

## [<sup>13</sup>C]Linoleic acid oxidation and transfer into milk in stunted lactating women with contrasting body mass indexes<sup>1-3</sup>

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### ABSTRACT

**Background:** The fat concentration of human milk is associated with maternal adiposity, but there is no clear understanding of the mechanisms controlling milk fat concentration.

**Objective:** We evaluated the effect of postpartum body mass index (BMI; in kg/m<sup>2</sup>) on the metabolic distribution of an oral dose of [<sup>13</sup>C]linoleic acid in lactating women.

**Design:** Ten lactating women stratified by BMI (either <22.5 or >23.5) at 5 mo postpartum received orally 2.5 mg [<sup>13</sup>C]linoleic acid/kg body wt. Exhaled air, milk, and plasma samples were collected in relation to tracer administration. Linoleic acid was determined by gas chromatography. Dietary intake, serum, milk composition, [<sup>13</sup>C]linoleic acid enrichment in milk and plasma, and exhaled <sup>13</sup>CO<sub>2</sub> (by isotope ratio mass spectrometry) were assessed.

**Results:** Women with a higher BMI exhaled more <sup>13</sup>CO<sub>2</sub> than did women with a lower BMI (22.8 ± 9.4% compared with 8.6 ± 3.5% of dose, *P* < 0.03). Cumulated 72-h transfer of [<sup>13</sup>C]linoleic acid to milk was not significantly different between groups (14.8 ± 6.5% compared with 17.7 ± 6.7% of dose). Within the first 9 h after dose administration, 51.6 ± 4.9% of the total isotope transfer into milk had passed in women with a higher BMI, but only 24.0 ± 15.3% had passed in those with a lower BMI (*P* = 0.02).

**Conclusions:** Women with a lower BMI, who were reputed as having less body fat, oxidized and secreted into milk less dietary linoleic acid within 12 h after tracer administration than did women with a higher BMI. In both groups, a large proportion of [<sup>13</sup>C]linoleic was retained in the maternal compartment, most likely fat tissue, in a slow turnover pool, and released slowly in later hours. *Am J Clin Nutr* 2001;74:827–32.

**KEY WORDS** [<sup>13</sup>C]Linoleic acid, human milk, lactation, milk fat, isotope ratio mass spectrometry, body mass index, BMI, body fat, stunted, Mexico

### INTRODUCTION

The lipid concentration in human milk is positively related to maternal body fat mass in women with low to normal BMIs living in both underprivileged (1–4) and affluent (5–7) societies, although no association between dietary composition and total milk lipid concentration has been shown (2, 5, 6). Controlled

longitudinal studies in a rural community of Mexico (8, 9) found significant differences in the milk fat concentration of women who were in the extreme ranges of adiposity, living within the same community, and eating diets with comparable composition. There is no clear understanding of the mechanisms controlling milk fat concentration. Some clues may be found in studies we conducted in the same community, which addressed the circadian variability of total milk fat and its fatty acid composition (10, 11). In these studies, higher total milk fat concentrations were seen in the absorptive state, which was associated with a higher proportion of long-chain saturated and monounsaturated fatty acids (presumably derived from direct intestinal absorption of dietary fats). In contrast, during the prolonged postabsorptive state resulting from the habitual schedule of 2 meals/d of this population, total milk fat concentrations were lower, coinciding with higher relative concentrations of saturated medium-chain fatty acids (most probably from de novo synthesis in the mammary gland) (12).

The latter pattern was not universal. Some subjects showed a permanent predominance of long-chain fatty acids in their milk, despite having similar variations in the total milk fat concentration as did other subjects. These results are not easy to interpret because the fatty acid composition in human milk is highly variable. The relative proportions of lipid and carbohydrate in the diet, the fatty acid composition of dietary fat, the maternal energy balance and the composition of maternal body fat stores influence such variation (13–15).

