Palmitate and Glycerol Kinetics during Brief Starvation in Normal Young Adult and Elderly Subjects

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Abstract

Data obtained in vitro suggest that the ability to mobilize fat decreases with age. We determined lipolytic rates in vivo in normal weight young adult (22–33 yr) and elderly (65–77 yr) subjects using a simultaneous infusion of [1,2-13C2]palmitate and [2H3]glycerol. The subjects were studied after a 12-h fast and again after 60–82 h of fasting. When lipolysis was expressed per unit of adipose tissue the values for the young adults were more than double those for the elderly (P < 0.05). However, the amount of body fat in the elderly was twice that of the young adults, so that lipolysis per unit of body weight was similar in both groups. These results demonstrate that lipolysis per unit of adipose tissue is lower in elderly subjects. This may be due to their increase in body fat, however, since the total amount of potential energy mobilized from adipose tissue was similar to that of the young adults.

Introduction

The process of aging is characterized by a continuous decline in the function of many physiological systems (1). A decreased response to beta adrenergic stimulation has been shown in a variety of tissues (2–4) including adipose tissue. The adipose tissue of the adult, than double those in young adults, has been suggested to be due to an increased body fat mass in the elderly, and is a significant clinical consequence during periods of food deprivation. This issue, however, has not been carefully studied and the lipolytic response to starvation in elderly humans is not known.

The purpose of this study was to evaluate lipolytic rates in young adult and elderly subjects during brief starvation, and to relate those rates to the plasma levels of epinephrine. Lipolytic rates were measured with [3H]glycerol, and the mobilization of FFA was measured simultaneously with [1,2-13C2]palmitic acid. Total body fat was determined by H218O dilution to enable the expression of lipolysis as a function of total body fat as well as total body weight.

Methods

Six normal young adult male students and four elderly adults (Table I) consented to the study, which was approved by the Massachusetts Institute of Technology (MIT) Committee on the use of Humans as Experimental Subjects and the Policy and Executive Committees of the Clinical Research Center (CRC). Subjects with evidence of diabetes mellitus, coronary artery disease, hypertension (blood pressure > 140/90), renal failure, liver disease, and those who were >10% above or below ideal body weight were excluded.

Prior to the study, each subject consumed a weight-maintaining free-choice diet. After an overnight (12 h) fast in the CRC, teloth catheters were placed percutaneously into the antecubital vein of one arm of each subject using local anesthesia (lidocaine, 1%) for infusions, and into a contralateral dorsal hand vein for arterialized venous sampling, using the heated hand technique (17). The subjects rested for 60 min after catheter placement before baseline blood samples were drawn. [1,2-13C2]Palmitic acid (99.5 atom percent, 1,2-13C; MSD Isotopes, Montreal, Canada) bound to essentially fatty acid-free human albumin (Cutter Laboratories, Emeryville, CA) (18) and [2H3]glycerol (99 atom percent deuterium; MSD Isotopes), dissolved in normal saline, were mixed and infused together using a calibrated syringe pump (Harvard Apparatus Co., Inc., Natick, MA). Both the palmitic acid and glycerol solutions were tested for sterility and the absence of pyrogens by a licensed commercial laboratory before infusion. The palmitic acid was administered by constant infusion at a rate of ~0.04 μmol/kg per min and the glycerol was infused via the primed-constant infusion technique (19, 20) using a priming dose of 1.6 μmol/kg and infusion rate of 0.11 μmol/kg per min. The infusion continued for 90 min while the subjects were kept supine and as undisturbed as possible.

