Influence of diet on the modeling of adipose tissue triglycerides during growth

Daniel Z. Brunengraber,1 Brendan J. McCabe,2 Takhar Kasumov,2 James C. Alexander,1 Visvanathan Chandramouli,2 and Stephen F. Previs3

Departments of 1Mathematics, 2Medicine, and 3Nutrition, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Submitted 26 March 2003; accepted in final form 6 June 2003

Brunengraber, Daniel Z., Brendan J. McCabe, Takhar Kasumov, James C. Alexander, Visvanathan Chandramouli, and Stephen F. Previs. Influence of diet on the modeling of adipose tissue triglycerides during growth. Am J Physiol Endocrinol Metab 285: E917–E925, 2003. First published June 10, 2003; 10.1152/ajpendo.00128.2003.—We have studied the accretion of lipids in growing mice. We measured the rates of synthesis and degradation of triglycerides in epididymal fat pads of mice maintained for 44 days on a low-fat, high-carbohydrate diet (I) or a high-fat, low-carbohydrate diet (II). 2H2O was added to the drinking water for 14 days. Rates of incorporation/washout of 2H to/from C1 of triglyceride-glycerol showed that triglyceride synthesis was greater than triglyceride degradation (net triglyceride balance was ~2.5 times greater in II than in I). The data also show that the contribution of de novo lipogenesis to triglyceride-bound palmitate was ~3 times greater in I than in II. This was consistent with a greater relative intake of carbohydrate in growing mice. We measured the profile of triglyceride-bound fatty acids, we observed a decrease in the relative abundance of triglyceride-bound palmitate and stearate and an increase in triglyceride-bound oleate and linoleate. This was observed in I and II. In summary, diet substantially affects the deposition and modeling of triglycerides in adipose tissue during growth. 2H2O can be used to examine the mechanisms responsible for the accumulation of triglycerides, e.g., factors that affect J) triglyceride synthesis and degradation and 2) the source of fatty acids that are used in esterification.

There is an alarming increase in the number of reported cases of pediatric obesity (26, 32). Like obese adults, obese children present with metabolic abnormalities (e.g., impaired glucose tolerance, dyslipidemia) that are associated with an increased risk of developing a chronic disease (e.g., diabetes, cardiovascular disease) (8, 9, 21, 24, 32). A special concern regarding obese children is that many will remain obese as adults, which further increases the likelihood of developing a chronic disease. Presumably, the development of effective treatments for pediatric obesity will benefit from knowledge of how specific biochemical reactions drive lipid accumulation during growth.

We hypothesized that, during growth, the nature of one’s caloric intake will affect the rate of, and the mechanism of, fat accumulation. For example, as the ratio of dietary fat to carbohydrate increases, the rate of triglyceride accretion will increase. Also, the relative amount of carbohydrate in the diet will affect the relative contribution of de novo lipogenesis to the pool of triglyceride-bound fatty acids. We developed the use of 2H2O to measure the rates of the reactions involved in triglyceride accumulation (e.g., triglyceride synthesis and triglyceride degradation and the contribution of de novo lipogenesis to the pool of triglyceride-bound fatty acids). This approach also allows us to determine the rate of remodeling of fatty acids that are bound to triglycerides.

Application of the isotope labeling procedures (i.e., the chemical methods) required that we formulate a mathematical model to calculate the rates of triglyceride synthesis and degradation during non-steady-state metabolic conditions (e.g., during periods of net accumulation or loss). The need for this model is best explained using a simple example. Briefly, rates of triglyceride synthesis and degradation are determined according to the following rationale. Subjects are maintained on 2H2O for several days. During that time, triglycerides in adipose tissue become labeled. The rate of synthesis is determined from the rate of incorporation of 2H into tissue triglycerides. 2H is then eliminated from body water. The rate of triglyceride degradation is determined by measuring the decay of labeled triglycerides. A problem arises, however, when the rate of degradation of triglycerides is calculated via the decrease in labeling. This occurs because triglycerides are continuously being synthesized. Because the newly synthesized triglycerides are unlabeled, the apparent isotope decay rate will overestimate the true decay rate to extent of dilution from the newly synthesized unlabeled molecules.

