
Mean	24.7	63.3	22.9	5916		
± SEM	1.8	3.7	0.9	347		

BMI, body mass index; EE, energy expenditure (kJ = 0.239 kcal).

Study design

A crossover design was used, with each subject being studied on two occasions separated by at least 3 days and not more than 26 days. This resulted in a period of at least 65 h between tracer feedings in the same subject, to prevent a measurable carry-over in the isotope enrichment of CO_2 (27, 28). The same formula diet was fed during each study, so that TAG composition would be the same on both days. On one occasion, [1-^{13}C]palmitate tracer was administered, and on the other, [1-^{13}C]oleate was administered. The order in which the two tracers were given was alternated within each sex category.

On the first day of each protocol, subjects reported to the General Clinical Research Center in a fasted state and spent the entire day and following night in the unit. During that time, they consumed only the liquid diet formulated for this experiment, described below. After an overnight fast, 30 to 60 ml of the liquid diet was administered every 20 min for a total of 9 h; the first 2 h without tracer, and the last 7 h with tracer. Feeding the diet without tracer for 2 h before the baseline samples were collected was done to prevent shifts in background $^{13}C/^{12}C$ ratio of CO_2, which can occur when substrate oxidation shifts from endogenous to exogenous nutrients (29, 30). The hourly rate of energy intake for each subject during each tracer feeding protocol was 150% of that individual's fasting resting energy expenditure, which was measured during the screening process.

During each protocol, baseline breath and blood samples were collected 2 h after ingestion of the first non-tracer meal, or nominal time = 0:00, and at 3:00, 4:00, 5:00, 6:00, 6:20, 6:40, and 7:00 h after the baseline sample. Additional breath samples were collected at 6:10, 6:30, and 6:50 h. Each blood sample was 7 ml, resulting in a total collection volume of 49 ml per protocol or 98 ml per subject.

Diet and tracer

The test diet was a liquid formula diet similar in composition to Pediasure (Ross Products Division of Abbott Laboratories, Columbus, OH), except that the lipid content was 91% palm oil, 6%

high-oleic acid safflower oil, and 3% phosphatidyl choline. It provided 46% of energy as carbohydrate, 14% as protein, and 40% as fat. By design, oleate and palmitate contributed 16.5 and 16.3%, respectively, to total energy of the test diet. By gas chromatography analysis, fatty acid composition by weight was: oleate 41.0%, palmitate 39.5%, linoleate 10.1%, stearate 4.9%, and other fatty acids 4.5%. The carbohydrate was 70% hydrolyzed cornstarch and 30% sucrose.

The [1-^{13}C]oleate and -palmitate tracers were purchased from Isotec, Inc. (Miamisburg, OH). Tracers were added to the test diet in amounts equal to 5% of their respective unlabeled dietary fatty acids. This resulted in a tracer dose (mean ± SEM) of 1.79 ± 0.06 mg/kg per h each, or 6.95 ± 0.23 μmol/kg per h palmitate and 6.31 ± 0.21 μmol/kg per h oleate. By weight, each tracer equaled about 2% of the total fatty acid content of the test diet + tracer mixture. Oleate tracer was stored at −80°C. Palmitate tracer was stored at 4°C.

Tracer fatty acids were emulsified into the diet on the day before each protocol. Fifteen grams of granular phosphatidylcholine was dissolved in one cup of water at room temperature using a blender. Oleate tracer, which is liquid at room temperature, was added directly to this mixture and blended on low power. Palmitate has a melting point of about 58°C. Therefore, in order to emulsify this tracer, the lecithin mixture was heated to about 60°C, and the metal blender jar was also heated to about 100°C before adding the lecithin solution and palmitate. This mixture was then added to the test diet, divided into 21 equal parts, sealed in plastic cups, and refrigerated.

Fatty acid analysis

Blood samples were drawn from a small forearm vein via an indwelling catheter and arterialized using a flexible heating pad on the high setting, applied for at least 10 min before each sample was drawn (31). Blood was collected in heparinized tubes and immediately placed in an ice bath. Samples were centrifuged within 1 h at 4°C for 10 min at 3,000 rpm. The separated plasma was stored at −20°C.

The CM fraction was isolated from plasma by ultracentrifugation (Model L8-55 with SW50.1 rotor, Beckman Instruments, Schaumburg, IL). CM were defined as particles with Svedberg flotation rate (S_f) >400 (32). Approximately 3.7 ml saline (d 1.0063 g/ml) with EDTA (0.1 mg/ml) was layered over 0.8 ml of plasma. Samples were centrifuged at 33,500 rpm for 30 min, the surface layer was removed by pipette (about 0.5 ml), and the total volume was restored by adding fresh saline. This procedure was repeated once. CM samples were stored at −20°C.

Because the upper end of the size distribution for hepatic lipoproteins may exceed S_f = 400 (32), the large CM fraction (S_f > 1000) was separated in a subset of samples. These samples were centrifuged at 33,500 rpm for 12 min and their fatty acid enrichments were compared with that of the S_f > 400 fraction from the same sample.

Fatty acids were isolated from CM and derivatized to methyl esters (FAMES) using 3 N methanolic HCl according to the method of Hellerstein et al. (33).

In a subset of samples, enrichment of NEFA was assessed to test the assumption that plasma NEFA would be derived primarily from hydrolyzed CM during our feeding protocol. NEFA were prepared according to the method of Wolfe (34) using 0.6 ml of plasma per sample. Fatty acids were isolated from plasma using thin-layer chromatography and derivatized to FAMES using 14% BF3-CH3OH.

Isotope enrichment of fatty acids was determined by gas chromatography/mass spectrometry in the Electron Impact Mode (Model 5971A, Hewlett-Packard Co., Palo Alto, CA). A Supelco Omegawax 250 column, 0.25-mm ID × 30 m, was used for GC separation of FAMES. For each FAME, we assessed the M + 0 and M + 1 peaks; ions 270 and 271 for palmitate, 296 and 297 for oleate. The (M + 1)/(M + 0) isotope ratio was corrected against a standard curve, and enrichment was defined as moles percent excess (MPE) (34, 35). Each enriched sample was analyzed in duplicate. Unenriched samples were analyzed in quadruplicate. Acceptable reproducibility was defined to be <0.5%, or a difference in enrichment of <0.15 MPE.

