Fat Oxidation and Adiposity in Prepubertal Children: Exogenous versus Endogenous Fat Utilization

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ABSTRACT

Fat balance plays an important role in fat mass regulation. The mechanisms by which fat intake and fat oxidation are controlled are poorly understood. In particular, no data are available on the origin, i.e. exogenous (meal intake) or endogenous (adipose tissue lipolysis), of fat oxidized during the postprandial period in children and the proportion between these two components. In this study we tested the hypothesis that there is a relationship between adiposity and the oxidative fate of fat taken with a mixed meal in a group of 15 children with a wide range of fat mass (9–64%). The combination of stable isotope analysis ([13C] enriched fatty acids added to a mixed meal) and indirect calorimetry allowed us to differentiate between the exogenous and endogenous resting fat oxidation rate over the 9-h postprandial period. During the 9 hours of the postprandial period, the children oxidized an amount of fat comparable to that ingested with the meal (26.8 ± 3.1 g vs. 26.4 ± 2.3 g, respectively, P = ns). On average, exogenous fat oxidation (2.99 ± 3.0 g/9 h) represented 10.8% (±0.9) of total fat oxidation. Endogenous fat oxidation, calculated as the difference between total fat oxidation and exogenous fat oxidation, averaged 23.4 ± 1.9 g/9 h and represented 88.2% (±0.9) of total fat oxidation. Endogenous fat oxidation as well as exogenous fat oxidation were highly correlated to total fat oxidation (r = 0.85, P < 0.001; r = 0.84, P < 0.001, respectively). Exogenous fat oxidation expressed as a proportion of total fat oxidation was directly related to fat mass (r = 0.56, P < 0.03), while endogenous fat oxidation expressed as a proportion of total fat oxidation was inversely related (r = −0.57, P < 0.03) to the degree of adiposity. The enhanced exogenous fat oxidation observed when adiposity increases in the dynamic phase of obesity may be viewed as a protective mechanism to prevent further increase in fat mass and hence to maintain fat oxidation at a sufficient rate when the body is exposed to a high amount of dietary fat, as typically encountered in obese children. (J Clin Endocrinol Metab 84: 654–658, 1999)

FAT balance, calculated as the difference between total dietary fat and total fat oxidation, has been suggested as the key factor of fat mass regulation (1). Several studies have reported that obese children, like obese adults, ingest more fat than nonobese children (2–4). Regarding the output factor of the fat balance equation, i.e. fat oxidation, evidence exists that the postabsorptive fat oxidation rate is higher in obese than in nonobese children (5, 6). The level of fat oxidation is associated with body fat mass both under postabsorptive conditions (5, 6) and, as suggested from data obtained in adults, over 24 h in a respiration chamber (7).

No data are available on whether or not exogenous fat oxidation is different in obese and lean individuals during the postprandial phase after consuming a mixed meal containing carbohydrates, fat, and protein. The use of stable isotope tracers (fatty acid labeled with [13C]) allowed us to divide total fat oxidation into its exogenous (fat load related) and endogenous (adipose tissue related) components (8); total fat oxidation can be assessed by indirect calorimetry and exogenous fat oxidation by fatty acid labeled with [13C] integrated into a balanced test meal.

The purpose of this study was to explore the relationship between postprandial fat oxidation, partitioned into its exogenous vs. endogenous origin, and the degree of adiposity in a group of prepubertal children with different levels of adiposity. Our particular goal was to determine if the “efficiency” of exogenous fat oxidation is related to the degree of adiposity.

Subjects and Methods

Subjects

Fifteen children with a wide range of fat mass (fat mass: 9–64%) participated in the study. Their physical characteristics are given in Table 1. None of the children had any overt diseases other than obesity. The mean values of postabsorptive baseline glucose and insulin plasma levels are shown in Table 1. None of the obese subjects were dieting at the time of the study, and all the children had an essentially stable body weight for at least 1 month before the study. None were on medication. The children arrived at the Department of Pediatrics (University of Verona, Verona, Italy) the evening of the day before the indirect calorimetric test, accompanied by their parents. Informed consent was obtained before taking part in the study. The protocol was in accordance with the Declaration of Helsinki of 1975, as revised in 1983.