In this report, we show that J) 2H2O can be used to determine the rate of dietary carbohydrate to fat.
(or other factors) affects the rates of the specific reactions involved in lipid accretion and 2) special care must be used when interpreting data that are collected during conditions of growth (i.e., non-steady-state metabolism), since substantial remodeling of adipose tissue triglycerides can occur. We found that, although there is net accumulation of fat during growth, both triglyceride synthesis and triglyceride degradation are highly active. Last, de novo lipogenesis appears to be very active during growth.

MATERIALS AND METHODS

Chemical Supplies

Unless specified, all chemicals and reagents were purchased from Sigma-Aldrich. \( \text{D}_2\text{O} \) (99.9 atom percent excess) and \( \text{D}_2\text{H}_2\text{O} \) (98.5 atom percent excess) were purchased from Isotec (Miamisburg, OH). Ion exchange resins and HPLC columns were purchased from Bio-Rad (Hercules, CA). Gas chromatography-mass spectrometry supplies were purchased from Agilent Technologies (Wilmington, DE). Enzymes were purchased from Roche (Indianapolis, IN).

Biological Experiments

Theoretical. \( \text{D}_2\text{O} \) is bound to carbon-1 (C1) of triose phosphates (i.e., dihydroxyacetone phosphate and glyceraldehyde 3-phosphate) during their formation and isomerization (25, 29) (Fig. 1). Reduction of dihydroxyacetone phosphate yields glycerol 3-phosphate; \( \text{D}_2\text{H} \) remains bound to C1. Because glycerol 3-phosphate is used in the synthesis of triglycerides, we hypothesized that triglyceride synthesis could be quantified via the \( \text{D}_2\text{H} \) labeling of triglyceride-glycerol. After the washout of \( \text{D}_2\text{H} \) from body water, the rate of triglyceride degradation could be calculated by measuring the breakdown of \( \text{D}_2\text{H} \)-labeled triglyceride-glycerol. It is well known that \( \text{D}_2\text{H} \) from \( \text{D}_2\text{H}_2\text{O} \) is incorporated into fatty acids during fatty acid synthesis (1, 10, 18, 19). Thus the contribution of de novo lipogenesis to lipid accumulation could be determined by measuring the labeling of triglyceride-bound fatty acids. Last, the rate of remodeling of triglycerides could be determined by measuring the rates of incorporation, and removal, of newly synthesized (i.e., \( \text{D}_2\text{H} \)-labeled) fatty acids into, and from, triglycerides.

Determination of triglyceride synthesis and degradation. Male C57BL/6J mice (∼5 wk old) were purchased from Jackson Laboratories (Bar Harbor, ME). On arrival, the mice were randomized to two groups. Each group was given free access to either diet I or diet II, purchased from Research Diets (New Brunswick, NJ; diet I, no. D12450B, kcal distribution of 20% fat, 70% carbohydrate, and 20% protein; diet II, no. D12451, kcal distribution = 45% fat, 35% carbohydrate, and 20% protein). After 10 days, the mice were given a single intraperitoneal injection of \( \text{D}_2\text{H}_2\text{O}-\text{saline} \) (16.25 μg/kg body wt, 0.9 g NaCl in 1,000 ml 99% \( \text{D}_2\text{H}_2\text{O} \); assuming that body water accounts for 65% body wt, this should establish 2.5% \( \text{D}_2\text{H} \) enrichment of body water). Mice were returned to their cages and allowed free access to their respective diet and drinking water enriched to 5% \( \text{D}_2\text{H} \).

Mice were killed after 5, 10, or 14 days on \( \text{D}_2\text{H}_2\text{O} (n = 3 \text{ per day per group}) \). On day 14, the remaining mice in each group were switched from \( \text{D}_2\text{H} \)-labeled drinking water to tap water. Mice were killed on days 20 and 34 (n = 3 per day per group). At the time they were killed, blood and tissue samples were quick-frozen in liquid nitrogen and stored at −80°C.

Analytical Procedures

\( \text{D}_2\text{H} \) labeling of body water. The \( \text{D}_2\text{H} \) labeling of body water was determined by exchange with acetone as described by Yang et al. (38), with minor modifications. First, it was not necessary to decrease the ionization energy below 70 eV to obtain a correct basal (M + 1)/M signal ratio in acetone. Presumably, the ionization effect(s) reported by Yang et al. do not occur on our instrument. Second, assays were performed using 40 μl of sample or standard, 2 μl of 10 N NaOH, and 4 μl of a 5% (vol/vol) solution of acetone in acetonitrile.