Gas chromatography with flame-ionization detection (GC-FID) (Varian Star 3600 GC, System ID 3600-01313, Varian Analytical Instruments, Sugarland, TX) was used to determine the relative concentrations of oleate and palmitate. For each study, samples analyzed were t = 0:00, t = 4:00, and one from the final hour (t = 6:00 through 7:00), or six samples per subject. Each sample was analyzed in duplicate, with acceptable reproducibility defined to be <10% in the ratio of oleate and palmitate peak areas.

Enrichment of breath CO_2

Expired air was collected in plastic collection bags (Quintron Instruments, Milwaukee, WI) and stored, for not more than 6 months, in non-siliconized sterile tubes (Venoject, Terumo Medical Corporation, Elkton, MD). The CO_2 was cryogenically purified, and the enrichment (atoms percent excess, APE) was measured by gas isotope ratio mass spectrometry (Model Delta E, Finnigan MAT, San Jose, CA) (36). Reproducibility in our lab is 0.24% for replicate (n = 5) samples prepared and analyzed in the natural abundance range of ^{13}C. Each baseline (t = 0:00) sample was analyzed twice, and the results were averaged. All other samples were analyzed once.

Indirect calorimetry

Oxygen consumption (VO_2) and CO_2 production (VCO_2) were measured via indirect calorimetry, using a metabolic cart (Deltatrac, Sensor Medics Corporation, Yorba Linda, CA), which samples expired air once each minute. A 1-h measurement of fasting, resting energy expenditure was performed during the screening process and used in calculating the amount of diet to be fed to each subject. Two 20-min measurements were performed during each feeding protocol, and the final 15 min of each were analyzed. One measurement was made in the fifth hour (between t = 2:00 and 3:00), and one in the ninth hour (between t = 6:00 and 7:00).

Computation of relative oxidation

The aim of this experiment was to test the hypothesis that more CM-derived oleate is oxidized than palmitate. The basis for this comparison was their respective fractional rates of oxidation, computed from rates of appearance of labeled CO_2 and relative rates of appearance of labeled fatty acids in CM. The derivation of the equation used is given as an appendix to this paper. Relative oxidation (Rel_{OX}) was computed for each subject as follows:

$$Rel_{OX} = \frac{IE \cdot CO_{2\,OA} \times A \cdot PA \times 18 \times IE \cdot PA}{IE \cdot CO_{2\,PA} \times A \cdot OA \times 16 \times IE \cdot OA},$$

where OA is oleate, PA is palmitate, IE is isotope enrichment, $A \cdot$PA and $A \cdot$OA denote, respectively, the peak areas for PA and OA, derived from GC-FID data, and 18 and 16 are the number of carbon atoms in one molecule of oleate or palmitate.

Data analysis

As discussed in the Appendix, our use of steady-state tracer methodology relies on attaining a steady metabolic state, but not really an isotopic steady state in the enrichment of the tracer in plasma fatty acids or in breath CO_2. It is, however, desirable to maximize absolute substrate enrichment and minimize rate of

change in enrichment in order to maximize signal-to-noise ratio. For these reasons, we designed the protocol to produce a relative plateau or shallow rate of increase in enrichment of breath CO_2 that was the same during both tracer studies in each subject.

Plateau enrichment of CO_2 was defined a priori as the average APE of breath CO_2 during the final hour of each protocol (7 data points, from t = 6:00 to 7:00). This was based on the results of a preliminary study using a similar protocol in which slopes in CO_2 enrichment of 8% and 3% of the hourly average were achieved during the sixth and seventh hours of palmitate tracer feeding respectively (data not shown).

The CO_2 enrichment data for each protocol were inspected visually. If this revealed a suspected outlier, a test for exclusion of outliers was performed (37). No data were excluded on this basis.

Plateau enrichment of oleate and palmitate was defined a priori as the average MPE of CM-derived fatty acids during the final hour of each protocol (4 data points). NEFA enrichment was assessed in 2–4 samples from each palmitate study and three oleate studies (30 data points altogether) and compared with enrichments of CM in the same plasma samples by paired *t*-test. This was done to test the assumption that CM would be the principal source of plasma NEFA in our protocol.

Relative amounts of oleate and palmitate in CM ($A{\cdot}OA/A{\cdot}PA$) were assessed in three samples per protocol and compared within each subject by *t*-test to test the assumption that the relative rates of appearance of the two substrates did not vary between the two protocols.

Data from the four 15-min measurement periods of indirect calorimetry (two per protocol) were compared within each subject by a 2-way analysis of variance (day × time-of-day) with repeated measures. This was done to test two assumptions. The first is that within-subject metabolic state, particularly VCO_2, was the same on the 2 study days. The second is that a metabolic steady state was produced during each tracer feeding protocol such that there was no progressive shift in substrate oxidation.

Percent of energy derived from fat oxidation (F%) was estimated from respiratory quotient according to the equation of Lusk (38). Average respiratory quotient (RQ) for each subject was defined as the ratio of average VCO_2 to VO_2. Protein oxidation was assumed to equal intake, and a protein RQ of 0.835 was used to compute non-protein RQ, as recommended by Livesey and Elia (39).

Label recovery for each tracer was estimated from average VCO_2 as follows:

$$\%\ \text{Recovery} = VCO_2\ (\mu\text{mol/min}) \times IE \cdot CO_2\ (\text{APE}/100)/\text{tracer intake}\ (\mu\text{mol/min}) \times 100.$$

All results are expressed as mean ± SEM Confidence intervals (CI) were calculated for the mean of the ten individual ratios of oleate versus palmitate data for each outcome measure. Coefficient of variation (CV) was computed as SEM/mean × 100. Statistical significance was defined to be $P < 0.05$.

