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Fatty acid oxidation and fatty acid synthesis in energy restricted rats

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Abstract

The importance of fat oxidation and fatty acid synthesis were examined in rats fed approximately one half their *ad libitum* food intake for a period of 13 days followed by 7 days of *ad libitum* feeding (refed rats). This study was undertaken because previous reports demonstrated that refed rats rapidly accumulated body fat. Our results confirmed this observation: refed rats accrued body fat and body weight at rates that were approximately 3 times higher than controls. Evidence for a period of increased metabolic efficiency was demonstrated by measuring the net energy requirement for maintenance over the refeeding period: refed rats had a reduced metabolic rate during the period of energy restriction (approximately 30% lower than control) and this persisted up to 2 days after the reintroduction of *ad libitum* feeding. The major factor responsible for the rapid fat gain was a depressed rate of fatty acid oxidation. Calculations of protein and carbohydrate intake over the refeeding period showed that the simplest explanation for the decrease in fatty acid oxidation is fat sparing. This is possible because of the large increase in dietary carbohydrate and protein intake during the refeeding period when metabolic rates are still depressed. The increased carbohydrate and protein may adequately compensate for the increasing energy requirements of the ER rats over the refeeding period affording rats the luxury of storing the excess dietary fat energy.

Keywords: Metabolism; Dieting; Body fat; Dietary fat; Fatty acid synthesis; Metabolic control

1. Introduction

Restricting energy intake is frequently used as a stratagem for losing weight but it is rarely successful [1]. Part of the problem is an apparent decrease in metabolic rate that accompanies energy restriction. This has been observed in fasted rats [2–4] as well as in energy restricted humans [5–7] and rodents [8–13] suggesting metabolic processes exist that can severely limit the effectiveness of any dieting regime. The decrease in metabolic rate appears to be responsible for rapid weight gain and body fat gain during the post-energy restriction phase when normal energy consumption resumes [4,7,10] because it appears to persist long after a return to normal energy consumption [4,11,12]. Thus, rapid weight and fat gains occur because of the difference between the persistent lower total energy expenditure of the energy restriction phase and total energy consumption of the post-energy restriction phase. This has been demonstrated by refeeding fasted rats with 50% and 75% of normal energy intake [4].

Evidence suggests that the diet-induced weight gain is a problem of fat imbalance in post-energy restricted rats because it can be influenced by the amount [11,13] and type [14] of dietary fat. Fat balance is dependent on several factors including: fat intake, *de novo* fatty acid synthesis and fat oxidation. Although net fat synthesis occurs to a very small extent in humans [15,16], it has been shown to be important in rats [17] and the fat synthesis pathway is highly responsive to changes in diet and endocrine conditions [18]. Fat oxidation is dependent on several factors, including the amount of carbohydrate and protein available for oxidation [19], enzyme preference for polyunsaturated fatty acids [20,21], previous exposure to high-fat diets [22], cellular concentrations of allosteric inhibitors such as malonyl coenzyme A [23], and the activity of enzymes affected by reversible phosphorylation control [24]. Thus, many different factors could contribute to the rapid fat gain in post-energy restricted rats.

In the present study we examined the metabolic processes underlying the rapid fat and body weight gain during the post-energy restriction period in rats with particular

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focus on fat metabolism. The aim was to determine the metabolic pathways involved in mediating the physiological changes associated with energy restriction and during the post-energy restriction phase. In addition, we examined several key regulatory enzyme activities in amino acid utilization, glycolysis, gluconeogenesis and fatty acid synthesis to try and identify the pathway loci that may be involved in mediating the metabolic rate depression. The results suggest that the rapid increase in body fat is due, primarily, to an imbalance in energy expenditure and food intake.

2. Materials and methods

2.1. Animals and protocol

Male Sprague-Dawley rats (approximately 3 wk post weaning) were obtained from Charles River Co. (Québec). Rats were randomly assigned to individual wire-bottom stainless steel cages and maintained on a 12 h light/dark cycle. The room temperature was 22 ± 1°C and the humidity was held between 45–55%. Rats were obtained in two shipments. The first shipment of rats ($N = 88$, 153.1 ± 3.8 g) were assigned to age-matched (AM) and energy restricted (ER) groups. The second shipment of rats ($N = 25$, 152.3 ± 3.5 g) were used as the weight-matched (WM) group. All rats were given free access to water throughout the experimental time course. Animals were fed differing amounts of a cereal-based rodent diet, Rodent Laboratory Chow 5001 (Ralston Purina Canada, Inc., Strathroy, ON) depending on the protocol.