The \( \text{D}_2\text{H} \) labeling of acetone was determined using an Agilent 5973N-MSD equipped with an Agilent 6890 GC system. A DB17-MS capillary column (30 m × 0.25 mm × 0.25 μm) was used in all analyses. The temperature program was: 60°C initial, increase by 20°C/min to 100°C, increase by 50°C/min to 220°C, and hold for 1 min. The split ratio was 40:1 with a helium flow of 1 ml/min. Acetone elutes at ~1.5 min. The mass spectrometer was operated in the electron impact mode. Selective ion monitoring of mass-to-charge ratios (m/z) 58–60 was performed using a dwell time of 10 ms/lon.

\( \text{D}_2\text{H} \) labeling of triglyceride-bound glycerol. Total glycerides were extracted from frozen tissues and hydrolyzed in ethanol-KOH at 70°C for 3 h (16). \( \text{D}_2\text{O} \) (2 ml) was added, and the solution was acidified to pH 1 by adding 6 N HCl. The pH was monitored by using a pH meter (Accumet 900; Fisher Scientific). Fatty acids were first extracted using diethyl ether (3 × with an equal volume). The pH of the aqueous solution was then adjusted to ~7.0 (using 10 N NaOH), and

\[

d = \frac{M}{M + 1}
\]

Fig. 1. Incorporation/washout of \( \text{D}_2\text{H} \) (D) to/from triglycerides. In the presence of \( \text{D}_2\text{H}_2\text{O} \) (D), \( \text{D}_2\text{H} \) is incorporated into the glycerol moiety of triglycerides. During de novo lipogenesis, \( \text{D}_2\text{H} \) is incorporated into newly synthesized fatty acids. Rates and mechanisms of triglyceride synthesis can be determined by measuring the incorporation of \( \text{D}_2\text{H} \) into the various parts of the triglyceride molecule. After the washout of \( \text{D}_2\text{H} \) from body water, rates of triglyceride breakdown can be determined by measuring the decay of \( \text{D}_2\text{H} \)-labeled triglycerides.
free glycerol was recovered by passing the solution over an ion exchange column, made by layering an AG 50W-X8 resin (formate form) over an AG 1-X8 resin (hydrogen form). Glycerol was recovered by washing with H2O.

Although glycerol appears as a symmetrical molecule, it has biological asymmetry (5, 30, 34). That is, glycerokinase selectively phosphorylates C3. In this study, we determined the labeling of 2H that is bound to C1 of dihydroxyacetone phosphate by aldolase. Note that the samples used in this analysis were obtained from mice that were maintained on 2H2O for 14 days. The 2H labeling of HMT was determined using an Agilent 5973N-MSD equipped with an Agilent 6890 GC system. A DB17-MS capillary column (30 m × 0.25 mm × 0.25 μm) was used in all analyses. The temperature program: 100°C initial, hold for 2 min, increase by 20°C/min to 220°C, and hold for 4 min. The split ratio was 15:1 with a helium flow of 1 ml/min. HMT elutes at ~6.1 min. The mass spectrometer was operated in the electron impact mode. Selective ion monitoring of m/z 270–272, 298–300, 296, and 294, respectively, to that of heptadecanoate (17:0) m/z 284. To account for possible differences in the ionization efficiency of each fatty acid, the profile was compared against standards prepared by mixing known quantities of each fatty acid.

Mathematical modeling. The modeling of triglyceride synthesis and degradation is done in two steps. First the concentration of triglyceride-bound fatty acids is modeled, and then the concentration of triglyceride-bound saturated fatty acids is modeled. The first step allows us to determine the net accumulation of triglycerides, whereas the second step allows us to determine the contribution of de novo lipogenesis vs. diet to the pool of triglyceride-bound fatty acids and whether there is a remodeling of triglyceride-bound fatty acids.

The incorporation of 2H from water into triglycerides in adipose tissue is modeled using a single-compartment model, assuming that the labeling of plasma water reflects that of water in adipose tissue. The time-dependent labeling of 2H in water is c(t). 2H is eliminated from plasma at a rate c’ . The parameters of basic interest, the rates of triglyceride synthesis and degradation (S and D, respectively, expressed in mg/day), are estimated from data using nonlinear least squares fitting.