RESULTS

Indirect calorimetry

No significant intra-subject differences were found between the 2 study days or between times of day for VCO_2 or VO_2, which was in agreement with the experimental assumptions. Data from both protocols were therefore pooled for each subject in estimating F% and label recovery (60 observations over four 15-min collection periods).

The F% was estimated based on assuming that the 60 min of indirect calorimetry data was representative of the two 7-h tracer feeding periods. For the entire study group, F% was 42 ± 4%, which is not significantly different from the 40% of energy provided by the dietary fat. The range of estimated F% was 15 to 61%.

Estimated rate of energy expenditure during the tracer feeding protocols was 12 ± 2% above fasting resting energy expenditure. Therefore, rate of food intake during tracer feeding was 38 ± 2% more than energy expenditure, with a range of 28–50%. This was consistent with our goal of feeding subjects at a rate moderately above their energy expenditure.

Enrichment of breath CO_2

Figure 1 shows the time course of breath $^{13}CO_2$ enrichment for all subjects. Calculated average slopes for all subjects combined during the final hour were 17% per hour for oleate and 16% per hour for palmitate, which supports the assumption of similarity in the timing of isotope appearance in expired CO_2 for the two substrates. This is approximately double the slope during the same period in a preliminary study. In that study, however, a primer dose of [^{13}C]bicarbonate was used, which presumably hastened the process of achieving a plateau.

Table 2 shows the average plateau values of breath enrichment for each subject. It should be noted that the molar dose of palmitate tracer was 10% greater than oleate because equal weights were fed. The CV for plateau APE data ranged from 1% to 5%. Individual values of

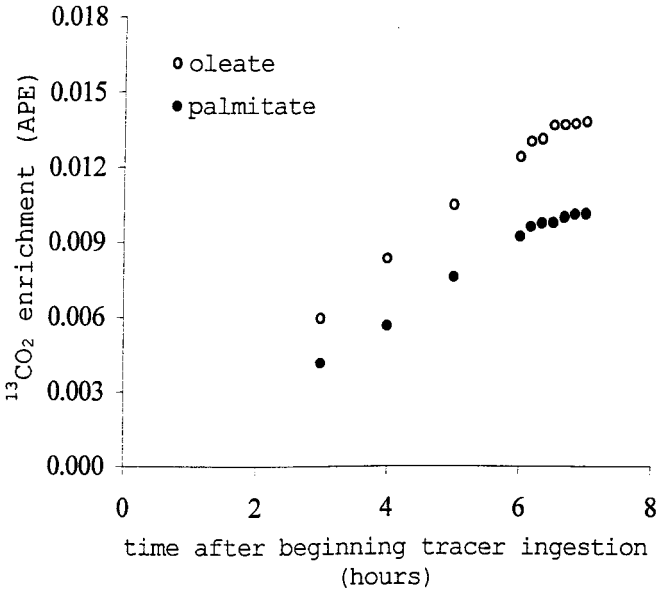

Fig. 1. Average isotope enrichment of breath CO_2 during oleate and palmitate tracer administration. 1-^{13}C-labeled fatty acids were fed in a liquid formula diet each 20 min for 7 h in a crossover design. Tracer dose was equal to 6.95 ± 0.23 μmol/kg per h palmitate and 6.31 ± 0.21 μmol/kg per h oleate. Error bars are not included because the data were analyzed by intrasubject comparison. Therefore, computing the standard error of the mean of all studies for each tracer would be misleading; ○ oleate tracer study; ● palmitate tracer study.

TABLE 2. Breath $^{13}CO_2$ enrichment and chylomicron-derived fatty acid ^{13}C enrichment

Subject	Oleate Tracer Study		Palmitate Tracer Study	
	IE·$CO_{2\,OA}$ (APE)	IE·OA (MPE)	IE·$CO_{2\,PA}$ (APE)	IE·PA (MPE)
3020	0.0137 ± 0.0002	3.42 ± 0.11	0.0108 ± 0.0002	2.84 ± 0.05
3022	0.0142 ± 0.0002	3.10 ± 0.11	0.0112 ± 0.0002	2.61 ± 0.08
3024	0.0112 ± 0.0002	2.32 ± 0.14	0.0096 ± 0.0004	2.71 ± 0.08
3025	0.0122 ± 0.0002	3.21 ± 0.15	0.0111 ± 0.0004	2.58 ± 0.13
3026	0.0157 ± 0.0003	3.62 ± 0.08	0.0126 ± 0.0002	2.57 ± 0.08
3027	0.0140 ± 0.0004	3.29 ± 0.08	0.0078 ± 0.0004	2.22 ± 0.04
3028	0.0107 ± 0.0001	3.19 ± 0.18	0.0096 ± 0.0001	2.56 ± 0.07
3029	0.0115 ± 0.0003	3.26 ± 0.06	0.0037 ± 0.0002	1.32 ± 0.07
3030	0.0149 ± 0.0003	4.12 ± 0.10	0.0080 ± 0.0001	3.44 ± 0.04
3031	0.0156 ± 0.0003	4.45 ± 0.18	0.0137 ± 0.0003	4.09 ± 0.08
Mean ± SEM	0.0134 ± 0.0006	3.40 ± 0.18	0.0098 ± 0.0009	2.69 ± 0.23

Individual values are averages during the final hour of tracer administration. 1-^{13}C-labeled fatty acids were fed in a liquid formula diet each 20 min for 7 h in a crossover design. Tracer dose was 5% of the unlabeled fatty acid in the diet, or 1.79 ± 0.06 mg/kg per h. IE·$CO_{2\,OA}$ and IE·$CO_{2\,PA}$ are breath $^{13}CO_2$ enrichment during final hour of oleate and palmitate tracer ingestion, respectively (n = 7 per protocol). IE·OA and IE·PA are ^{13}C enrichment of chylomicron-derived oleate and palmitate during the final hour of tracer ingestion, respectively (n = 4 per protocol). APE, atoms per cent excess; MPE, moles per cent enrichment.