To investigate the potential relation between maternal body fat mass and milk fat concentration, we compared the metabolic distribution of [<sup>13</sup>C]linoleic acid in 2 groups of stunted, lactating women stratified by body mass index (BMI). [<sup>13</sup>C]Linoleic acid

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was administered as an oral bolus to trace the metabolic fate of dietary fatty acids so that we could assess what proportion is converted to carbon dioxide and what proportion is transferred into milk fat. The rationale to use linoleic acid as a tracer was based on the fact that the relative concentration of linoleic acid is 22% of the total breast milk fat and 25% of the total lipids in the habitual diet of lactating women in this community (16). We investigated the working hypothesis that women with lower BMIs would oxidize more and transfer less [ $^{13}\text{C}$ ]linoleic acid to their milk than would those with a higher adiposity.

## SUBJECTS AND METHODS

### Subjects

Ten healthy women from the local prenatal clinic in San Mateo Capulhuac, Mexico, who exclusively breast-fed their infants, entered the study at 5–6 mo postpartum. They fulfilled the following inclusion criteria: uneventful pregnancy, delivered singletons weighing >2500 g, aged 18–34 y, parity <4, height between 145 and 154 cm (stunted), free of any chronic disease, and no regular consumption of any medication or alcohol. Women were stratified by their BMI ( $\text{kg}/\text{m}^2$ ) into 2 groups of 5 subjects each: a low BMI group (<22.5; L-BMI) and a high BMI group (>23.5; H-BMI). These cutoff points were derived from a previous survey of 256 lactating women in the community and represent  $\pm 1$  SD of the BMI distribution. In this way, it is more likely that significant differences may be detected than if individuals contained within  $\pm 1$  SD were included.

The characteristics of the village have been described elsewhere (2). In short, it is located in a mountainous area 150 km northwest of Mexico City, is 2800 m above sea level, and has 5500 inhabitants. The community lives on subsistence agriculture, with maize as their main staple food source. The habitual dietary intake of lactating women includes  $201.72 \pm 25.5$  kJ/kg body wt ( $48.2 \pm 6.1$  kcal/kg) for energy, and  $0.8 \pm 0.1$  g protein/kg. Carbohydrates provide  $\approx 75\%$  and lipids 17% of the energy intake. Cooking oil (mostly sesame seed and sorghum) is the main source of dietary lipid intake (16, 17).

The study protocol was reviewed and approved by the Ethics Committee of Instituto Mexicano del Seguro Social. Written, informed consent was obtained from all subjects after the nature, procedures, and burdens of the study were explained to them.

### Methods

Subjects were admitted to the local health facility for 5 d on the evening of day 0. After 12 h of resting and fasting, the women received one dose of uniformly labeled [ $^{13}\text{C}$ ]linoleic acid (2.5 mg/kg body wt) (Martek Bioscience, Columbia, NY). The dose was offered as free acid embedded in a piece of bread along with breakfast the morning of the first study day (14). Before and after the administration of the tracer, breath, milk, and blood samples were obtained, and total carbon dioxide production was determined by indirect gas calorimetry. All women were encouraged to maintain their habitual daily activity pattern, ie, cooking, house cleaning, laundry, etc, within the grounds of the health center.

#### *Habitual dietary intake and design of the controlled diet*

The subjects' usual dietary intake was assessed during the week before the study by a combination of test weighing for 2 d and 24 h recall for 1 d in subjects' households. Macronutrients and

energy intakes were calculated by comparison with Mexican food-composition tables (18). Based on the latter information, subjects received a controlled diet during the 4th d of the study, which resembled the composition and energy density of their habitual diets. Duplicates of this controlled diet were collected, homogenized, and frozen at  $-20^\circ\text{C}$  until analysis of total fat, by gravimetry, and linoleic acid concentration, by gas chromatography.

#### *Milk samples for [ $^{13}\text{C}$ ]linoleic acid enrichment and fat concentration*

To determine [ $^{13}\text{C}$ ]linoleic acid enrichment, milk samples were collected at 0, 6, 9, 12, 15, 24, 36, 48, and 72 h, relative to the time the tracer was ingested. To include variations in the fat content of milk, the samples were composed of 2 fractions of 5 mL each and were pumped from the same breast (Egnell breast-pump; Egnell, Inc, Cary, IL) before and after the infants suckled until satiation. Milk samples were pooled and stored at  $-70^\circ\text{C}$  until analysis of the isotopic enrichment of linoleic acid by isotope ratio mass spectrometry.