Experimental design

The study lasted for 10 consecutive hours during which the children were under medical supervision. During the days preceding the test, no attempt was made to influence the usual diets of the children (each of whom had access to a free diet), but none of them was on a hypocaloric diet. The day immediately before the test, they did not eat any food with a high abundance of [13C] (sugar cane, maize, pop-corn, millet, polenta, pineapple, or any tropical fruit), nor did they perform any intense physical activity. Each child arrived at the Department of Pediatrics the day before the calorimetric test. The children consumed their last meal in the evening at 1900 h. The day of the test a blood sample was obtained in postabsorptive conditions from an antecubital vein to measure plasma glucose and insulin levels. Plasma glucose was measured by the glucose


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oxidase method; plasma insulin was determined by a commercialized enzyme immunoassay kit. Continuous respiratory exchange measurements assessed by indirect calorimetry began at 0800 h. For the duration of the test, each child was laying down on a hospital bed in a comfortable temperature- (~24 C) and humidity-controlled environment. Two complete urine samples were obtained, once before test meal ingestion and once again during the entire postprandial period.

Anthropometry and body composition

Anthropometric assessments (weight, height, and four skinfold thicknesses at the biceps, triceps, suprailiac, and subscapular sites) were carried out in each child. Skinfold thickness was measured to the nearest 0.1 mm in triplicate with a Harpenden skinfold caliper (CSM Weighing Equipment Ltd., London, UK). The formulae of Slaughter et al (9) for this age category were used to estimate relative body fat.

Dietary intakes

On the day of the study, after a 30 min baseline calorimetric period, the children were given one single food to consume (a vanilla ice cream). The test meal energy (2.5 ± 0.2 ml) was equivalent to 40% of each individual postabsorptive metabolic rate, calculated over 24 h, as determined by indirect calorimetry at baseline. Expressed as a percentage of total energy value, the test meal contained 9% protein (12.1 ± 0.8 g), 40% fat (26.8 ± 1.6 g), and 51% carbohydrate (77.5 ± 5.1 g) energy. This was eaten under supervision at 0830. In order to assess the rate of exogenous fat oxidation in response to the ingestion of the food, the ice cream was previously enriched with [13C] by adding corn oil plus a mixture of fatty acids artificially enriched with [13C]. Twenty grams of cold pressed corn oil (naturally enriched with [13C]) was mixed with 750 mg of a mixture of fatty acid enrichment (98% AP). The same lot was used for the whole study (Martek Bioscience Corp., Columbia, MD). The extrinsically labeled fat was thoroughly mixed with the slightly warmed corn oil. Then, 2.5 g of the homogenized mixture (20.75 ml) was mixed with thawed ice cream flavored with vanilla (Motta, Milan, Italy), which provided each child with an equivalent of 68 mg of a [13C]-fatty-acid-lipid mixture in the food served. The ice cream was refrigered before consumption.

The rationale for adding artificially enriched lipids was the low natural abundance of [13C] in corn oil (essentially equal to corn starch ~1.092% AP) and the fact that a large proportion of ingested lipids is stored in the body (and not directly oxidized, as in the case of carbohydrates), yielding a low postprandial [13C]O2 abundance in the breath. This precluded the use of corn oil as the sole source of exogenous [13C]. In addition, we decided against using a single fatty acid labeled with [13C] (typically 1-13C-palmitic acid), oxidation of which may not reflect the total amount of fatty acid oxidized by the body. The extrinsically labeled fatty acid covered a large range of fatty acids from C16:0 (22% of total fatty acid) to C18:1 (10%), C18:2 (27%), and C18:3 (12%). The fatty acid pattern of the mixture was checked by gas-liquid chromatography, whereas the actual level of enrichment was measured by gas chromatography immunoradiometric assay.

Assessment of exogenous and endogenous fat oxidation

Calculation of exogenous fat oxidation was based on the simple concept of [13C] balance. This is defined as the difference between the amount of ingested [13C] as lipid carbon minus excreted [13C] in exhaled air as [13CO2].