IE·$CO_{2\,OA}$/IE·$CO_{2\,PA}$ were adjusted for differences in the number of molecules of carbon dioxide generated in oxidizing each tracer and are shown in **Table 3**. Average rate of label recovery in breath in the final hour of each protocol, based on extrapolated VCO_2 data, was 18 ± 1% for oleate and 12 ± 1% for palmitate. The two recoveries were significantly different by paired *t*-test ($P < 0.01$).

The difference in intrasubject baseline (t = 0:00) $^{13}CO_2$/$^{12}CO_2$ ratios for the two protocols (second minus first) ranged from −4E-6 to +34E-6. In six subjects there was an interval of at least 6 days between tracer protocols. In the remaining four subjects, whose tracer protocols were separated by 65 h, the difference in baseline values ranged from +2E-6 to +23E-6. Therefore, if the background ^{13}C was elevated by residual label from the first study, this effect was comparable to that of random changes in baseline caused by recent consumption of foods relatively high in ^{13}C. When enrichment is computed, baseline values are subtracted from enriched values, which controls for both effects as long as the rate of change of background is negligible.

For duplicate analyses of the 20 baseline samples, the standard deviation of the difference was 6E-6. In terms of signal-to-noise ratio, this compares favorably with the 116E-6 average change from baseline in the final hour of tracer feeding (i.e., plateau APE/100) observed herein.

Enrichment of oleate and palmitate

Figure 2 shows the time course of ^{13}C enrichment of CM-derived OA and PA for all subjects. Table 2 shows the average plateau values of CM-derived fatty acid enrichment for each subject. The CV for plateau MPE data ranged from 1% to 6%. Calculated average slopes for all subjects combined during the final hour were 5.7% per hour for oleate and 5.4% per hour for palmitate, which

TABLE 3. Individual ratios used in computing relative oxidation of oleate versus palmitate

Subject	IE·$CO_{2\,OA}$/IE·$CO_{2\,PA}$ × (18/16)[a]	IE·OA/IE·PA	*A*·OA/*A*·PA	Rel_{OX}
3020	1.43	1.20	1.06	1.11
3022	1.42	1.19	1.05	1.14
3024	1.30	0.86	1.10	1.38
3025	1.23	1.24	0.89	1.11
3026	1.40	1.41	0.98	1.01
3027	2.02	1.48	1.12	1.21
3028	1.25	1.25	0.99	1.02
3029	3.48	2.47	1.04	1.36
3030	2.10	1.20	1.13	1.55
3031	1.28	1.09	1.00	1.17
Mean ± SEM	1.69 ± 0.22	1.34 ± 0.14	1.04 ± 0.02	1.21 ± 0.05

Non-esterified 1-^{13}C-labeled fatty acids were emulsified in a liquid formula diet fed each 20 min for 7 h in a crossover design. TAG content of the diet was identical during the two protocols. Tracers made up 5% of their respective tracees, or 2% of total dietary fatty acids. IE·$CO_{2\,OA}$ and IE·$CO_{2\,PA}$ are breath $^{13}CO_2$ enrichment during final hour of oleate and palmitate tracer ingestion, respectively (n = 7 per protocol). IE·OA and IE·PA are ^{13}C enrichment of chylomicron-derived oleate and palmitate during the final hour of tracer ingestion, respectively (n = 4 per protocol). *A*·OA and *A*·PA are GC-FID peak areas of oleate and palmitate in CM samples (n = 6 total; 3 from each protocol). Rel_{OX} = (IE·$CO_{2\,OA}$ × *A*·PA × 18 × IE·PA)/(IE·$CO_{2\,PA}$ × *A*·OA × 16 × IE·OA).

[a] Corrects for difference in molar production of CO_2 from palmitate (16) versus oleate (18).

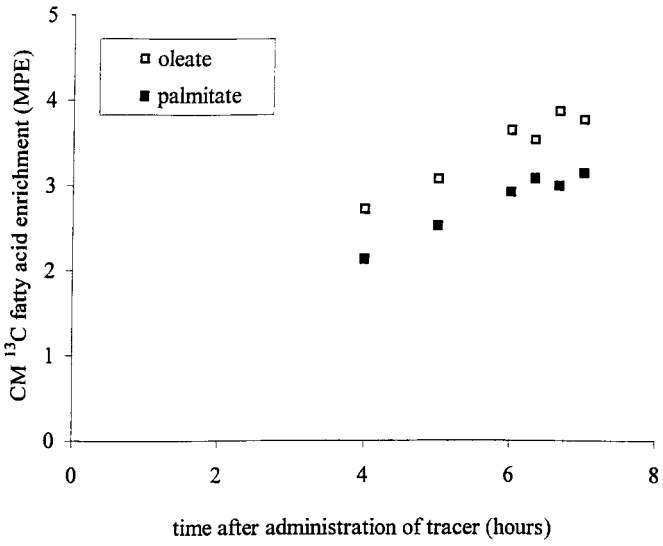

Fig. 2. Average isotope enrichment of chylomicron-derived oleate and palmitate during tracer administration. 1-^{13}C-labeled fatty acids were fed in a liquid formula diet each 20 min for 7 h in a crossover design. Tracer dose was approximately 5% of tracee (unlabeled) fatty acid in the diet, or 1.79 ± 0.06 mg/kg per h each. Error bars are not included because the data were analyzed by intra-subject comparison. Therefore, computing the standard error of the mean of all studies for each tracer would be misleading; □ oleate tracer study; ■ palmitate tracer study.

supports the assumption of similarity of timing of incorporation of the two substrates into CM. Individual values of IE·OA/IE·PA are given in Table 3. This ratio was significantly greater than 1.0 (95% CI: 1.03–1.64), indicating greater enrichment of CM-derived oleate than palmitate in contrast to the equal dietary enrichments. Average enrichment of oleate was 26% higher than palmitate, but 32% less than the calculated dietary oleate enrichment.