Rats assigned to the AM and WM groups had free access to rodent laboratory chow 5001 (Ralston Purina Co., St. Louis, Missouri). The chow contained 23.4% protein, 4.5% fat, 5.8% fibre and 49% carbohydrate. Rats assigned to the ER group were fed *ad lib* for the first week, and then fed 12 g of chow (half the measured food consumption of days 5 and 6) per day for 2 weeks. After this time, the rats were again given free access to food.

At various times throughout the protocol, six animals from each group were assessed for their ability to oxidize palmitic acid (see below). Six other animals were injected with tritiated water and used to measure the rate of fatty acid synthesis (see below). Livers from these latter animals were also removed and clamped in liquid nitrogen-cooled tongs. Tissues were stored at −80°C until used for measurement of enzyme activities.

2.2. Measurement of de novo fatty acid synthesis

At indicated times, six animals from each group were injected i.p. with 4 mCi ^3H_2O (Amersham, Mississauga, ON) in 0.3 ml water. Animals were killed one hour later by a 1 min exposure to O_2 followed by a 3 min exposure to CO_2. Injecting with ^3H_2O enabled the measurement of ^3H incorporation into fatty acids synthesized *de novo* [25,26]. Blood was withdrawn from the heart (for measurement of ^3H_2O specific activity) and the liver was rapidly dissected and clamped in liquid nitrogen-cooled tongs. As indicated above, livers were removed and stored for measurement of enzyme activity. Carcasses were frozen at −20°C until processed. Carcasses and livers were processed separately and total fatty acid synthesis rates were obtained by adding the liver and carcass values. Carcasses were homogenized with an equal volume of water using a Waring blender until smooth. A 40 mL sample was further processed using a Brinkman homogenizer to ensure sample homogeneity. Livers were homogenized with an equal volume of water. Samples were extracted with $CHCl_3$, dried and counted in a scintillation counter to measure the incorporation of tritium into fatty acids.

2.3. Measurement of fatty acid oxidation

The methodology for measuring fatty acid oxidation was essentially that of Reed et al. [22]. At indicated times, six animals from each group were fed, by gavage, 1.5 μCi of uniformly labelled palmitic acid in 0.5 mL/100 g body weight of corn oil. Animals were immediately placed in open-flow metabolic chambers through which ambient air was drawn with a vacuum pump. Airflow was maintained at 1.1 L/min. The CO_2 in the expired air was trapped by bubbling through vials containing approximately 30 g of Carbo-Sorb E (Packard Chemicals, Mississauga, ON) placed between the cage and the vacuum pump. Traps were replaced after 2 h and 6 h for a total of 3 vials/24 h. Approximately 66% of the total recovered radioactivity was found in the first vial. The remaining two vials contained about 16% each.

2.4. Enzyme assays

All chemicals were obtained from either Boehringer-Mannheim Co. (Montreal, P.Q.) or Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated. Activities were measured after preparation of crude homogenates. The procedure involved grinding frozen tissue 1:4 in 50 mM imidazole, 5 mM EDTA, 5 mM EGTA, 100 mM NaF, and 30 mM β-mercaptoethanol (pH 7.0) and centrifuging for 15 minutes in an Eppendorf centrifuge (12,000 × g). The supernatant was removed and stored on ice until assay. The EDTA-EGTA-NaF buffer prevented changes in enzyme phosphorylation by inhibiting phosphatase action (NaF) and by chelating free magnesium and calcium ions.