The young subjects continued to fast for another 72 h (82 h total), while the elderly subjects fasted for another 48 h (60 h total). They were given only water, vitamins, KCI, and NaCl orally. On the second day of fasting, blood samples were obtained before and 4 h after the oral administration of H218O (10.45 atom percentage excess, ICON Isotopes, Inc., Summit, NJ) to measure total body water (TBW). The H218O was administered at a dose calculated to produce an increase of 40 parts per thousand in body H218O concentration, and was weighed to the nearest 0.1 mg. The infusion protocol was repeated at the end of the starvation period before the resumption of caloric intake. Since there are no pub-

1. Abbreviations used in this paper: BF, body fat; GCMS, gas chromatography-mass spectrometry; LPM, lean body mass; MPE, mole percent excess; Rn, rate of release; TBW, total body water.
lished reports of experimental fasting in elderly individuals for longer than 24 h and in vitro data suggest an impairment in lipolysis, it was decided to limit the duration of the fast in this age group to a maximum of 60 h.

Baseline blood samples were drawn before the onset of the stable isotope infusion and again after isotopic equilibrium was established at 45, 60, 70, 80, and 90 min. Two baseline blood samples for catecholamine determination were collected 5 min apart in ice tubes containing heparin and reduced glutathione. These were centrifuged immediately at 4°C and the supernatant divided into two 2.5-ml aliquots, which were frozen in an acetone bath containing dry ice. The samples were stored at −70°C until the time of analysis. Plasma norepinephrine and epinephrine concentrations were measured by reverse-phase liquid chromatography (21).

Plasma levels of insulin were determined by radioimmunoassay (22, 23) using an Amersham kit (code IM.78, Amersham Corp., Arlington Heights, IL) for the younger subjects and a Micromedic kit (Micromedic Systems, Inc., Horsham, PA) for the elderly. Whole-blood glucose and beta hydroxybutyrate were determined enzymatically (24).

Blood for analysis of [1,2-13C2]palmitic acid enrichment was collected in heparinized tubes and immediately placed in a 4°C ice bath. This procedure effectively prevents the in vitro hydrolysis of endogenous triglycerides by heparin-activated lipoprotein lipase (25). The plasma was promptly separated by centrifugation and stored at −20°C until the time of analysis. The samples were processed for quantitative gas chromatography as described by McDonald-Gibson and Young (26). After conversion to the methyl esters, [1,2-13C2]palmitic acid enrichment was determined by gas chromatography and mass spectrometry (GCMS) as described by Wolfe et al. (18) using a GCMS system (Hewlett-Packard 5895, Palo Alto, CA). Gas chromatographic separation of the fatty acid methyl esters was performed on a 3 ft x 2 mm glass column packed with 5% diethylene-glycolsuccinate on a 100–200 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) with the column temperature at 190°C and the helium carrier gas flow rate at 30 ml/min. Ions at mass-to-charge ratios of 270.2 and 272.2, representing the molecular ions of unlabeled and [1,2-13C2]palmitic acid methyl esters, respectively, were selectively monitored at 0.1-s intervals and their corresponding peaks integrated. Palmitic acid concentration was quantitated separately on a gas chromatograph.

The blood for glycerol evaluation was collected in the same manner as that for palmitate. Plasma proteins were precipitated with Ba(OH)2 and ZnSO4 and the resultant supernatant passed through anion (Dowex AGI-X8) and cation (Dowex AG-50W-X8) exchange columns. The trimethylsilyl derivative was formed and 1H-enrichment determined by GCMS (27). Glycerol concentration was measured on an aliquot of the preinfusion sample by the internal standard technique using [1H3]glycerol as the internal standard and GCMS analysis to determine concentration.

TBW was measured by H218O dilution as described by Schoeller et al. (28). 18O enrichment was analyzed on a triple inlet, triple collector, isotope ratio mass spectrometer (Nuclide Corp., State College, PA).

