Lipid Metabolism in Diet-Induced Obese Rabbits Is Similar to That of Obese Humans\(^1,2\)

Xiao-jun Zhang,\(^3,4^{*}\) David L. Chinkes,\(^3,4\) Asle Aarsland,\(^3,5\) David N. Herndon,\(^3,4\) and Robert R. Wolfe\(^3,4,6\)

\(^3\)Metabolism Unit, Shriners Hospital for Children, and Departments of \(^4\)Surgery and \(^5\)Anesthesiology, University of Texas Medical Branch, Galveston, TX 77550

Abstract

Whereas diet-induced obese rabbits have been used to study various aspects of obesity, alterations of lipid metabolism in this model have not been clarified. This study aimed to compare plasma nonesterified fatty acid (NEFA) and triglyceride (TG) kinetics in obese and lean rabbits by means of U\(^{13}\)C\(_{16}\)-palmitate infusion. Young female rabbits consumed either a high-fat diet (49% energy from fat) ad libitum to develop obesity (\(n = 6\)) or a normal diet (7.9% energy from fat) as lean control (\(n = 5\)). After 10 wk of feeding, the body weight of obese rabbits (5.33 ± 0.05 kg) was greater (\(P < 0.001\)) than that of lean rabbits (3.89 ± 0.07 kg). The obese rabbits had higher concentrations of plasma NEFA and TG and a greater rate of fatty acid (FA) turnover. Whereas the fractional secretion rates of hepatic TG did not differ, 100% of hepatic secretory TG was synthesized from plasma NEFA in the lean rabbits compared to 59% in the obese rabbits (\(P < 0.001\)). In the lean rabbits, hepatic lipase-mediated hydrolysis of lipoprotein TG did not contribute to the FA pool for synthesis of secretory TG, consistent with the naturally occurring deficit in hepatic lipase in this species. We conclude that lipid metabolism in diet-induced obese rabbits is similar to that in obese humans. The deficiency in hepatic lipase in rabbits simplifies the quantitation of hepatic lipid kinetics. J. Nutr. 138: 515–518, 2008.

Introduction

Over the last decade, diet-induced obese rabbit models have been used to research obesity (1–5). Carroll et al. (2) reported that young female rabbits consuming a high-fat diet ad libitum gained weight rapidly, >86% of which is fat. Such obese rabbits have similar hemodynamic and neurohumoral changes as in obese humans and this model has been used for the study of obesity-associated disorders (2,3,6,7). However, this obese model has not been used for studying lipid kinetics, although elevated serum lipid concentrations have been reported (2). Because lipid dysfunction is essential in the etiology and pathology of obesity, it is important to characterize lipid kinetics to use this model for the study of obesity.

This study was designed to investigate alterations in lipid metabolism in diet-induced obese rabbits using stable isotope techniques. We hypothesized that obese rabbits are characterized by increased lipid turnover similar to obese humans and therefore would be a suitable model for the study of obesity. On the other hand, the rabbit is known to be naturally deficient in hepatic lipase (1), so its effect on hepatic lipid metabolism needs to be recognized.

Materials and Methods

Animals. Female New Zealand White rabbits (Myrtle’s Rabbitry), weighing ~3.5 kg, were used for this study. The rabbits were housed in individual cages and were given 100–120 g/d of unpurified diet (Lab Rabbit Chow 5326, Purina Mills) for 1 wk for acclimation. This diet provided 203–244 kcal/d (850–1022 kJ/d) with 23% from protein, 7.9% from fat, and 69% from carbohydrate. They were then randomly assigned to 2 groups: lean and obese. This protocol complied with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals, and was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

Experimental design. The rabbits in the lean group were given the same diet as in the acclimation phase. The rabbits in the obese group consumed a high-fat diet ad libitum consisting of the same unpurified solid grain diet mixed with additional 10% corn oil and 8% lard; the energy sources consisted 13% from protein, 49% from fat, and 38% from carbohydrate. After 10 wk of feeding, the rabbits were food-deprived for 24 h before tracer infusion to ensure that there was no exogenous fat entry.