Breath collection was made every hour from the baseline period up to the end of the study, that is, 9 h after the ingestion of the meal whose fat was labeled with [13C]. This was performed to assess the isotopic [13C]/[12C] ratio in breath CO2 and to obtain an estimate of the exogenous fat oxidation during the postprandial period. At each time point, triplicate samples of exhaled air were collected in 10 ml evacuated, air-tight glass tubes (Europa Scientific Inc., Crew, UK). The [13CO2] isotopic enrichment of breath samples was determined by continuous flow isotope-ratio mass spectrometry (CF-IRMS, Tracer Mass, Europa Scientific). Food sample [13C] enrichment was determined by continuous flow isotope-ratio mass spectrometry after combustion in a furnace at 1000 C (Roboprep CN, Europa Scientific). As shown in Fig. 2, the abundance of [13C] in exhaled CO2 was expressed in atom percent excess (APE), according to the following equation: \( APE = \left( \frac{[13C]}{[12C]} \right) - 1 \) in exhaled CO2, test-[13C] CO2 in exhaled CO2, baseline, from the ratio of excreted [13C], which corresponded to the breath [13CO2] in postmeal minus premeal period, multiplied by VCO2 and divided by the ingested [13C] (corresponding to the net amount of [13C] in naturally enriched corn oil plus the artificially enriched mixture of fatty acids mentioned above). We calculated the fractional exogenous fat oxidation. This corresponds to the proportion of ingested [13C] that is excreted as [13CO2]. This can be calculated after prior transformation into mass (gram) mole or equivalent volume (l). The amount of exogenous fat oxidized was obtained from the total amount of lipid ingested in the test meal multiplied by the fractional exogenous fat oxidation. Endogenous fat oxidation was calculated as the arithmetic difference between total fat oxidation minus exogenous fat oxidation.

Statistical analysis

All results presented are expressed as mean and the standard error of the mean (SEM). Relationships between two variables were assessed by simple regression analysis. A multiple regression analysis was per-
formed to assess the relationships between the rates of exogenous and endogenous fat oxidation (independent variables) and fat mass, postabsorptive plasma insulin levels, total fat oxidation, and fat intake (independent variables) during 9 h of REE recording. A probability level of $P < 0.05$ was used to indicate statistical significance. Statistical analyses were done using JMP 2.0 software (SAS Institute, Inc., NC).

**Results**

The rate of appearance of $^{13}$C in exhaled carbon dioxide after meal intake is shown in Fig. 1. In response to the labeled $^{13}$C fat contained in the ice cream, $^{13}$C in the breath progressively increased. Peak values of $^{13}$C in the exhaled air were reached 7 h after the test meal, with a slow decline in the subsequent hours. The duration of the study (9 h) was not sufficient to observe the return of the $^{13}$C levels to the background baseline abundance.

During the 9 h of postprandial energy intake, the children oxidized an amount of fat comparable to that ingested with the ice cream [26.8 (±2.31) g vs. 26.4 (±2.3) g, $P = ns$]. Exogenous fat [2.99 (±3.0) g/9 h] represented 10.8% (±0.9) of total fat oxidation. Endogenous fat, calculated as the difference between total fat oxidation and exogenous fat oxidation averaged 23.4 (±1.9)g/9 h and represented 88.2% (±0.9) of total fat oxidation.

Endogenous fat oxidation and exogenous fat oxidation were highly correlated to total fat oxidation ($r = 0.83, P < 0.001; r = 0.84, P < 0.001$, respectively). Fat mass was related to total fat oxidation ($r = 0.86, P < 0.001$). Fat mass was also related to endogenous fat oxidation ($r = 0.83, P < 0.001$) and to exogenous fat oxidation ($R = 0.84, P < 0.001$) (Fig. 2). Exogenous fat oxidation expressed as a proportion of total fat oxidation was directly related to fat mass ($r = 0.56, P < 0.03$), while endogenous fat oxidation expressed as a proportion of total fat oxidation was inversely related ($r = -0.57, P < 0.03$) to the degree of adiposity. When a multiple regression analysis (with exogenous fat oxidation as the dependent variable and fat mass, total fat oxidation, fat intake, and the postabsorptive plasma insulin level as independent variables) was performed with the stepwise procedure, none of the independent variables except fat mass was statistically important, and none were included in the final equation: [Exogenous fat oxidation (g/9 h) = 0.053 [mult] fat mass (kg) (se $\beta = 0.01$) + 1.302 (se $\beta = 0.296$); $R^2 = 0.705$].