The total fat concentration, assessed by gravimetry, and the linoleic acid percentage, assessed by gas chromatography, were determined in a pooled milk sample that was representative of 24 h milk secretion. The pooled milk sample was obtained by completely expressing the milk content of the left breast, which the infant was not allowed to suckle for 2 h, at 1000, 1400, and 1800 by use of an electric breast pump (Egnell, Inc). Aliquots were drawn and the remainder of the milk was offered to the infant.

Total milk production was measured by test-weighing the infant before and after nursing for 2 consecutive days by using an electronic balance (Sartorius 3862MP8; Sartorius, Göttingen, Germany) with a precision of 0.1 g. To reduce variability, the balance was equipped with an electronic device that averaged 100 weighing signals as a final reading.

#### *Total carbon dioxide production and [ $^{13}\text{C}$ ]enrichment in breath samples*

The 24-h total carbon dioxide production and oxygen consumption were measured on the second day of the study with a Delta-Trac metabolic monitor (DeltaTrac, Inc, Yorba Buena, CA). Measurements were made in a thermoneutral environment with the subject lying in a supine decubitus position. The basal metabolic rate was measured after the subjects slept for 12 h and subsequent measurements were performed every hour for 12 h. Subjects did not rest before measurements to include increments of carbon dioxide production and oxygen consumption associated with physical activity and of thermogenesis associated with food. Breath samples were collected before and after the administration of the tracer at hourly intervals for the first 12 h and then every 24 h for the following 3 d. Exhaled air was collected initially in breath Douglas bags and aliquots were then transferred into evacuated glass tubes and stored at  $4^\circ\text{C}$  until analysis of the [ $^{13}\text{C}$ ]enrichment of carbon dioxide.

#### *Maternal anthropometry*

Body weight, at the beginning and at the end of the study, and height were measured in fasting mothers who wore light clothing by using an electronic balance with a precision of 0.1 kg (Tanita, Tokyo) and a stadiometer with a precision of 1 mm (Holtain Limited, Crymch, United Kingdom). Triceps, biceps, subscapular, and suprailiac skinfold thicknesses were measured with a Lange-type caliper (Cambridge Scientific Industries, Cambridge, MA)



calibrated to the nearest millimeter. Waist, hip, arm, and calf circumferences were measured by using a nonextensible measuring tape. All anthropometric measurements were performed by the same trained observer (AL) and standardization was conducted as recommended by Habicht (19). The percentage of body fat was calculated from the skinfold thicknesses by using the equation of Durnin and Womersley (20).

#### Gas chromatography and isotope ratio mass spectrometry

The [<sup>13</sup>C]enrichment of carbon dioxide was determined by isotope ratio mass spectrometry (Delta S; Finnigan MAT, Bremen, Germany) after chromatographic purification of the carbon dioxide in a continuous flow inlet system, as previously described (14).

Total fat was extracted into organic solvents from milk and diet samples and was quantified gravimetrically (21). Fatty acids were transmethylated with methanolic hydrochloric acid (3 mol/L) at 85°C for 1 h. The transmethylated fatty acids were stored in hexane and 2 g butylhydroxytoluene/L, which served as an antioxidant. Isotopic analysis and quantification of individual fatty acids by gas chromatography were performed on aliquots of this solution (22).

Individual fatty acids were quantified with an HP5890 Series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector. Separation of fatty acid methyl esters was performed with a BPX70, 50-m column (SGE; Weiterstadt, Germany), as described elsewhere (14). Results are expressed as weight percentages of fatty acids with chain lengths from 14 to 24 carbon atoms.

Methyl esters of [<sup>13</sup>C]linoleic acid in milk and plasma lipids were measured by gas chromatography–combustion isotope ratio mass spectrometry (Hewlett-Packard gas chromatograph interfaced to a Finnigan MAT delta S mass spectrometer) by using chromatographic conditions as described above. Under these conditions, the separated fatty acid methyl esters were combusted online after they were eluted from the column, and the <sup>13</sup>C:<sup>12</sup>C ratio was determined by the resulting carbon dioxide. The δ<sup>13</sup>C values were obtained by comparing the <sup>13</sup>C:<sup>12</sup>C ratio in the sample to the international Pee Dee Belemnite standard (23).