Isotope infusion protocol. Surgical procedures were performed to insert catheters into the carotid artery and jugular vein under general anesthesia (8). The arterial line was used for drawing blood and monitoring arterial blood pressure and heart rate; the venous line was used for infusing a stable isotope tracer and anesthetics. Tracheotomy was performed for placement of a tracheal tube, which was connected to a hood filled with oxygen-enriched room air. U\(^{13}\)C\(_{16}\)-palmitate (99%
enriched; Cambridge Isotope Laboratories), bound to albumin in a 5% solution, was infused at 0.11 μmol·kg⁻¹·min⁻¹ for 8 h. Blood samples were taken before the start of the tracer infusion and every hour during the tracer infusion. The blood samples were collected in prechilled tubes containing EDTA; plasma was separated by centrifugation and stored at −20°C. During the tracer infusion, heart rate, mean arterial blood pressure, and rectal temperature were maintained stable by adjusting the infusion rates of anesthetics and physiological saline and by using heating lamps. At the end of tracer infusion, the rabbits were killed by i.v. injection of 5 mL saturated KCl under general anesthesia.

**Sample analysis.** Blood glucose concentration was measured with a glucose analyzer (model 2300; Yellow Springs Instruments). To measure concentrations in plasma of nonesterified fatty acids (NEFA) and triglycerides (TG), heptadecenoic acid and triheptadecanoin were added to the plasma as internal standards. NEFA and TG fractions were isolated by TLC (9). Seven fatty acids (FA) in plasma NEFA were measured using a GC system with flame ionization detection (model 6890, Agilent); their individual contributions to the total 7 FA were expressed as percents. The 7 FA were myristate (14:0), palmitate (16:0), palmitoleate [16:1(n-1)], stearate (18:0), oleate [C18:1(n-9)], linoleate [18:2(n-6)], and linolenate [(18:3(n-3)]. Palmitate enrichment in the NEFA and TG fractions were measured using a GC-MS (MSD system, Agilent). Ions were selectively monitored at mass-to-charge ratios of 270, 285, and 286 for palmitate.

**Calculations.** The rate of appearance (Ra) of plasma palmitate was calculated by tracer dilution. The equation is

\[ R_a = \frac{F}{E}. \]  
(Eq. 1)

where \( F \) is U⁻¹³C⁻¹⁻palmitate infusion rate in μmol·kg⁻¹·min⁻¹ and \( E \) is plasma nonesterified palmitate enrichment at plateau.

Plasma TG-bound palmitate enrichment was calculated by the following equation:

\[ \text{Enrichment} (t) = A + B \times \exp(1 \times t), \]  
(Eq. 2)

where enrichment \( t \) is plasma TG-bound palmitate enrichment at time \( t \), \( A \) is the plateau enrichment, \( B \) is the increase in enrichment from baseline to plateau, and \( I \) is the initial slope. The equation used to calculate percent of secretory TG synthesis from plasma NEFA is

\[ \% \text{TG}_{\text{palmitate}} = \left( \frac{E_{\text{TG}}}{E_{\text{palmitate}}} \right). \]  
(Eq. 3)

where \( E_{\text{TG}} \) is plateau enrichment of plasma TG and \( E_{\text{palmitate}} \) is plateau enrichment of plasma nonesterified palmitate.

The fractional secretion rate (FSR) of hepatic secretory TG from plasma NEFA was calculated from the following equation:

\[ \text{FSR}_{\text{palmitate}}(\%)/h = \left( \frac{E_{\text{TG}} - E_{\text{t}}}{E_{\text{TG}}(t_2 - t_1) \times (t_2 - t_1)} \right), \]  
(Eq. 4)

where \( E_{\text{TG}} - E_{\text{t}} \) is the increment of plasma TG-bound palmitate from time 1 to time 2; \( E_{\text{TG}}(t_2 - t_1) \) is the mean plasma nonesterified palmitate enrichment from time 1 to time 2.

Total FSR of plasma TG was calculated from the equation:

\[ \text{FSR}_{\text{total}}(\%)/h = \left( \frac{\text{FSR}_{\text{palmitate}}}{\% \text{TG}_{\text{palmitate}}} \right), \]  
(Eq. 5)

where FSRpalmitate is calculated from Eq. 4 and % TGpalmitate is calculated from Eq. 3.

Values are expressed as means ± SEM. Differences comparing 2 parameters were tested by \( t \) test either paired (within 1 group) or nonpaired (between 2 groups). Differences dealing with multiple time points within 1 group (comparisons to time 0) or between 2 groups (comparison between each pair of corresponding time points) were evaluated using 2-way repeated measures ANOVA followed by Bonferroni’s multiple comparison test as appropriate. A P-value <0.05 was considered significant.