There was no significant difference in enrichment of the $S_f > 1000$ samples which were analyzed versus their corresponding $S_f > 400$ samples, indicating that the decrement in CM versus diet enrichment was not due to dilution of CM particles by larger lipoproteins of hepatic origin (32).

NEFA enrichment in a subset of 30 samples (2–6 per subject) was not significantly different from that of CM-derived fatty acids from the same plasma samples, assessed by paired *t*-test. On average, NEFA enrichment was 93 ± 11% of CM fatty acid enrichment, indicating minimal endogenous release of NEFA from adipose stores.

Chylomicron fatty acid composition

No significant intra-subject differences were found in CM composition (A·OA/A·PA)($P > 0.05$). This supports the assumption that feeding equal dietary TAG resulted in similar rates of appearance of unlabeled substrate during both protocols. Table 3 shows the average value for each subject. This ratio was not significantly different from 1.0 for all subjects combined (95% CI: 0.98–1.09). However, it was a significant factor in the final experimental outcome, because there was a difference of up to 13% between oleate and palmitate content of CM on an individual basis.

Relative oxidation of oleate versus palmitate

The relative oxidation of CM-derived oleate versus palmitate (Rel_{OX}) was 1.21 ± 0.05 with a 95% CI of 1.10–1.32 and a 99.5% CI of 1.03–1.39 (Table 3). Thus, on average, 21% more of the oleate delivered to plasma in CM was being oxidized than was the palmitate in CM. The range in Rel_{OX} was 1.01–1.55.

DISCUSSION

This study demonstrates higher fractional oxidation of CM-derived oleate than palmitate in healthy adults under certain dietary conditions. Two novel experimental approaches were used in comparing oxidation of these fatty acids. One was the use of frequent small meals to produce a steady state in metabolism, thus permitting use of steady-state tracer methodology. The other was to focus exclusively on metabolic processing which occurs downstream from enteral synthesis of CM. This was achieved by correcting the ^{13}C enrichment of CO_2 for the appearance of 1-^{13}C-labeled fatty acids in CM, and was intended to generate results that would complement those generated by other methods in which such corrections were not made.

Two previous studies have compared oxidation of these fatty acids in rats, with conflicting results. In one, 1-^{14}C-labeled tracers were dissolved in 0.2 ml olive oil and given to fasting rats by stomach tube (11). Over the following 24 h, 57 ± 2% of the oleate label versus 32 ± 3% of palmitate label was recovered in breath. Absorption of tracer was not assessed. By contrast, in the other study 1-^{14}C-labeled tracers were transesterified to soy oil TAG and given in 5.5 g of a liquid diet (13). Recoveries of the two labels were similar throughout the 51-h protocol, with total recovery being 66 ± 4% of oleate and 70 ± 2% of palmitate label. Absorption of both tracers was >96%. Differences in methods of tracer administration in those two studies may account for their conflicting results.

No human studies have compared oleate and palmitate oxidation. However, Jones, Pencharz, and Clandinin (9) compared oleate, linoleate, and stearate oxidation in a protocol in which encapsulated tracers were given in a mixed meal, and recovery of label from breath was assessed over the next 9 h using area-under-the-curve techniques. In that study, recovery of absorbed oleate tracer was 15 ± 6%, linoleate recovery was 10 ± 4%, and stearate recovery was 3 ± 2%.

In the present research, CM fatty acid enrichment and composition were used to control for potential differences in digestion and absorption of both the tracers and the unlabeled, tracee fatty acids. One major consideration in making this choice of methodology was potential malabsorption of the tracers, which were in the form of NEFA, as compared with dietary tracees, which were in the form of TAG. Previous research has shown wide variations in NEFA absorption. For example, in rats given NEFA in an otherwise fat-free diet, absorption of [1-^{14}C]palmitate was only 30% after 8 h and 40% after 24 h (15). By contrast, when NEFA were dissolved in olive oil, [1-^{14}C]palmitate

absorption in rats was 60% after 6 h in one study (10), and 97% after 24 h in another study (11). Dissolving the NEFA tracers in oil may have caused them to be assimilated similarly to the TAG. When tracers were esterified to TAG in another study, both palmitate and oleate were 96% absorbed after 51 h (13).

In humans, fat absorption can only be assessed by fecal collection, so its time course cannot be measured. However, when Jones et al. (9) fed healthy adults encapsulated NEFA tracers with a mixed meal, absorption of [1-^{13}C] stearate was 78% whereas absorption of dietary stearate was 92%. Absorption of capsule-fed non-esterified [1-^{13}C] oleate was 97% in that study, or virtually complete, just as it was in rats fed [1-^{14}C]oleate in TAG form (13). In a study designed to assess intra-subject variability in absorption of non-esterified palmitate, [1-^{13}C]palmitate was mixed with butter and fed to healthy women with bread and juice. Tracer absorption was 86 $\pm$ 10% in the first trial and 68 $\pm$ 25% in a second trial in the same subjects (40). Fecal excretion of tracer was unrelated to total lipid excretion in that study. Thus, while oleate tracer absorption is almost consistently complete, there is considerable inter- and intra-subject variation in absorption of non-esterified palmitate.

These literature findings support our conjecture that the low enrichment of CM-palmitate versus oleate reported in our study was due, at least in part, to reduced absorption of palmitate tracer relative to oleate tracer.

A further consideration in analyzing CM is the dilution of dietary fat and tracers by endogenous fatty acids, which are evidently substantial both in rats (18, 19) and humans (20). Because of this, we could not assume that lower fatty acid enrichment of CM relative to diet was due to malabsorption of tracer relative to dietary TAG. Dilution by endogenous fatty acids would also reduce enrichment.