#### Calculations

The daily secretion of linoleic acid in milk was calculated by multiplying the 24-h milk volume by the total fat concentration and then multiplying by the percentage weight of linoleic acid. The amount of [<sup>13</sup>C]linoleic acid secreted into milk in a given time was calculated as the percentage of <sup>13</sup>C in linoleic acid multiplied by the moles of carbon secreted as linoleic acid during a given period of time. The moles of [<sup>13</sup>C]linoleic acid secreted in milk were expressed as a percentage of the dose administered in recovery studies, and as μmol/h in kinetic studies.

The change from baseline calculated by subtracting the δ[<sup>13</sup>C] values from baseline samples of those in individual samples obtained after the administration of the tracer. The percentage of <sup>13</sup>C in linoleic acid was calculated from these δ[<sup>13</sup>C] values. The area under the curve for the [<sup>13</sup>C]linoleic acid secreted as <sup>13</sup>CO<sub>2</sub> and as [<sup>13</sup>C]linoleic acid in milk was calculated by standard procedure.

#### Statistical analysis

The SPSS statistical software program (version 9; SPSS Inc, Chicago) was used for data analysis. Differences between means for descriptive variables were analyzed with Student's *t* test. Significance was defined as a *P* value < 0.05. All values are presented as means ± SDs.

**TABLE 1**

Some anthropometric characteristics of the lactating women<sup>1</sup>

	BMI < 22.5 ( <i>n</i> = 5)	BMI > 23.5 ( <i>n</i> = 5)	<i>P</i>
Age (y)	22.2 ± 3.7	20.6 ± 3.21	NS
Height (cm)	149.2 ± 2.7	149.5 ± 3.0	NS
Weight (kg)	45.7 ± 3.8	54.3 ± 5.1	0.01
BMI (kg/m <sup>2</sup> )	20.5 ± 1.2 (19.0–21.6) <sup>2</sup>	24.3 ± 1.7 (23.3–25.9)	0.004 —
Circumferences (cm)			
Waist	74.6 ± 2.7	82.6 ± 6.1	0.04
Hip	87.0 ± 1.7	94.2 ± 2.8	0.002
Arm	23.6 ± 1.5	26.6 ± 2.8	0.07
Calf	29.5 ± 1.4	31.7 ± 1.2	0.03
Skinfold thicknesses (mm)			
Biceps	7.3 ± 2.7	13.0 ± 2.5	0.01
Triceps	10.5 ± 2.2	19.3 ± 2.1	0.0003
Subscapular	15.5 ± 3.3	22.6 ± 4.7	0.02
Suprailiac	10.3 ± 2.9	17.3 ± 2.4	0.004
Estimated total body fat (%)	24.3 ± 2.7	31.5 ± 1.0	0.002

<sup>1</sup> $\bar{x} \pm \text{SD}$ .

<sup>2</sup>Range in parentheses.

Repeated measurement analysis was used to evaluate changes in time for the kinetic studies of exhaled <sup>13</sup>CO<sub>2</sub> and for [<sup>13</sup>C]linoleic acid secreted in milk. Comparisons among time and group were conducted with the general linear model procedure, including the evaluation of interactions. For multiple comparisons, Bonferroni's correction was applied. Comparisons of differences between slopes were performed by using *t* test analysis.

#### RESULTS

Characteristics of the mothers are summarized in **Table 1**. Body weight and BMI were significantly different between groups, as expected by the study design. The mean body weight of women in the H-BMI group was 9.6 kg greater than that of women in the L-BMI group. These differences corresponded with a larger body fat mass of the women in the H-BMI group, as indicated by higher body circumferences, skinfold thicknesses, and calculated percentage of body fat. Most women fell below 2 SDs of the mean National Center for Health Statistics reference for 18-y-old women and thus were considered as having stunted growth.