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**Results**

The initial body weights did not differ between the lean (3.54 ± 0.05 kg) and obese (3.48 ± 0.07 kg) rabbits. After 10 wk of feeding, body weights increased \( (P < 0.001) \) in both groups and were greater \( (P < 0.001) \) in the obese (5.33 ± 0.05 kg) than in the lean (3.89 ± 0.07 kg) rabbits.

During the tracer infusion, the rectal temperature was similar in the lean and obese rabbits (Table 1). Heart rate and mean arterial pressure were higher in the obese than in the lean rabbits (Table 1). Plasma concentrations of glucose (Fig. 1A), NEFA (Fig. 1B), and TG (Fig. 1C) were higher \( (P < 0.05) \) in the obese than in the lean rabbits (Table 1). Palmitate accounted for 32.4 ± 0.2 and 24.8 ± 0.3% of the total 7 NEFA in the lean and obese groups, respectively \( (P = 0.05) \). These values were used to convert plasma nonesterified palmitate concentration to total NEFA concentration.

Plasma nonesterified palmitate enrichment was greater \( (P < 0.05) \) in the lean rabbits for the first 4 h of tracer infusion \( (P < 0.01-0.05) \) (Fig. 2A). Plasma TG-bound palmitate enrichments increased over time and then reached plateau in both groups (Fig. 2B). Because during the second 4 h of tracer infusion, plasma concentrations of NEFA and TG plasma enrichments of nonesterified and TG-bound palmitate were different from those in the first 4 h, we calculated \( R_a \) of plasma palmitate during these 2 periods. In the obese group, \( R_a \) of plasma palmitate was similar throughout the first \( 2.30 ± 0.19 \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} \) and second 4 h of tracer infusion \( 2.31 ± 0.13 \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} \). In the lean group, \( R_a \) of palmitate was greater \( (P < 0.01) \) in the second 4 h \( 2.08 ± 0.20 \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} \) than in the first 4 h \( 1.66 ± 0.17 \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} \). The difference in \( R_a \) of palmitate between the 2 groups was significant \( (P < 0.05) \) only during the first 4 h. \( R_a \) of plasma palmitate over the entire 8 h infusion was greater \( (P < 0.05) \) in obese \( 2.31 ± 0.16 \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} \) than in lean rabbits \( 1.78 ± 0.18 \mu \text{mol·kg}^{-1} \cdot \text{min}^{-1} \).

Percent of plasma TG from plasma NEFA was greater \( (P < 0.001) \) in the lean group \( (100 ± 5\%) \) than in the obese group \( (59 ± 6\%) \). Total TG FSR and the TG FSR from plasma NEFA were 10 ± 2 and 10 ± 2%/h in the lean group, respectively, which did not differ from the corresponding values of 13 ± 2%/h and 7 ± 1%/h in the obese group.

**Discussion**

Obese rabbits had higher concentrations of plasma NEFA and TG compared with their lean counterparts. The \( R_a \) of plasma palmitate in the obese rabbits was 30–40% greater than that in the lean rabbits, indicating increased lipolysis. These are typical alterations in lipid metabolism observed in both human obesity (10–14) and high-fat-diet obese rodent models (15,16). Thus, lipid metabolism in the diet-induced obese rabbits is similar to that in obese humans.

Hyperlipidemia and elevated NEFA turnover are the primary alterations of systemic lipid metabolism in obesity. This reflects increased lipolysis in conjunction with relatively insufficient plasma clearance, leading to fat accumulation. In human obesity, the rate of lipolysis may increase by 20–30%, when expressed as per unit of fat-free mass compared with that of lean counterparts (11,13). In obese animals, plasma NEFA and TG concentrations are reported to be elevated (2,15,16); however, the lipid kinetics have not been adequately measured. We found that the \( R_a \) of NEFA (reflecting the rate of lipolysis) in the obese rabbits was ~30 or 40% greater than that in lean rabbits during the total 8 h or the first 4 h of tracer infusion. These results are...
consistent with the previous report of insulin resistance in obese rabbits (2), because insulin would normally be expected to limit lipolysis and thus \( R_{\text{NEFA}} \).