The same statistical analysis, using endogenous instead of exogenous fat oxidation as the dependent variable, showed that just total fat oxidation was included in the final equation: [Endogenous fat oxidation (g/9 h) = 0.827 [mult] Total fat oxidation (g/9 h) (se $\beta = 0.029$) + 1.568 (se $\beta = 0.805$); $R^2 = 0.984$].

**Discussion**

In adults, Leibel RL et al (12) have demonstrated that humans tend to maintain body weight by means of adaptation mechanisms which cause an increase in energy expenditure as a consequence of overeating or a reduction of energy expenditure in case of undereating. The three components of energy expenditure (basal metabolic rate, dietary induced thermogenesis, and energy expenditure for activity) change when body weight and body composition change. Because energy expenditure depends upon nutrient oxidation, body weight and body composition variations might promote changes in nutrient oxidation rates. Protein balance as well as carbohydrate balance have been shown to be efficiently self-regulating both in animals and adults (13).
contrast, fat balance is imperfectly regulated. Studies conducted in adults have shown that acute fat intake does not promote fat oxidation (14). This characteristic makes fat balance a key factor in body fat regulation. Fat mass may be viewed as an energetic buffer (15). Previous studies performed in obese children and adults reported that postabsorptive fat oxidation is proportional to body fat mass even after FFM has been statistically adjusted (5, 6). Little information is available on the postprandial fat oxidation rate in children. The results of the present study, in which a common food (vanilla ice cream) was used as test meal, have shown that postprandial fat oxidation measured over a 9 h interval was also associated with fat mass. Moreover, both components of total fat oxidation (exogenous and endogenous) were greater in obese than in nonobese children.

The results of this study show that the rate of exogenous fat oxidation in proportion to total fat oxidation increased with adiposity in children (Fig. 2). The great advantage of stable isotopes for studying nutrient metabolism in children is that they are not radioactive and are safe. In the present study, a correct interpretation of the data requires the following assumptions to be made:

1. The absorption of lipids does not vary substantially between subjects. Recent data in children suggest that the correction of the excretion of labeled $^{13}$C in breath for differences in absorption does not alter the variability observed between individuals or the differences between groups (16).

2. There is a negligible retention of labeled carbon dioxide within the body bicarbonate pool. In the present study, no correction factor due to the incomplete $^{13}$C recovery of the administered dose was used because of the uncertainty of the value to be used (typically 0.8). The latter heavily depends on the nature of the labeled substrate (17) and the type of individual fatty acid considered. As a result, exogenous fat oxidation may be underestimated, particularly in the early post-meal period, but cross-comparison between subjects still remains valid.

3. A last assumption is that $\beta$-oxidation of fatty acids proceeds to completion once it has been initiated. We point out that due to the wide range of uniformly labeled fatty acids, no assumption is necessary regarding the random distribution of a single labeled fatty acid within triglycerides during the process of absorption across the intestinal lumen.

The experimental design and situation of our study differs from previous investigations conducted in adults. In particular, because the rate of exogenous fat oxidation obtained reflects a larger pattern of fatty acids than in experimental studies where a single fatty acid was labeled with $^{13}$C (18). Moreover, considering that we worked with children, it was unreasonable to further prolong the duration of $^{13}$CO$_2$ measurements. In previous studies on adults, the measurement was usually made over a shorter period of time, i.e. 6 h (16). Finally, the meal contained all three macronutrients rather than a single one (pure lipid), so that an effect on postprandial insulin secretion response was assured.