The reported habitual energy intake per kilogram body weight of women in the H-BMI group tended to be lower than that of women in the L-BMI group, although the difference was not significant. During the study period, the control diet was adjusted to contain 167.4 kJ·kg<sup>-1</sup>·d<sup>-1</sup> (40 kcal·kg<sup>-1</sup>·d<sup>-1</sup>) when the reported intake was below this amount. The proportion of energy derived from fat (17%) remained constant and was not significantly different between groups (**Table 2**). The fat intake of these Otomi women was low compared with the fat intake of lactating women living in Western countries (5, 6, 14, 24). The dietary intake of linoleic acid represented ≈25% of the total fat intake in both the H-BMI and L-BMI groups.

The milk volume of women in the L-BMI group was significantly higher than that of women in the H-BMI group, but total lipid concentration and 24-h secretion of milk did not differ significantly between the groups (**Table 2**). Although the proportion of linoleic acid in milk was not significantly different between these 2 groups of Otomi women, it was higher than that

**TABLE 2**  
Dietary intake and milk composition of lactating women stratified by BMI<sup>1</sup>

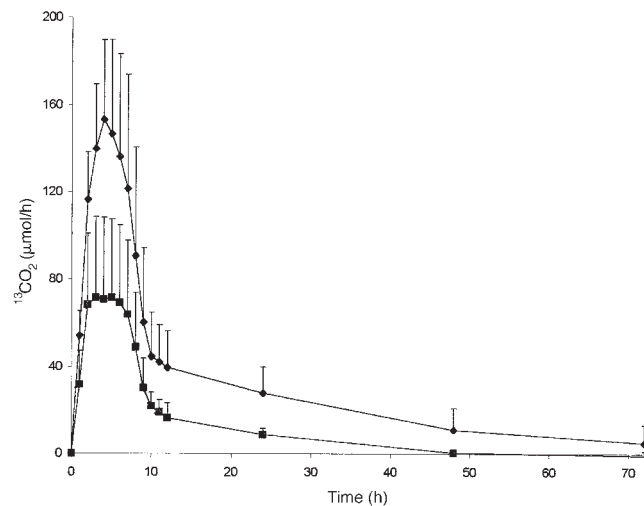
	BMI (kg/m <sup>2</sup> )	
	<22.5 (n = 5)	>23.5 (n = 5)
Dietary intake <sup>2</sup>		
Total energy		
(MJ/d)	9.27 ± 1.6	9.74 ± 1.49
(kJ/kg)	198.4 ± 34.3	175.8 ± 14.2
Total fat		
(g/d)	43.0 ± 6.1	51.0 ± 14.3
(% of energy)	17.6 ± 1.9	19.5 ± 3.1
Linoleic acid		
(g/d)	9.9 ± 2.3	13.0 ± 3.8
(% by wt)	23.5 ± 7.6	26.4 ± 2.6
Milk composition		
Volume (g/d)	763 ± 93	676 ± 149 <sup>3</sup>
Total lipid (g/L)	27.0 ± 6.0	36.0 ± 12.0
Lipid secretion (g/d)	20.4 ± 6.0	23.4 ± 6.0
Linoleic acid		
(g/d)	5.8 ± 1.7	6.2 ± 1.9
(% by wt)	28.5 ± 1.5	26.1 ± 3.1

<sup>1</sup> $\bar{x} \pm$  SD.

<sup>2</sup>Data obtained by calorimetry, gravimetry, and gas chromatography analysis of diet composites.

<sup>3</sup>Significantly different from women with a BMI < 22.5,  $P < 0.05$ .

reported for American or European women (13–15). Accordingly, the proportion of oleic acid was lower. The daily secretion of linoleic acid in milk corresponded to 50–60% of the daily maternal intake of linoleic acid, but there was no correlation between secretion and intake ( $R^2 = 0.2$ ,  $P = 0.9$ ).