Because the mass of triglycerides (expressed in mg) increases linearly with time (m, expressed in mg/day), the total amount of labeled triglycerides at time t satisfies the differential equation

\[ \frac{dm(t)}{dt} = Sc(t) - \frac{Dm(t)}{mt + b} \]

where \( h \) equals the 2H labeling of triglycerides in adipose tissue (expressed in mg 2H). The parameter c(t) is essentially constant while mice are maintained on 2H-labeled drinking water, yet it decays exponentially (at rate c’) when mice are switched to tap water. The parameter c’ is estimated from the data.

Differential equations can then be solved in closed form for “before” and “after” removal of 2H-labeled drinking water. Those equations are
The only remaining parameters that require estimation are the rate of synthesis (S) and the rate of degradation (D), which equals $S - m$ and $v_0$. A Levenberg-Marquardt nonlinear fit (equal weights on all data points) is done for these parameters.

Once the parameters of triglyceride synthesis and breakdown are determined, the synthesis of fatty acids is then modeled. Here, an additional compartment is needed, since fatty acids can be created de novo or incorporated from the diet. Accordingly, a new parameter $p$, the proportion of de novo production, is introduced.

$$\frac{dh(t)}{dt} = pSc(t) - \frac{Dh(t)}{mt + b}$$

The remaining formulas are similar to those described above, and a similar nonlinear least squares fit is made to the data.

Calculations. Unless noted, data are expressed as means ± SE. Statistical differences were calculated using a $t$-test.

RESULTS

Figure 2 shows the body weight and epididymal fat pad weight of growing mice maintained on different diets. Body weight was similar in the two groups until the last time point (Fig. 2A). However, the weights of the epididymal fat pads (2 pads were pooled from each mouse) were different at all time points (Fig. 2B).

Figure 3 shows the change in the total amount of triglyceride-bound glycerol and triglyceride-bound fatty acids from the epididymal fat pads. Regression analyses showed a linear increase in the quantity of triglyceride-glycerol (diet I: $y = 4.3x + 179, r^2 = 0.996$; diet II: $y = 11.4x + 271, r^2 = 0.992$) and triglyceride-fatty acids (diet I: $y = 15.8x + 537, r^2 = 0.971$; diet II: $y = 36.6x + 766, r^2 = 0.992$).

$\text{D}_2\text{O}$ was used to quantify the rates of synthesis and breakdown of lipids. Figure 4 shows that the labeling of body water remained constant while mice were maintained on $\text{D}_2\text{O}$-labeled drinking water. The $\text{D}_2\text{O}$ enrichment of plasma water was $\sim 50\%$ that of the drinking water. The apparent exchange/dilution of label is in agreement with other reports (1) and presumably occurs via respiration and digestion of food. Between days 14 and 20, $\text{D}_2\text{O}$ was washed out of body water by changing the drinking water from $\text{D}_2\text{O}$ to $\text{H}_2\text{O}$. On day 20, the $\text{D}_2\text{O}$ labeling of plasma water decreased to $\sim 0.45\%$ $\text{D}_2\text{O}$. The calculated $t_{1/2}$ of body water is $\sim 2.5$ days. This is consistent with experiments in which the elimination of body water was measured over the course of 1 wk (4).

Figure 5 shows the $^2\text{H}$ labeling of triglyceride-glycerol and triglyceride-palmitate. Data are shown as the total labeling of $^2\text{H}$ bound to C1 of triglyceride-glycerol and the total labeling of triglyceride-bound palmitate. While the mice were maintained on $\text{H}_2\text{O}$ there was continuous labeling of triglycerides; after switching to $\text{D}_2\text{O}$, the labeling in triglycerides decreased.

Figure 6 shows a fit of the data in Figs. 3–5 after application of the equations presented in MATERIALS AND METHODS. There was a good fit between the observed data and the computed parameters.

Table 1 contains the rates of triglyceride turnover (i.e., rates of triglyceride synthesis and degradation,
contribution of de novo lipogenesis, and rates of incorporation and removal of newly synthesized palmitate into and from triglycerides). Regardless of diet, the rates of triglyceride synthesis were greater than the rates of triglyceride degradation. The accumulation of triglycerides is consistent with the fact that growing mice were studied. The net accumulation of triglycerides was \( \frac{1}{10} \) times greater on diet II compared with diet I. The contribution of de novo lipogenesis was about three times greater on diet I than on diet II.

Regardless of diet, there was net accumulation of palmitate. The accumulation of palmitate was \( \frac{1}{7.5} \) times greater on diet I than on diet II.