The difference in FA sources used for hepatic secretory TG synthesis between the groups was striking. In the lean rabbits, 100% of the hepatic secretory TG was synthesized from plasma NEFA. In contrast, only 59% was synthesized from plasma NEFA in the obese rabbits. Hepatic secretory TG synthesis may use FA from plasma NEFA, de novo synthesized FA in the liver, FA released from hepatic TG degradation, and portal vein delivery of chylomicra and omental FA. Because the rabbits in this study were food deprived for 24 h before the tracer infusion, the contributions from hepatic de novo synthesis and from portal vein delivery of chylomicra can be excluded. Therefore, in the obese rabbits, the balance of hepatic TG synthesis (41%) was likely due to either hepatic TG degradation and/or portal vein delivery of omental FA.

Hepatic TG degradation consists of 2 potential components: plasma lipoprotein TG hydrolysis catalyzed by hepatic lipase in the sinusoids and hydrolysis of hepatic TG stored in the cytosol of hepatocytes. The latter is referred to as the delayed secretory pathway (17). The rabbit lacks hepatic lipase (18), which in other species binds to the sinusoidal surface of hepatic endothelial cells and plays a fundamental role in hydrolyzing the TG in lipoproteins and chylomicron remnants (19–21). Therefore, plasma lipoprotein TG hydrolysis can be excluded, meaning that hepatic TG degradation is exclusively attributable to the hydrolysis of storage TG. This is a unique characteristic of rabbits, because in lean fasted humans, plasma NEFA accounts for 90–94% of FA in VLDL-TG (22,23). Considering the complex nature of hepatic lipid metabolism, the deficiency in hepatic lipase in rabbits could be advantageous in quantification of hepatic lipid kinetics in future experiments because of a simplification of the necessary calculations.

In summary, the diet-induced obese rabbits had high plasma NEFA and TG concentrations and increased plasma NEFA

**TABLE 1** General characteristics of lean and obese rabbits

<table>
<thead>
<tr>
<th></th>
<th>Rectal temp, °C</th>
<th>HR, bpm</th>
<th>MAP, mm Hg</th>
<th>Plasma NEFA, mmol/L</th>
<th>Plasma TG, mmol/L</th>
<th>Serum cholesterol, mg/L</th>
<th>Plasma glucose, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean (n = 5)</td>
<td>38.6 ± 0.1</td>
<td>135 ± 4</td>
<td>61 ± 2</td>
<td>0.32 ± 0.03</td>
<td>295 ± 38</td>
<td>519 ± 29</td>
<td>10.3 ± 0.2</td>
</tr>
<tr>
<td>Obese (n = 6)</td>
<td>38.8 ± 0.1</td>
<td>166 ± 7*</td>
<td>69 ± 1*</td>
<td>0.63 ± 0.6*</td>
<td>471 ± 75*</td>
<td>575 ± 46</td>
<td>15.8 ± 1.3*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM. Rectal temperature (Rectal temp), heart rate (HR) in beats per minute (bpm), mean arterial pressure (MAP) are means of 17 measurements during tracer infusion. Plasma concentrations of NEFA, TG, and blood glucose are means of 8-h measurements. *Different from lean, \( P < 0.01 \); †Different from lean, \( P < 0.05 \).

2 Data from (2); to convert mg/L to mmol/L, divide by 386.

**FIGURE 1** Plasma glucose (A), NEFA (B), and TG (C) concentrations in lean and obese rabbits during the 8 h tracer infusion. Values are means ± SEM, \( n = 5 \) (lean) or 6 (obese). *Different from the lean group, \( P < 0.05 \); †Different from 0 h within a group, \( P < 0.05 \).

**FIGURE 2** Isotopic enrichments of plasma nonesterified palmitate (A) and TG-bound palmitate (B) in lean and obese rabbits. Values are means ± SEM, \( n = 5 \) (lean) or 6 (obese). *Different from the lean group, \( P < 0.05 \); †Different from 1 h within a group, \( P < 0.05 \).
turnover rate. These alterations in lipid kinetics are similar to those observed in obese humans. The deficiency of hepatic lipase in the rabbit provides a potential advantage in quantification of hepatic lipid metabolism, because it excludes hydrolysis of lipoprotein TG as a source of FA for synthesizing secretory TG.

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Literature Cited