**FIGURE 1.** Mean ( $\pm$ SD) exhaled breath  $^{13}\text{CO}_2$  after the administration of an oral dose of [ $^{13}\text{C}$ ]linoleic acid to lactating women with body mass indexes (BMIs; in  $\text{kg}/\text{m}^2$ ) <22.5 (■) or >23.5 (◆).  $n = 5$  per group.  $^{13}\text{CO}_2$  excretion was significantly higher ( $P < 0.04$ ) in the high-BMI group than in the low-BMI group 4–7 h after the dose. After a rapid decline in  $^{13}\text{CO}_2$  from 5 to 9 h after tracer administration, the slope of the enrichment curve became slower from 10 to 72 h ( $P = 0.01$ ), decreasing to basal values at 72 h. There was an interaction between BMI group and time ( $P < 0.001$ ) that was more significant in the high-BMI group ( $P < 0.001$ ) from 12 to 24 h and from 24 to 48 h.

**TABLE 3**  
Cumulated recovery of [ $^{13}\text{C}$ ]linoleic acid in milk of lactating women 72 h after tracer administration<sup>1</sup>

	Percentage of dose		<i>P</i>
	BMI < 22.5 (n = 5)	BMI > 23.5 (n = 5)	
	%		
Breath carbon dioxide	8.6 ± 3.5	22.8 ± 9.4	<0.05
18:2n-6	15.2 ± 6.4	13.2 ± 5.6	0.70
Total recovered	23.7 ± 17.5	36.0 ± 13.1	NS

<sup>1</sup> $\bar{x} \pm$  SD.

Exhaled  $^{13}\text{CO}_2$  was higher in women with high BMIs than in women with low BMIs, 4–7 h after the administration of the tracer (**Figure 1**). After the tracer peaked  $\approx 5$  h after administration, exhaled  $^{13}\text{CO}_2$  concentrations declined rapidly in both groups from 5 to 9 h, and then declined at a significantly slower rate from 10 to 72 h (slope differences: 5–9 h compared with 10–72 h,  $P = 0.01$ ), returning to baseline values at 72 h. Women in the H-BMI group produced higher amounts of cumulated  $^{13}\text{CO}_2$  than did women in the L-BMI group (**Table 3**), which was not due to a higher total production of carbon dioxide ( $281.6 \pm 12.9$  compared with  $297.6 \pm 20.8$  L/d, respectively). The area under the curve showed significant differences by BMI group ( $P < 0.001$ ) and time ( $P < 0.001$ ) that was greater in the H-BMI group ( $P < 0.001$ ), from 12 to 24 h and from 24 to 48 h.

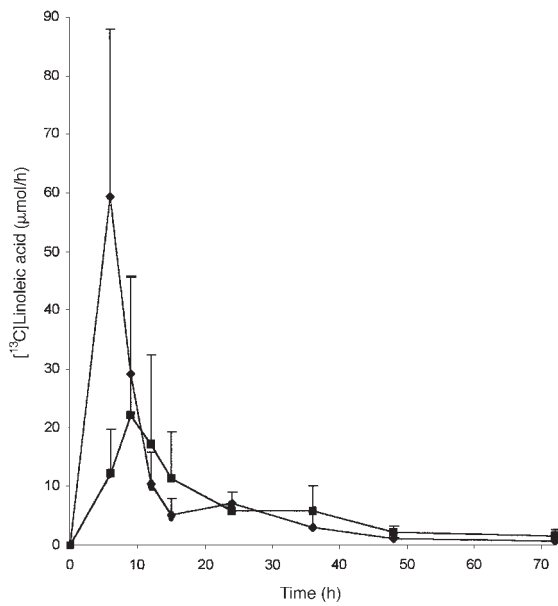
The enrichment of [ $^{13}\text{C}$ ]linoleic acid in milk (**Figure 2**) peaked 6 h after tracer administration in the H-BMI group and after 9 h in the L-BMI group. Differences between BMI groups were significant only at 6 h. Thereafter, the enrichment declined rapidly; by 48 h it returned to basal values in both groups. The 72-h cumulated recovery of [ $^{13}\text{C}$ ]linoleic acid in milk was not significantly different between BMI groups (**Table 3**); however, the transfer of [ $^{13}\text{C}$ ]linoleic acid into the milk of women in the H-BMI group occurred earlier than it did in women in the L-BMI group. Within the first 9 h of isotope administration,  $51.6 \pm 4.9\%$  and  $24.0 \pm 15.3\%$  of the total amount of recovered isotope had passed in the milk of women in the H-BMI group and L-BMI group, respectively (**Table 4**). The area under the curve for linoleic acid in milk was significantly different between the H-BMI and the L-BMI group ( $P = 0.035$ ). The interaction between BMI group and time ( $P < 0.001$ ) was greater, from 0 to 9 h, in the H-BMI group.