Enzymes were measured using a microplate reader with a 340 nm filter (25°C, Dynatech Labs, Chantilly, VA). Kinetic analyses were performed with third party software [27]. Glucose 6-phosphate dehydrogenase, malic enzyme, ATP-citrate lyase, fatty acid synthetase, glucokinase, aldolase, lactate dehydrogenase, phophoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, phosphofructokinase, pyruvate kinase and fructose 2,6-bisphosphate were

Table 1
Body weight, body composition and body energy

Group	Time (d) refeeding	Body weight (g)	Body fat (g)	Lean body mass (g)	Body energy (kcal)
ER	0RF[1]	217.3 ± 6.0[b]	8.9 ± 2.1[b]	62.2 ± 3.7[b]	326.5 ± 31.7[b]
	2RF	263.8 ± 8.2[c]	12.2 ± 1.0[a]	66.2 ± 1.4[c]	375.8 ± 9.6[c]
	7RF	315.8 ± 16.8[d]	16.4 ± 2.4[c]	79.7 ± 4.7[d]	469.1 ± 39.2[d]
AM	0RF	330.3 ± 10.4[d]	21.0 ± 2.1[d]	84.5 ± 2.2[e]	534.5 ± 27.5[e]
	7RF	360.2 ± 12.7[e]	23.1 ± 3.1[d]	93.5 ± 4.5[f]	590.0 ± 41.0[f]
WM	2RF	237.7 ± 4.8[a]	13.5 ± 1.0[a]	57.8 ± 2.0[a]	357.0 ± 16.6[a,b]
	7RF	283.7 ± 7.2[f]	16.7 ± 2.0[c]	74.4 ± 2.2[g]	452.0 ± 26.8[d]

[1] This group was also used as the ORF value for the WM animals. RF; refed. Values in the same column with different superscripts are significantly different at the $P < 0.05$ level as determined by the LSD test. Body energy was calculated as body fat × 10.26 + LBM × 3.78 [30]. Values are means ± SD for $N = 6$ rats.

measured as described by Brooks and Lampi [28]. Pyruvate dehydrogenase was measured as described by Brooks and Storey [29]. Activation time courses (performed at room temperature) were followed to ensure maximal activation. Aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, serine dehydratase, and branched chain amino acid dehydrogenase were measured according to Brooks and Lampi [30].

2.5. Other methods and statistics

Protein concentrations were measured by Biuret after solubilizing in 5% SDS in 0.5 N NaOH as this produced the most accurate determination of carcass protein content [31]. Body fat was measured after chloroform-methanol extraction [32]. Water content was measured after freeze-drying. Samples were corrected for added water. All statistical analyses were performed using the LSD test after ANOVA analysis (STATISTICA for Windows, StatSoft, Inc., 1997, Tulsa, OK). All values are reported as means ± SD. The variance of the calculated values was estimated as follows.

$$\sigma(x \pm y) = \sigma(x) + \sigma(y) \quad (1)$$

$$\sigma(x \times y) = \bar{x}^2 \times \sigma(y) + \bar{y}^2 \times \sigma(x) \quad (2)$$

$$\sigma(x/y) = \frac{\bar{x}^2 \times \sigma(y) + \bar{y}^2 \times \sigma(x)}{\bar{y}^4} \quad (3)$$

In some cases, the variance of the data was proportional to the square of the mean. When this was true, data were transformed using a $\log_{10}$ function prior to statistical analysis [33]. This procedure always normalized the data. Percentages were normalized by an arcsin ($\sqrt{y/100}$) transformation [33]. Differences were considered significant if the probability was less than 0.05.

3. Results

3.1. Food intake, body weight, body composition and blood chemistry

The ER rats were compared to both AM and WM animals to control for a potential effect of body size. During the 14 d energy restriction period, the ER rats were fed approximately half their normal intake of rat chow (12 g/d). Over this time period, ER rats neither gained nor lost weight (Table 1, Fig. 1) but their body composition changed significantly so that the percentage body fat was lower and the percentage body water higher than that of AM rats. The loss of fat exactly balanced an increase in body water so that the ER rats weighed the same before and after the energy restriction period. The decrease in body fat paralleled a decrease in the weight of peri-renal and epididymal fat deposits (data not shown) and a loss in the total fat content (Table 1). The percentage of body mass due to protein did not change over the entire experimental period (Fig. 1) although total body protein and lean body mass (total body weight − fat − water) increased (Table 1). Food consumption during the refeeding phase of the experiment showed some small differences between groups. The WM controls consumed 24.9 ± 1.1 g/d, which was significantly less than the AM, controls (27.9 ± 1.4 g/d and the ER rats. These values represent means of 2RF and 7RF animals since no significant differences were noted between these two time periods. As such, the values represent means of 12–24 animals.