**Calculations**

The R of palmitate and glycerol into plasma were calculated according to Steele's equation (29) as it applies to isotopic steadystate conditions. Thus, the R of the substrate is:

\[ R_d (\mu mol/kg per min) = \frac{F}{IE} \]

where F is the isotope infusion rate (\( \mu \)mol/kg per min) and IE is the isotopic enrichment at plateau. Since the infusion of stable isotopes contributed up to 2% to the mass of the substrate pool the above equation is corrected to:

\[ R_d (\mu mol/kg per min) = \left( \frac{1}{IE_p} - 1 \right) \times F, \]

where \( R_d \) is the rate of appearance of palmitate or glycerol, IE is the isotopic enrichment of the infused (atom percent excess) and IE\( _p \) is the isotopic enrichment of plasma (atom percent excess) at isotopic equilibrium.

When substrate concentrations are not changing and when isotopic equilibrium is reached:

\[ Substrate\ uptake = \frac{R_d (\mu mol/kg per min)}{MPE} \]

and the clearance rate of a substrate:

\[ Substrate\ uptake = \frac{Substrate\ plasma\ concentration}{\text{Substrate}\ uptake} \]

Since palmitate is thought to be typical of other long-chain fatty acids (30, 31), the flux of all fatty acids is assumed to be similar to that of palmitate.

The concentration of plasma glycerol was determined as follows:

\[ \text{Unlabeled glycerol (\( \mu \)mol/ml)} = \frac{0.007 \mu mol/ml}{\text{MPE}} - 0.007 \mu mol/ml \]

where 0.007 \( \mu \)mol/ml is the concentration of isotope of \([\text{H}_3\text{glycerol}]) added to 1 ml of the preinfusion, baseline plasma sample, and MPE is the mole percent excess equal to R/R + 1, where R is equal to the final isotopic enrichment of the plasma minus the enrichment before 0.007 \( \mu \)mol of labeled glycerol was added.

The estimation of body fat was derived from the calculation of TBW as follows:

\[ \text{TBW (kg)} = \frac{D \times APE}{\text{MW} \times 100} \times 18.02 \times \frac{1}{R_{\text{sat}} + \Delta H^{18O}} \]

where D is the dose of \( \text{H}_3\text{O} \) (g), MW is the molecular weight of the \( \text{H}_3\text{O} \), APE is the atom percentage \( \text{H}^{18O} \) enrichment of the \( \text{H}_3\text{O} \), F is the \( \text{H}^{18O} \) fractionation between \( \text{CO}_2 \) and \( \text{H}_2\text{O} \) (1.0407), R\( _{\text{sat}} \) is the ratio of \( \text{H}^{18O}\text{O} \) in the standard (0.002045) and \( \Delta H^{18O} \) is the difference in enrichment in the plasma water resulting from the isotopic administration. Once having calculated TBW, then:

\[ \text{Lean body mass (kg)} = \text{TBW/0.72} \]

\[ \text{Body fat (kg)} = \text{Body weight} - \text{lean body mass} \]

**Statistical analysis**

The Student's t test for paired comparisons was used to test the significance of changes in palmitate flux, glycerol flux, insulin levels, and substrate concentrations before and after fasting. Differences between the young adults and elderly were evaluated by the Student's t test for independent samples. An analysis of variance (ANOVA) was used to test the significance of changes in norepinephrine and epinephrine levels before and after fasting (32). One-sided P values were used when evaluating changes in palmitate and glycerol release since these processes should always increase with starvation. All other reported P values are based on two-sided tests.

**Results**

**Young adults.** Approximately 16% of total body weight consisted of adipose tissue (Table I). This is similar to the average fat