Regardless of the differences between dietary and CM fatty acids, CM are the end product of enteral processing of diet-derived fat, and therefore present a useful opportunity to study its further metabolism. This assumes, however, that dietary oleate and palmitate are not transported directly to portal circulation from enterocytes. Mansbach, Dowell, and Pritchett (41) found that when rats were infused with 135 μmol/h of [^{3}H]triolein into the duodenum, 39% of labeled oleate entered the portal vein directly. In a later study, 16.5% of a 27 μmol/h [^{3}H]triolein infusion entered the portal circulation (42). However, when phosphatidylcholine was added to the infusate, this dropped to 0.5% at the lower rate of infusion, and 1.4% at the higher rate (42). In the present research, diet TAG and tracers were emulsified with phosphatidylcholine such that the rate of intake (moles/kg body weight) was similar to that used in rats. The ingestion rate of TAG herein was also comparable to the lower infusion rate used in rats on the basis of body weight. On the basis of estimated metabolic rate (43), our subjects' fat ingestion was roughly one-third that of the higher rate used by Mansbach and Dowell (42). Therefore, it is unlikely that significant tracer was transported to portal circulation in this experiment.

A final assumption which should be addressed is that metabolism of tracer fatty acids in CM is identical to that of their tracees. Most palmitate in palm oil is on the *sn*-1 or *sn*-3 positions of the glycerol backbone of TAG, and a majority of oleate is in the *sn*-2 position (44). During fat digestion, about 75% of the 2-position of dietary TAG is conserved in CM TAG (45, 46), so it is likely that a majority of both tracers were incorporated in the *sn*-1 or *sn*-3 positions in CM. This would not cause differences in metabolism of the two tracers, or differences between palmitate tracer and its tracee. However, to the extent that oleate tracer was over-represented on the *sn*-1 and *sn*-3 positions relative to its tracee, there was an opportunity for differential fates of tracer and tracee oleate in the periphery. Although lipoprotein lipase only hydrolyzes the 1- and 3-positions of TAG (47) hydrolysis of fatty acids from TAG in plasma appears to be complete, so that no 2-monoacylglycerol is taken up (48).

Another difference between oleate tracer and tracee due to stereopositional isomerism was the potential for greater incorporation of tracer into phospholipids or cholesteryl esters, which make up 3–6% and 2–5% of CM, respectively (49). However, when oleate and palmitate tracers were given to rats, 94–98% were recovered in TAG of CM (18).

Whenever repeated tracer protocols are performed in the same subject, there is concern as to whether the label is adequately washed out between studies. Our data do not support the conclusion that there was a significant effect of residual label from the first protocol on baseline CO_2 enrichment in the second protocol. Such an effect would not, however, alter our computed APE values, because the rate of change of background enrichment would be negligible. The half-life of labeled CO_2 after [1-^{13}C]palmitate infusion, in fasting adults at rest, has been reported to be 107 min (28). Thus, $^{13}CO_2$ excretion should return to within 1% of baseline by 46 h after delivery of labeled substrate to plasma ceases in fasting subjects confined to bed. In a preliminary study, we found that APE fell by 20% during the period between 40 and 60 min after ingestion of the final tracer dose (data not shown). Some portion of the tracer was also presumably stored in adipose tissue. Total tracer dose per protocol ranged from 0.6 to 0.9 g, and the average adult has body fat stores of 10–20 kg. Therefore, whole-body dilution of stored tracer would be $<$1:10,000. Even if some endogenous fat were oxidized during a subsequent feeding protocol, stored tracer oxidation would not be detectable.

If there are differences in fatty acid oxidation, it is logical to search for mechanistic explanations. Fatty acid specificities have been reported for several enzymes and transporters. In plasma, NEFA are transported bound to albumin. Differences in affinities for oleate versus palmitate have been reported in three binding sites of bovine serum albumin (50). The process by which fatty acids cross the cell membrane is apparently saturable, but the evidence suggests that oleate and palmitate do not compete (51). However, the distribution of binding between inner and outer membrane at saturation is remarkably different, with ole-

ate having an inner/outer ratio of 0.45 while the ratio for palmitate is 4 to 5 (51). Within the cell, fatty acids are thought to be transported by the family of fatty acid binding proteins (FABP). Oleate and palmitate binding affinities have been similar in the human FABP studied thus far, including liver, muscle (52), and adipose tissue (53).

Net uptake of plasma fatty acids is a function of interactions between albumin, membrane transport, and intracellular processes, including FABP binding. Thus, no single isolated binding specificity can be considered meaningful in isolation. Studies, which have measured fasting fractional uptake of fatty acids by human heart (54), liver (55), or forearm (55) or perfused rat liver (56) have failed to demonstrate any difference between palmitate and oleate.

Several intracellular enzymes regulate aspects of fatty acid metabolism. Acyl-CoA synthetases from rabbit heart and liver (57) have marked substrate specificities, although human data are lacking. In one study, the rabbit heart enzyme had a higher affinity for oleate than palmitate, but a higher maximal activity for palmitate (57). Carnitine palmitoyl transferase I catalyzes the rate limiting step in fatty acid oxidation. Rat liver carnitine palmitoyl transferase I was found to have a lower affinity for oleoyl-CoA than palmitoyl-CoA in one study (58). In another, enzyme activity towards oleoyl-CoA was more sensitive to inhibition by malonyl-CoA than was activity towards palmitoyl-CoA (59). Both of these findings would favor oxidation of palmitate.

The present finding of unequal fractional oxidation has implications for research in which palmitate tracers are used to study whole-body fat oxidation. That is, in many studies, fractional oxidation of all fatty acids is assumed to be equal, and absolute palmitate oxidation can therefore be extrapolated to compute total fatty acid oxidation. This approach has been shown to provide a reasonably accurate estimation of fat oxidation when compared with indirect calorimetry results in fasting subjects at rest or during moderate exercise (60, 61). This may be because fractional palmitate oxidation is intermediate among all fatty acids. That is, if oleate fractional oxidation is higher, but that of stearate and longer-chain fatty acids is lower (9), then palmitate might coincidentally provide an accurate average value for absolute oxidation. On the other hand, the oxidation of CM-derived fatty acids in the present fed-state study cannot be directly generalized to fasting subjects, whose fat oxidation is primarily derived from NEFA released from adipose stores.