**DISCUSSION**

Lipid accretion is an important component of normal growth. For example, between the ages of \( \sim \)5 and 20 yr, healthy individuals accumulate comparable amounts of protein and fat. Unfortunately, epidemiological data show a dramatic increase in the number of obese children (26). We suspect that determining how specific biochemical reactions affect lipid accretion during growth is an important first step in the development of treatments for preventing and/or reversing pediatric obesity (and thereby reducing the risk of developing future disease).

We developed the use of \( ^2\text{H}_2\text{O} \) to study the influence of diet on the biochemical basis of triglyceride accumulation during growth. First, we discuss the development of this method. Second, we discuss the implications of our data on future studies.

**Development of the \( ^2\text{H}_2\text{O} \) Method**

A prerequisite for using \( ^2\text{H}_2\text{O} \) was to establish and maintain a steady-state labeling of body water (the precursor pool). This is easily achieved by administering a priming bolus of \( ^2\text{H}_2\text{O} \) and then allowing free access to \( ^2\text{H} \)-labeled drinking water (Figs. 1 and 4). As shown, \( ^2\text{H} \) is readily washed out of body water by switching mice to tap water. Therefore, it is possible to calculate multiple parameters related to triglyceride turnover by measuring the incorporation/washout of \( ^2\text{H} \) to/from lipids.
Rates of triglyceride synthesis are calculated after determining the precursor-to-product labeling ratio (7). Although determination of the precursor labeling is straightforward (i.e., measure the labeling of body water), we questioned the correct “product labeling.” For example, we (17) previously reported that the ratio of 2H bound to C3 vs. C1 of VLDL triglyceride-glycerol is 0.93; however, the ratio of 2H bound to C3 vs. C1 of blood glycerol is 0.72 (Ref. 17 and Tables 2 and 3 therein, respectively). Those data suggest that the labeling of different carbons of triglyceride-glycerol may not be equivalently labeled. In the present study, we observed that the labeling of 2H that is bound to C3 of triglyceride-glycerol obtained from adipose tissue of mice is 75% that of C1. This was true whether we examined samples that were obtained from mice maintained on diet I or diet II (not shown). Presumably, incomplete equilibration of 2H on the different carbons of glycerol reflects the fact that glycerol 3-phosphate, used in the synthesis of triglycerides, can be derived from glucose and/or phosphoenolpyruvate carboxykinase (PEPCK; i.e., glycero genesis). For example, phosphoglucoisomerase incorporates 2H on C1 glucose 6-phosphate to yield [1-2H]fructose 6-phosphate. Conversion to [1-2H]fructose 1,6-bisphosphate and hydrolysis yields two triose phosphates; during hydrolysis and equilibration, 2H is incorporated on C1 dihydroxycetone phosphate and C1glyceraldehyde 3-phosphate. Reduction to glyceral 3-phosphate yields two molecules of glyceral 3-phosphate with 2H on C1; only one of those molecules also has 2H on C3 (i.e., that which was bound to C1 of fructose 1,6-bisphosphate). The conversion of substrates to glyceral 3-phosphate via PEPCK to glyceral 3-phosphate could also preferentially label C1triglyceride-glycerol vs. C3triglyceride-glycerol (6).

Rieder and Rose (25) and Rose et al. (29) demonstrated that the two hydrogens bound to C1 of dihydroxyacetone phosphate derive from different reactions. Therefore, after establishing that the labeling of C1triglyceride-glycerol reflects total synthesis (above), we examined whether the hydrogens bound to C1 triglyceride-glycerol are equally labeled. We analyzed samples obtained from mice that were maintained for 14 days on 2H2O. The total labeling of C1triglyceride-glycerol was ~2.21% (diet I) and ~2.50% (diet II; Fig. 5). Samples treated with triose phosphate isomerase had an enrichment of ~1.03% (diet I) and ~1.17% (diet II). Thus, regardless of diet,
the two hydrogens of C1 triglyceride-glycerol reached ~88% equilibrium.

On the basis of the observations described above (i.e., the different labeling on C1 triglyceride-glycerol vs. C3 triglyceride-glycerol and the equilibration of 2H between the two hydrogens of C1 triglyceride-glycerol), we concluded that the correct product labeling is the labeling of 2H bound to C1 triglyceride-glycerol divided by 2.