The decreased proportion of endogenous fat oxidation with increased fat mass indicates that the postprandial endogenous lipolysis process must be down-regulated somehow (probably via the secretion of insulin). Due to the large size of fat mass, this can be viewed as a process to avoid an excessive amount of free fatty acid released into circulation. We know that doubling fat mass (i.e. from 20 kg to 40 kg) decreases the proportion of endogenous to total fat oxidation from 90% to 87.5%, suggesting a rather small sensitivity of this process.

There have been a limited number of studies in which exogenous fat (fatty acid) oxidation was assessed with $^{13}$C-labeled fatty acids, all of them referred to adulthood (18, 19). They unequivocally showed that most exogenous fat is stored in adipose tissue during the postprandial phase, whereas a limited fraction (~10%) is directly oxidized. Our study on children confirms these findings, as exogenous fat oxidation represented 11% of the total fat eaten in the ice cream. However, the interindividual variability was large (5–18%), and we were able to identify one major factor that explains this variation of fat mass.

Exogenous fat oxidation rate in proportion to total fat oxidation increased with adiposity in children. This finding may be interpreted as an attempt on behalf of the organism to counteract a further increase in body fat mass during the dynamic phase of obesity. Other metabolic modifications
seem to accompany this process; in particular, insulin resistance, which was identified as a protecting factor for fat gain in adults because it enhances fat oxidation (20). In a longitudinal study conducted in the Pima Indians, individuals with a low insulin sensitivity, as assessed by a hyperinsulinemic euglycemic glucose clamp, showed a lower risk to further weight (fat) gain over a 4-yr period (20). Therefore, the higher proportion of exogenous fat oxidation may be associated with insulin resistance. Obese children have an increased postabsorptive plasma insulin concentration, which reflects peripheral insulin resistance (21). Postabsorptive insulin levels were slightly but significantly related to both total fat oxidation ($r^2 = 0.34$) and absolute endogenous fat oxidation ($r^2 = 0.36$). Insulin stimulates glucose oxidation directly by enhancing glucose transport in insulin-sensitive cells, by activating the glycolytic pathway at several regulatory enzyme steps, and by activating the enzyme complex pyruvate dehydrogenase, which accelerates entry of glucose-derived acetyl CoA into the Krebs cycle (22). Insulin also suppresses lipolysis and inhibits lipid oxidation. The efficacy of insulin in reducing lipolysis is much more powerful than in suppressing lipid oxidation (25). This finding is (indirectly) confirmed by the lack of association between postabsorptive plasma insulin levels and postprandial total fat oxidation found in this sample of children (Fig. 3). Unfortunately, for ethical reasons, it was not possible to take repeated blood samples, so the pattern of change in postprandial glycemia and insulinemia (as well as other substrates and hormones of interest) could not be assessed in these children.

In summary, most of the fat ingested ($\approx 90\%$) was stored in adipose tissue in the postprandial phase; the amount directly oxidized represented a small fraction of the total fat ingested and oxidized ($\approx 10\%$). Both postabsorptive and postprandial fat oxidation were related to fat mass in prepubertal children. The enhanced total fat oxidation observed in obesity was explained by increases in both exogenous and endogenous fat oxidation. The proportion of exogenous $75\%$ endogenous oxidation increased with adiposity, suggesting an increase in the “efficiency” of endogenous fat oxidation. The degree of control of the partition between exogenous $75\%$ endogenous fat utilization appeared to be somehow limited, particularly considering the potential error of the methodology employed.

Taken together, the results of this study suggest that relatively blunted oxidation of endogenous fat may be viewed as a protective mechanism to prevent further increase in fat mass and hence to maintain fat oxidation at a sufficient rate, when the body is exposed to exogenous fat combined in a meal.

In conclusion, the use of exogenous fat after the consumption of a commonly eaten food load was found to be greater with increasing adiposity. In contrast, endogenous fat, coming from the lipolysis of adipose tissue, accounts for a lower proportion of total fat oxidation in obese rather than in nonobese children. Insulin resistance may partially explain these findings. Preferential oxidation of exogenous fat might constitute an important adaptive mechanism, which tends to protect the organism from further fat gain and may result from a chronic exposure to excess dietary fat intake.

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**References**