In conclusion, we have found evidence in healthy adults for preferential oxidation of CM-derived oleate compared to palmitate when both are present in similar quantities and fed in small, frequent meals. The results from this and similar studies may be of relevance to the differential effects of oleate and palmitate on disease risk factors. For example, if a diet higher in oleate enhanced fat oxidation during digestion, storage of carbohydrate as glycogen might be enhanced. Even a subtle shift in fat oxidation might produce a significant effect on glycogen when integrated over the course of a day. Further studies are required to investigate this possibility.

APPENDIX

Use of steady-state equations

When absolute substrate oxidation is calculated in a steady-state protocol, it is necessary to achieve a quasi-steady state, or plateau, in excretion of labeled CO_2. Otherwise, absolute oxidation is underestimated. In that case, two distinct types of steady state are required: an unchanging and well-characterized metabolic state and a quasi-steady rate of excretion of label from the CO_2 pool. The term "quasi-steady state (rate)" is used because a true isotopic steady state will not occur until the isotopic enrichment of the element of interest (in this case, carbon) matches that of the "environment" (in this case, the ^{13}C enrichment of the diet and tracer mixture). At that point in time, tracer conditions no longer exist. However, it is important in tracer studies to demonstrate, in a relative sense, that the enrichment of a particular substrate (e.g., fatty acid) or end product (e.g., CO_2) is a consequence of metabolic processes of importance such as dilution by unlabeled "tracee" or transfer of label from one compound (e.g., fatty acid) to another (e.g., CO_2) (i.e., oxidation) and not due to "mere" delays in the delivery of the isotope to relevant metabolic pools and subsequent mixing. The "plateau" or quasi-steady state only can be defined operationally in terms of the relative variation in isotopic enrichment at the sampling time used for the pertinent calculation(s). Often, this is done by inspection or by observing that the slope does not differ significantly from zero. Obviously, using the slope approach, there is considerable Type II error with only a few points, so slopes are usually not significantly different from zero. In our study, we were much more interested in demonstrating similar slopes for the change over time in isotopic enrichment of CM-derived PA or OA or CO_2. This rationale is amplified in the next paragraph.

In the present experiment, fractional oxidation of two substrates was compared under conditions of metabolic steady state. In this case, if the timing of the appearance of label into CO_2 is the same for the two substrates, then the ratio of the two enrichments remains constant at each time point in the protocol, so that it is not necessary to achieve steady-state label excretion. For example, if the $^{13}CO_2$ enrichment at the end of the protocol was 80% of the "true" steady-state value, when the ratio of the two enrichments is taken, the two values of 0.80 will cancel. Expressed mathematically, $IE \cdot a / IE \cdot b = (IE \cdot a \times 0.80) / (IE \cdot b \times 0.80)$.

Derivation of relative oxidation equation

The expression used herein for relative fractional fatty acid oxidation is derived as follows. In a metabolic steady state, the absolute rate of oxidation of a given fatty acid (FA_{OX}) is equal to its rate of appearance in the precursor pool for oxidation ($Ra \cdot FA$) multiplied by the fraction of substrate that is oxidized ($FA_{fract\ OX}$) (34). In this experiment the precursor pool is CM, by definition. Fractional oxidation, then, can be expressed:

$$FA_{fract\ OX} = \frac{FA_{OX}\ (\mu mol/min)}{Ra \cdot FA (\mu mol/min)}. \qquad Eq.\ 1)$$

When tracers are infused at a constant rate, designated *F*, the rate of infusion can be used to determine Ra·FA, because isotope enrichment of the substrate precursor pool, IE·FA, is equal to the fraction of the precursor pool that is composed of tracer. This fraction is in turn equal to the ratio between rates of appearance of labeled and unlabeled substrate. Therefore:

$$\text{IE} \cdot \text{FA} = \frac{F(\mu\text{mol/min})}{\text{Ra} \cdot \text{FA}(\mu\text{mol/min})}, \qquad \textit{Eq. 2})$$

If the fraction of tracer oxidized, $\text{TR}_{\text{fract OX}}$, is assumed to equal the fraction of unlabeled substrate oxidized, then fractional substrate oxidation can be computed from the rate of label excretion, V^{13}CO_2, relative to the infusion rate. Thus,

$$\text{FA}_{\text{fract OX}} = \text{TR}_{\text{fract OX}} = \frac{\text{V}^{13}\text{CO}_2(\mu\text{mol/min})}{F(\mu\text{mol/min}) \times c}.$$

The isotope exchange correction factor, *c*, accounts for the temporary loss of a portion of labeled carbon due to exchange with endogenous pools of unlabeled CO_2 and tricarboxylic acid cycle intermediates (28, 34, 62). Rearranging the equation above, rate of label excretion in breath CO_2 can be expressed:

$$\text{V}^{13}\text{CO}_2(\mu\text{mol/min}) = \text{FA}_{\text{fract OX}} \times F(\mu\text{mol/min}) \times c. \qquad \textit{Eq. 3})$$

However, the rate of label excretion is also equal to the total rate of excretion of CO_2 in breath, VCO_2, multiplied by enrichment of breath $^{13}CO_2$, designated $\text{IE}\cdot\text{CO}_2$. (Note that the logic for this relationship is similar to that of equation 2.)

$$\text{V}^{13}\text{CO}_2(\mu\text{mol/min}) = \text{VCO}_2(\mu\text{mol/min}) \times \text{IE} \cdot \text{CO}_2. \qquad \textit{Eq. 4})$$

Therefore, equations 3 and 4 can be set equal and rearranged to become:

$$\text{FA}_{\text{fract OX}} = \frac{\text{VCO}_2(\mu\text{mol/min}) \times \text{IE} \cdot \text{CO}_2}{F(\mu\text{mol/min}) \times c}. \qquad \textit{Eq. 5})$$