Application of the 2H2O Method

There is uncertainty regarding how the ratio of dietary fat to carbohydrate affects the development of obesity (14, 36). We hypothesized that the relative distribution of one’s caloric intake would substantially affect the pathway(s) of lipid accretion during growth. 2H2O was used to test our hypothesis. Although the predominant difference between the diets was the ratio of dietary fat to carbohydrate, the caloric intake was slightly greater in mice that were fed a high-fat, low-carbohydrate diet (diet II) compared with those fed a low-fat, high-carbohydrate diet (diet I) (~10.5 vs. ~9.6 kcal·mouse⁻¹·day⁻¹, respectively, P < 0.075).

We observed that, during growth, the rate of accumulation of triglycerides in epididymal fat pads (i.e., the balance between the rates of synthesis and breakdown) is related to the intake of dietary fat (i.e., 4.4 μmol/day on diet I vs. 10.9 μmol/day on diet II; Table 1). Although our overall observation is consistent with the literature [i.e., percent dietary fat affects rate of lipid accretion (35)], these data are the first direct measurements of the rates of triglyceride synthesis and degradation in vivo. More importantly, the data show that, despite the very active triglyceride synthesis, triglyceride degradation also occurs at a considerable rate (i.e., degradation occurs at ~50% of the rate of synthesis). Our findings agree with the original work of Schoenheimer and Rittenberg (31), who concluded that ~50% of dietary fat moves through adipose tissue each day. This also supports the hypothesis put forth by Frayn (11), that adipose tissue may act as a buffer for daily lipid flux.

Using 2H2O allowed us to examine how the relative amount of dietary carbohydrate affects the relative contribution of lipogenesis (1, 10, 18, 19, 37). This was done by dividing the total enrichment of palmitate [i.e., M1 + (2 × M2), where M1 and M2 refer to the percentage of excess palmitate molecules with one or two 2H atoms, respectively] by the enrichment of body water times 22 (Fig. 6) [i.e., the average number of hydrogens incorporated during synthesis (1, 10)]. We found that the contribution of de novo lipogenesis to the pool of triglyceride-bound palmitate was about three times greater in mice maintained on diet I than on diet II (Table 1). Similar data were obtained by measuring the labeling of stearate (not shown). The observation is consistent with the fact that mice on diet I consume more carbohydrate than mice on diet II. One intriguing observation is that the type of carbohydrate also varied between the diets. For example, the ratio of complex to simple carbohydrates was greater in diet I than in diet II.

---

### Rates of Lipid Synthesis and Breakdown

<table>
<thead>
<tr>
<th>Diet</th>
<th>Triglyceride synthesis</th>
<th>Triglyceride breakdown</th>
<th>Net</th>
<th>Contribution of lipogenesis (%)</th>
<th>Incorporation into triglycerides</th>
<th>Removal from triglycerides</th>
<th>Net</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9.8</td>
<td>5.4</td>
<td>4.4</td>
<td>96</td>
<td>10.4</td>
<td>9.6</td>
<td>0.8</td>
</tr>
<tr>
<td>II</td>
<td>23.9</td>
<td>13.0</td>
<td>10.9</td>
<td>33</td>
<td>11.3</td>
<td>5.3</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Rates of triglyceride synthesis and breakdown were determined from the labeling of 2H bound to carbon 1 of triglyceride-glycerol. Units are in μmol/day. Contribution of de novo lipogenesis was determined from the labeling of triglyceride-bound palmitate. Rates of incorporation and removal of palmitate into and from triglycerides were determined from the labeling of triglyceride-bound palmitate.
Although the intake of simple carbohydrates is usually more closely associated with increased lipogenesis (14, 36), our data show that de novo lipogenesis is very active during growth despite the type of carbohydrate(s) being consumed (Table 1).