<table>
<thead>
<tr>
<th>Characteristics (Mean±SE) of the Young Adult and Elderly Subjects</th>
<th>Young adults</th>
<th>Elderly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24±2</td>
<td>70±3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181±2</td>
<td>171±2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72±3</td>
<td>72±3</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>60±3</td>
<td>46±3</td>
</tr>
<tr>
<td>Total body fat (kg)</td>
<td>11±1</td>
<td>23±2</td>
</tr>
<tr>
<td>Fat (% body wt)</td>
<td>16±1</td>
<td>33±2</td>
</tr>
</tbody>
</table>
content reported previously in young adult men (33). The changes in various metabolic and hormonal factors in response to several days of fasting are shown in Table II. Weight loss was progressive throughout the fasting period. The mean (±SE) concentration of blood glucose decreased from 5.15±0.25 to 3.14±0.10 mmol/liter with continued fasting (P < 0.001), as did the level of insulin (from 8.1±0.9 to 3.0±0.8 μU/ml) (P < 0.05). These data are in agreement with previous reports (34, 35). The concentration of beta hydroxybutyrate (Table II) increased markedly with accelerated lipolysis from 0.08±0.04 mmol/liter to 3.01±0.64 mmol/liter (P < 0.005).

The overall mean (±SE) Ra of palmitate after the overnight fast (Table II), was 1.86±0.18 μmol/kg per min and doubled to 4.02±0.13 μmol/kg per min after 82 h of fasting (P < 0.001). When lipolysis is expressed per unit of body fat (BF), the mean Ra of palmitate increased from 12.73±2.03 μmol/kg BF per min after an overnight fast to 27.48±3.29 μmol/kg BF per min with starvation (P < 0.005). The mean (±SE) amount of palmitate released into the bloodstream for possible utilization by the LBM was 2.24±0.23 μmol/kg LBM per min after the overnight fast and 4.76±0.16 μmol/kg LBM per min after 82 h of starvation (P < 0.001).

The mean (±SE) Ra of glycerol after the overnight fast was 2.59±0.22 μmol/kg per min (24 g/d), which doubled to 5.04±0.28 μmol/kg per min (46 g/d) with prolonged fasting (P < 0.001). When lipolysis was expressed per unit of BF, the mean Ra of glycerol increased from 17.04±1.16 to 34.21±4.21 μmol/kg BF per min after an overnight fast to 5.29±0.33 μmol/kg BF per min with starvation (P < 0.005). The mean (±SE) of glycerol per kg LBM was 3.08±0.26 and 5.97±0.33 after the overnight and 82-h fasts, respectively (P < 0.001).

Although the mean concentration of plasma glycerol doubled from 0.040±0.007 to 0.087±0.011 μmol/ml (P < 0.01) and the mean total plasma palmitate increased threefold from 0.052±0.008 to 0.162±0.012 μmol/ml (P < 0.001), there was no statistically significant change in the relative rates of plasma glycerol or palmitate clearance (Table II). The rate of uptake of glycerol and palmitate thus increased in proportion to the change in plasma concentrations of these substrates.

The mean levels of plasma catecholamines are also displayed in Table II. Plasma epinephrine increased by 43%, from 53±7 to 76±11 pg/ml with continued starvation (P = 0.001). Plasma norepinephrine also increased slightly (by 16%), but the difference was not statistically significant.

Elderly subjects. The elderly subjects fasted for a total of 60 h, 22 h less than the young adult subjects. In general, the elderly group tolerated the fast quite well without major complaints of hunger or weakness. The percentage of body weight as fat (33%) was twice that of the young adults (Table I). This is similar to the average fat content in elderly subjects reported previously (33). Table III summarizes the various metabolic changes occurring in this group during starvation. None of the factors listed in this table, after an overnight fast, were significantly different from the younger group. There was little decrease in body weight during the 60-h fast. The decrease in blood glucose (P = 0.001) and increase in beta hydroxybutyrate (P < 0.05) were similar to those observed in the younger subjects. The mean plasma insulin concentration declined from 6.1±0.5 to 4.6±0.2 μU/ml with continued fasting (P < 0.05). The plasma concentrations and plasma clearances of glycerol and palmitate after the overnight and 60-h fasting periods were similar to those found in the younger subjects.