Because the aim herein was to compare the oxidation of two substrates, some additional annotation of terms is required. In formulating a single expression for relative oxidation, it is necessary to denote which tracer was being fed in each of the terms. Modifying equation 5 for fractional oleate oxidation yields:

$$\text{OA}_{\text{fract OX}} = \frac{\text{VCO}_{2\,\text{OA}}(\mu\text{mol/min}) \times \text{IE} \cdot \text{CO}_{2\,\text{OA}}}{F_{\text{OA}}(\mu\text{mol/min}) \times c_{\text{OA}}}. \qquad \textit{Eq. 6a})$$

Similarly, for fractional palmitate oxidation:

$$\text{PA}_{\text{fract OX}} = \frac{\text{VCO}_{2\,\text{PA}}(\mu\text{mol/min}) \times \text{IE} \cdot \text{CO}_{2\,\text{PA}}}{F_{\text{PA}}(\mu\text{mol/min}) \times c_{\text{PA}}}. \qquad \textit{Eq. 6b})$$

Relative oxidation of the two fatty acids, Rel_{OX}, was defined as the ratio of the two fractional oxidation rates, or $\text{OA}_{\text{fract OX}}/\text{PA}_{\text{fract OX}}$. If one assumes that metabolic conditions remain constant between the two protocols, then $c_{\text{OA}} = c_{\text{PA}}$ and $\text{VCO}_{2\,\text{OA}} = \text{VCO}_{2\,\text{PA}}$. These terms then cancel, and the simplified expression becomes:

$$\text{Rel}_{\text{OX}} = \frac{\text{IE} \cdot \text{CO}_{2\,\text{OA}}/F_{\text{OA}}}{\text{IE} \cdot \text{CO}_{2\,\text{PA}}/F_{\text{PA}}}. \qquad \textit{Eq. 7})$$

When a tracer is infused directly to the circulation, its rate of entry, *F*, is known precisely. In the present protocol, however, there is uncertainty in this value due to the possibility of incomplete incorporation of ingested tracers into CM. Therefore, an expression of *F* is needed that enables the computation of Rel_{OX} from the available data. Rearranging equation 2 and modifying the notation to reflect an oleate tracer study yields:

$$F_{\text{OA}}(\mu\text{mol/min}) = \text{Ra} \cdot \text{OA}_{\text{OA}}(\mu\text{mol/min}) \times \text{IE} \cdot \text{OA}. \qquad \textit{Eq. 8a})$$

Similarly, for a palmitate tracer study:

$$F_{\text{PA}}(\mu\text{mol/min}) = \text{Ra} \cdot \text{PA}_{\text{PA}}(\mu\text{mol/min}) \times \text{IE} \cdot \text{PA}. \qquad \textit{Eq. 8b})$$

Substituting equations 8a and 8b into equation 7:

$$\text{Rel}_{\text{OX}} = \frac{\text{IE} \cdot \text{CO}_{2\,\text{OA}}/[\text{Ra} \cdot \text{OA}_{\text{OA}}(\mu\text{mol/min}) \times \text{IE} \cdot \text{OA}]}{\text{IE} \cdot \text{CO}_{2\,\text{PA}}/[\text{Ra} \cdot \text{PA}_{\text{PA}}(\mu\text{mol/min}) \times \text{IE} \cdot \text{PA}]}. \qquad \textit{Eq. 9})$$

Like *F*, the rates at which oleate and palmitate in CM enter the circulation are not known. However, in a steady-state protocol the ratio between these two rates is equal to the ratio of their concentrations in plasma CM. This is based on the assumption that fractional turnover rates of the two substrates in plasma are identical because they are both in the same CM particles, rather than circulating independently of each other as is the case for NEFA. A second assumption that must be made is that Ra of each unlabeled substrate in CM is the same during both protocols. That is, $\text{Ra}\cdot\text{OA}_{\text{OA}} = \text{Ra}\cdot\text{OA}_{\text{PA}}$, and $\text{Ra}\cdot\text{PA}_{\text{OA}} = \text{Ra}\cdot\text{PA}_{\text{PA}}$. This is based on the feeding of identical dietary TAG during both protocols. Therefore:

$$\frac{\text{Ra} \cdot \text{OA}(\mu\text{mol/min})}{\text{Ra} \cdot \text{PA}(\mu\text{mol/min})} = \frac{\text{C} \cdot \text{OA}(\mu\text{mol/min})}{\text{C} \cdot \text{PA}(\mu\text{mol/min})}. \qquad \textit{Eq. 10})$$

Where C·OA and C·PA represent the concentrations of CM-derived oleate and palmitate in plasma, respectively.

The amounts of oleate and palmitate in a given sample can be determined by GC-FID. Within a given GC-FID sample run, the response factor relating peak area to carbon mass remains constant, and therefore cancels when the ratio of two peak areas is taken. In addition, when the two substrates are measured within the same sample, the plasma volume terms cancel. Hence, the ratio of oleate carbon versus palmitate carbon present in a given CM sample can be expressed $A\cdot\text{OA}\ (\mu\text{g})/A\cdot\text{PA}(\mu\text{g})$, where $A\cdot\text{OA}$ and $A\cdot\text{PA}$ are the GC-FID-derived peak areas of oleate and palmitate, respectively.

At this point, it only remains to convert the ratio of areas to the basis of μmoles rather than carbon mass. This can be accomplished by dividing the peak area terms by the number of carbon atoms per molecule of substrate, which is 18 for oleate and 16 for palmitate. Therefore:

$$\frac{\text{Ra} \cdot \text{OA}_{\text{OA}}}{\text{Ra} \cdot \text{PA}_{\text{PA}}} = \frac{A \cdot \text{OA}/18}{A \cdot \text{PA}/16}. \qquad \textit{Eq. 11})$$

Substituting equation 11 into equation 9 and rearranging gives the final expression for relative oxidation:

$$\mathrm{Rel}_{OX} = \frac{IE\ CO_{2\,OA} \times A\ PA \times 18 \times IE \cdot PA}{IE\ CO_{2\,PA} \times A \cdot OA \times 16 \times IE \cdot OA}. \quad \textit{Eq. 12})$$

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