Finally, we were able to determine the rate of remodeling of triglyceride-bound fatty acids. This was done by measuring the rates of incorporation and removal of newly synthesized (i.e., $^2$H-labeled) palmitate into and from triglycerides. Initial inspection of the labeling data suggested that there must be a major remodeling. For example, if triglycerides are accumulating at 4.4 mol/day (e.g., Table 1, diet I), then 13.2 mol of fatty acids must be incorporated per day. Because palmitate typically accounts for a large percentage (e.g., ~35%) of the total fatty acids found in adipose tissue, one should expect that palmitate would accumulate at ~4.6 mol/day (i.e., 13.2 x 0.35). We found that the net palmitate flux was substantially less than what was expected on the basis of the net triglyceride flux (e.g., net palmitate flux was only 0.8 mol/day; Table 1, diet I). This was observed in both groups (Table 1). On measuring the distribution of the triglyceride-bound fatty acids, we found that the relative amount of palmitate decreased over time (Fig. 7). These observations are consistent with the original data presented by Hirsch (15). Namely, triglycerides in adipose tissue are in a dynamic exchange with the diet; the remodeling that we observed reflects the fact that the fatty acid content of diet that the mice were raised on (at Jackson Laboratories) is not the same as what we fed them (purchased from Research Diets, New Brunswick, NJ). Also, these data suggest that tracing the turnover of triglycerides via glycerol labeling has an advantage over using a labeled fatty acid (28), since various fatty acids have different fates (3, 33). Therefore, measuring the incorporation of one fatty acid into triglyceride may not accurately reflect the fate of other fatty acids.

In summary, our data show that diet influences 1) the rate of triglyceride accumulation and 2) the pathway(s) that affects lipid accretion. Also, although growth is characterized by net accumulation of lipid, lipid breakdown is very active, and substantial remodeling of triglycerides can occur. Because future experiments are aimed at studying the regulation of triglyceride turnover in adipose tissue, we reviewed the literature to identify parameters that could affect these measurements. In particular, several studies have examined compartmentation/heterogeneity of adipose tissue. We discuss the significance of those reports below.

First, Rodbell (27) demonstrated that adipose tissue is contaminated with vascular tissue. If unaccounted for, this may affect measurements of protein and DNA content of fat tissue. However, because vascular tissue contains virtually no triglyceride (27), quantitation of glyceride kinetics in adipose depots should be possible after a tissue biopsy without purification from vascular tissue.

Second, the lipid composition of a tissue can affect measurements of turnover. However, because ~90% of the glycerides found in adipose tissue are triglycerides, it does not seem that one needs to separate various glyceride species (e.g., triglyceride, diglyceride, phospholipids). Also, pulse-chase studies have shown that the small pool of diglycerides is in rapid equilibrium with the large pool of triglycerides (39).

Third, Angel (2) studied the intracellular structural heterogeneity. This author demonstrated that >90% of all lipid in adipocytes is localized in the cytoplasmic pool. A minor part of the total pool (<10%) is associated with other compartments (e.g., mitochondria, microsomes, etc.).

Fourth, chemical heterogeneity has been described. This can occur within the triglyceride pool because of the positional specificity of the lipases (12, 22). For example, lipoprotein lipase, which hydrolyzes extracellular triglycerides before entry into adipocytes, has different specificity from hormone-sensitive lipase, which hydrolyzes intracellular triglycerides before release from adipocytes.

Finally, vascularization can affect heterogeneity of adipose tissue (13). For example, investigators have observed variations in the number of, and area of, blood vessels within a single adipose tissue. Presumably, the greater the degree of perfusion, the greater the metabolic activity.

In summary, $^2$H$_2$O can be used to measure the total rates of triglyceride synthesis and degradation in adipose tissue in vivo. $^2$H$_2$O is uniquely suited for investigations in free-living conditions because catheterization is not required. Although the dose of $^2$H$_2$O used in our experiments is safe for rodents (23), to administer less $^2$H and not compromise analytical sensitivity we recently developed an alternative approach to processing gas chromatography-mass spectrometry data (20).

In conclusion, the ratio of dietary fat to carbohydrate affects the rates of, and the mechanisms of, triglyceride accumulation in adipose tissue. Our data show that triglycerides in adipose tissue are in a dynamic state during growth. Although future studies by our laboratory are aimed at studying how different interventions may affect the biochemical basis of lipid accumulation during growth, $^2$H$_2$O is also ideal for studying how to affect lipid kinetics in other conditions (e.g., obese or lipodystrophic subjects).

We thank Dr. Timothy Kern for generously donating mice that were used in several pilot studies and Dr. Vernon Anderson for helpful discussions regarding the stereoselectivity of $^2$H incorporation into triose phosphates via enzymatic reactions.

DISCLOSURES

This project was supported by funds from the Mt. Sinai Health Care Foundation of Cleveland, the Diabetes Association of Greater Cleveland (Grant no. 449-01), and Merck Pharmaceuticals. V. Chandramouli was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-14507.

REFERENCES

21. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, and Kahn CR. Role of glucose and insulin resis-