The mean (±SE) Ra of palmitate (Table III) was 1.93±0.30 μmol/kg per min after an overnight fast and doubled to 3.91±0.33 μmol/kg per min after 60 h of starvation (P < 0.05). This response was similar to that found for young adults, despite a somewhat shorter fast. The mean (±SE) Ra of palmitate per unit of LBM was also similar to the young adults (Table III). When lipolysis was expressed per unit of body fat, however, the release of palmitate was less than half that of the young adults (P < 0.05). The mean Ra of palmitate was 5.30±1.22 and 11.64±0.62 μmol/kg BF per min after the overnight and 60-h fasts, respectively (P < 0.05).

As in the younger subjects, the mean Ra of glycerol was about equivocally to that of palmitate. The average Ra of glycerol was 2.19±0.16 μmol/kg per min after the overnight fast and increased to 3.84±0.36 μmol/kg per min after 60 h of fasting (P = 0.005).
Table III. Response of Metabolic Factors (Mean±SE) to a 60-h Fast in Elderly Subjects

<table>
<thead>
<tr>
<th></th>
<th>Overnight fast</th>
<th>60-h Fast</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>71.8±2.8</td>
<td>71.2±2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/liter)</td>
<td>5.26±0.24</td>
<td>3.14±0.17</td>
<td>0.001</td>
</tr>
<tr>
<td>Beta hydroxybutyrate (mmol/liter)</td>
<td>0.07±0.001</td>
<td>3.01±0.80</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>6.1±0.5</td>
<td>4.6±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Epinephrine (µg/ml)</td>
<td>41±8</td>
<td>63±10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Norepinephrine (µg/ml)</td>
<td>307±56</td>
<td>396±77</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glycerol (µmol/ml)</td>
<td>0.048±0.004</td>
<td>0.086±0.016</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitate (µmol/ml)</td>
<td>0.075±0.020</td>
<td>0.194±0.040</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>$R_n$ of glycerol (µmol/kg per min)</td>
<td>2.19±0.16</td>
<td>3.84±0.36</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>$R_n$ of glycerol (µmol/kg BF per min)</td>
<td>7.03±0.64</td>
<td>12.54±0.49</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>$R_n$ of glycerol (µmol/kg LBM per min)</td>
<td>3.49±0.30</td>
<td>6.32±0.65</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>$R_n$ of palmitate (µmol/kg per min)</td>
<td>1.93±0.30</td>
<td>3.91±0.33</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>$R_n$ of palmitate (µmol/kg BF per min)</td>
<td>5.30±1.22</td>
<td>11.64±0.62</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>$R_n$ of palmitate (µmol/kg LBM per min)</td>
<td>2.52±0.34</td>
<td>6.00±0.95</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Glycerol clearance (ml/kg LBM per min)</td>
<td>72±5</td>
<td>68±11</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitate clearance (ml/kg LBM per min)</td>
<td>58±5</td>
<td>40±1</td>
<td>NS</td>
</tr>
</tbody>
</table>

* One-sided P value.

When the $R_n$ of glycerol is expressed per unit of body fat, it is again less than half that of the young adults ($P < 0.05$). The mean $R_n$ of glycerol was 7.03±0.64 and 12.54±0.49 µmol/kg BF per min after the overnight and 60-h fasts, respectively ($P < 0.05$).

The concentration of plasma catecholamines increased significantly after 60 h of starvation when compared to that after the overnight fast. The mean concentration of plasma epinephrine increased by 54% from 41±8 to 63±10 pg/ml ($P < 0.01$) and that of norepinephrine by 29% from 307±56 to 396±77 pg/ml ($P < 0.01$).

Discussion

The results of this study are consistent with observations made previously in isolated rat adipocytes (5–9). Lipolysis per unit of adipose tissue was much lower in the elderly subjects than in the normal volunteers after both the overnight and the more prolonged period of fasting. As in rats (36), however, the percentage of body fat in the elderly (33%) was more than twice that of the young adults (16%), so that whole-body lipolytic rates were similar in both groups. Furthermore, the amount of potential energy supplied from fat in relation to the LBM was also similar in both groups.