The enrichment of [ $^{13}\text{C}$ ]linoleic acid in plasma (**Figure 3**) peaked 2 h after tracer administration in both BMI groups. Thereafter, the enrichment declined slowly in both groups, remaining above basal values until 48 h.

The transformation of labeled linoleic acid into long-chain polyunsaturated fatty acids and its secretion into milk was shown in a study by Del Prado et al (25). Briefly, 0.01% of the [ $^{13}\text{C}$ ]linoleic acid administered in the study was secreted as arachidonic acid in milk.

## DISCUSSION

In this sample of lactating women living in underprivileged conditions, those with less body fat oxidized [ $^{13}\text{C}$ ]linoleic acid at a lower rate than did those with a higher body fat mass, despite consuming a diet of similar composition. In the present study, the amount of [ $^{13}\text{C}$ ]linoleic acid (15–17%) transferred into milk



**FIGURE 2.** Mean ( $\pm$ SD) [<sup>13</sup>C]linoleic acid secreted into the milk of women with body mass indexes (BMIs; in kg/m<sup>2</sup>) <math>< 22.5</math> (■) or >23.5 (◆) after an oral dose of [<sup>13</sup>C]linoleic acid.  $n = 5$  per group. [<sup>13</sup>C]Linoleic acid peaked at 6 h in the high-BMI group and at 9 h in the low-BMI group; there was a significant difference at 6 h ( $P < 0.001$ ). After a rapid decline from 6 to 12 h, the slope became slower, decreasing to basal values at  $\approx 48$  h in both groups. The area under the curve was significantly greater in the high-BMI than in the low-BMI group from 0 to 9 h ( $P = 0.035$ ), with an interaction between BMI group and time ( $P < 0.001$ ).

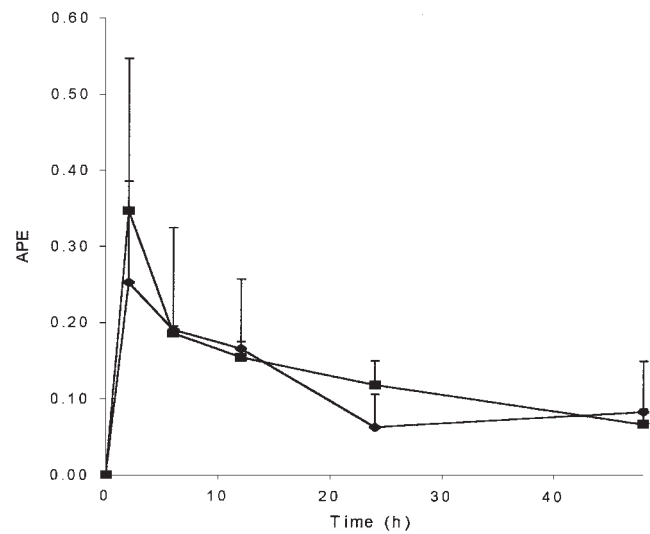
was slightly higher than that reported for well-nourished women. Demmelmair et al (14) and Hachey et al (26) found that  $\approx 12\%$  of labeled fatty acids administered orally to lactating subjects was transferred into milk.

Throughout the 72 h, the percentage of [<sup>13</sup>C]linoleic acid expired as <sup>13</sup>CO<sub>2</sub> by the H-BMI group (23%) was similar to values reported previously for German women (18–24%) (14); however, <sup>13</sup>C recovery in breath was almost one-half that in the L-BMI group (8.6%). The difference in exhaled <sup>13</sup>CO<sub>2</sub> between groups was not due to differences in the total carbon dioxide production. The amount of [<sup>13</sup>C]linoleic acid transferred into the milk of women in the L-BMI group was almost 2-fold the amount of tracer <sup>13</sup>C recovered in breath (15.2% and 8.6%, respectively),

**TABLE 4**  
Cumulated fractional recoveries of [<sup>13</sup>C]linoleic acid secreted into milk<sup>1</sup>

Time after dose (h)	BMI < 22.5	BMI > 23.5	P
	(n = 5)	(n = 5)	
	%		
6	7.3 $\pm$ 6.2	29.2 $\pm$ 4.3	0.002
9	24.0 $\pm$ 15.3	51.6 $\pm$ 4.9	0.02
12	40.1 $\pm$ 14.0	62.1 $\pm$ 2.0	0.03
15	53.6 $\pm$ 18.9	68.4 $\pm$ 2.0	NS
24	65.3 $\pm$ 17.7	83.4 $\pm$ 3.4	NS
36	80.9 $\pm$ 16.6	91.4 $\pm$ 1.3	NS
48	90.0 $\pm$ 10.3	95.6 $\pm$ 1.0	NS
72	100	100	—

<sup>1</sup> $\bar{x} \pm$  SD. Values are expressed as percentages of the 72-h recovery in milk.




**FIGURE 3.** Mean ( $\pm$ SD) plasma concentrations of [<sup>13</sup>C]linoleic acid [expressed as atoms in percent excess (APE)] peaked 2 h after dosing. APE diminished slowly, but by 48 h it was still above the basal concentration of enrichment. No significant differences were found between groups.

whereas in the H-BMI group, the amount of [<sup>13</sup>C]linoleic acid transferred into milk was one-half the amount of tracer <sup>13</sup>C recovered in breath (13.2% and 22.8%, respectively). It is tempting to speculate that triacylglycerols in chylomicrons and VLDLs are taken up more actively by the mammary glands of women with low BMIs, leaving less dietary triacylglycerols available for oxidation. In a study conducted in women from the same village with the same stratification as the present study, those with higher BMIs had a significantly larger amount of fat-free mass than did women with lower BMIs (9). Extrapolating those differences in fat-free mass to women in the present study, it is reasonable to conclude that the larger amount of fat-free mass in the women with higher BMIs contributed to higher <sup>13</sup>CO<sub>2</sub> production because of the direct relation between skeletal muscle mass and the rate of triacylglycerol oxidation (27). Further, the measured respiratory quotient was not significantly different between the L-BMI and H-BMI groups, indicating that the composition of the nutrient mixture oxidized was not different.

The enrichment curve of <sup>13</sup>CO<sub>2</sub> in both groups had 2 components: 1) a fast slope and 2) a slower slope with an inflection point at 10 h. This second component occurred several hours after the expected end of the absorptive period for the label, suggesting that the isotope had been recycled from an intermediate maternal pool. This corresponded with the observed kinetics of the label in plasma. The kinetics of [<sup>13</sup>C]linoleic acid in milk also showed a slow component from 12 to 48 h. All of this evidence suggests the participation of at least one maternal pool with a low turnover rate that releases [<sup>13</sup>C]linoleic acid into plasma several hours after the absorptive state. Such an interpretation is supported by the fact that [<sup>13</sup>C]linoleic acid was still high in most of the subjects' plasma 48 h after ingestion, and a considerable amount (60–70%) of the tracer had not been recovered as exhaled <sup>13</sup>CO<sub>2</sub>, biotransformed to long-chain polyunsaturated fatty acids, or secreted into milk during the 3 d of study, implying that it was retained in other maternal compartments.

In conclusion, our results show that women with low BMIs, who were reputed as having less body fat, oxidized and secreted

less dietary linoleic acid into their milk within the first 12 h of tracer administration than did women with high BMIs and greater adiposity. The body stores of both groups of lactating women could take up significant amounts of absorbed dietary fat and release it slowly for several hours. Studies in lactating rats (28, 29) showed that lipogenesis and lipoprotein lipase activity are low in fat tissue, although such activity occurs simultaneously with intense lipolysis. There is little information about the metabolic activity of adipose tissue in lactating women. Further studies addressing the ability of human adipose tissue to take up and hydrolyze triacylglycerols during lactation are necessary to improve the understanding of the role of adipose tissue in controlling the concentration of fat in human milk. 

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