The mechanism regulating this process is not known but could be related to the energy needs of the LBM. Therefore, the lower rate of lipolysis per unit of adipose tissue in the elderly may be a reflection of their proportionate increase in body fat rather than their increase in age. Without some regulatory mechanism, individuals with excess body fat would release large amounts of FFA into the bloodstream during fasting. This would not only exceed energy requirements, but could become hazardous if fatty acid binding sites on albumin were overwhelmed (37). In vivo studies performed in young adult obese subjects revealed similar results (38). Lipolysis per unit of BF was much lower in the obese when compared with normal weight subjects. The amount of fatty acids released per unit of LBM was similar, however, in both groups.

The mean release of palmitate from adipose tissue into plasma approximately doubled with the 60- or 80-h fasts. Since palmitate is typical of other fatty acids and usually constitutes ~25% of total circulating fatty acids (31), the rate of lipolysis at the end of the fasting period represents ~4.0 kcal of potential energy released into the circulation per day. This exceeds daily energy needs and supports previous observations that only a portion of the FFA flux is oxidized for fuel (39, 40).

The average $R_n$ of glycerol after an overnight fast amounts to 24 g released into the plasma per day in the young subjects and 21 g/d in the elderly. This correlates well with other reports using different methodology (41). Glycerol release increased after further starvation, but was still less than 50 g/d. Even if all of the glycerol released from adipose tissue were used solely for gluconeogenesis, this would amount to the production of <25 g of glucose/d. The amount of glucose utilized by the brain and other tissues during this period of starvation is much higher (42). This explains, in part, why nitrogen loss continues throughout starvation, since gluconeogenesis from amino acids must occur to meet the body's glucose requirement.

Even though the plasma concentration of palmitate and glycerol increased during starvation, plasma clearance for these substrates did not change. This confirms that the higher concentrations of palmitate and glycerol resulted from an increase in release from adipose tissue and not from a decrease in their clearance.

The increase in plasma epinephrine during fasting is consistent with the view that fasting stimulates the secretion of epinephrine from the adrenal medulla, which results in an increase in lipolysis (14). However, the level of plasma epinephrine observed in this study remained below the reported threshold level for lipolysis (43, 44). If this modest increase in plasma epinephrine does stimulate lipolysis, the lipolytic response to epinephrine must be increased. It has been shown that starvation increases epinephrine-induced lipolysis in humans (45) and increases epinephrine-stimulated adenylate cyclase activity in rats (46). Furthermore, the decrease in plasma insulin may enhance epinephrine-induced lipolysis. When insulin is deficient as in strepto-
zotocin-induced diabetic rats, adipose tissue is 5–10 times more sensitive to the lipolytic action of epinephrine than adipose tissue from normal rats (47).

The plasma concentration of norepinephrine increased with starvation in both the young and old subjects, but this increase was only statistically significant in the elderly. It is unlikely that the sympathetic nervous system is involved in lipolysis during starvation. Since norepinephrine functions mainly as a neurotransmitter in sympathetic nerve endings, dynamic measurements of norepinephrine turnover may be required to assess sympathetic nervous system activity (48). A decrease in norepinephrine turnover has been demonstrated in animals during starvation (49) and in humans during hypocaloric feeding (50). Increased urinary excretion of norepinephrine may occur during starvation but is eliminated by adrenalectomy (14). Therefore, the increase of plasma norepinephrine levels observed in the present study may be explained by an increased secretion of norepinephrine from the adrenal medulla.

In conclusion, adipose tissue can supply all of the daily caloric needs during starvation in both young adult and elderly subjects by increasing the rate of lipolysis. Although lipolysis per unit of adipose tissue is lower in elderly subjects during brief starvation, this may reflect their increase in body fat since the whole-body release of fatty acids and glycerol was similar to the young adults.

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References