Serum concentrations of glucose, triglycerides, β-hydroxybutyric acid, protein, and albumin were measured to determine the metabolic state of the rats at the end of the energy restriction period. Serum glucose concentrations were significantly lower in ER rats (7.8 ± 1.2 mmol/L, $N = 6$) as compared to all other groups (10.4 ± 1.2 mmol/L, $N = 42$ pooled values). No changes in the concentration of triglycerides (1.66 ± 0.52 mmol/L, $N = 48$), β-hydroxybutyric acid (134 ± 53 μmol/L, $N = 48$), protein (58.7 ± 6.6 g/L, $N = 48$) or albumin (41.7 ± 5.0 g/L, $N = 48$) were observed. In addition to these parameters, liver glycogen levels were also measured to determine if the rats had depleted their carbohydrate stores at the end of the energy restriction period. Liver glycogen remained constant at 190 ± 44 μmol/gww ($N = 48$) throughout the experiment.

During the refeeding phase, the rate at which ER rats regained body weight depended on the time of measurement. Over the first 2 days, the ER rats gained weight at a

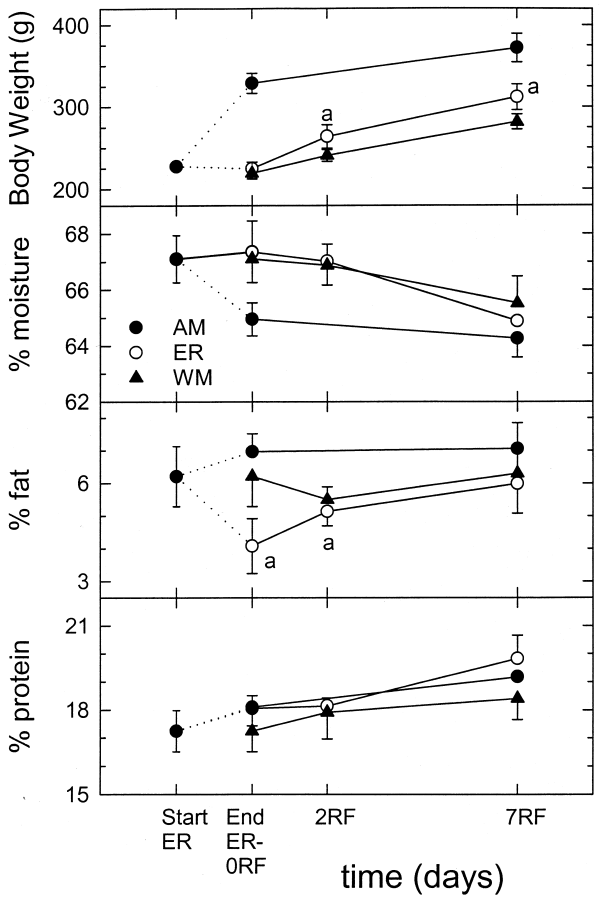

Fig. 1. Live body weight and body composition as a function of energy restriction in energy restricted (ER; open circles), age-matched (AM; filled circles) and weight-matched (WM; filled triangles) rats. See Table 1 for the absolute fat and lean body mass values. Values are means ± SD for $N = 6$ rats. (a) Significantly different from ORF-AM rats at the $P < 0.05$ level. RF; refeeding period.

rate that was 5.4 fold higher than the AM rats and approximately 30 fold higher than the WM rats, which failed to gain a significant amount of weight over this time period (Table 1). Total fat increased by 1.6 g/d in the refed ER animals whereas no significant accumulation was observed in AM or WM animals. Body energy increased at a rate that was approximately three times that of the AM animals but identical to that of the WM animals (Table 1).

Over the next 5 days post energy restriction, the rate of weight gain of ER rats was comparable with that of WM rats but was higher than that of the AM animals (2.4 fold; Table 1). Similarly, the rate of body fat and body energy accretion was comparable to WM rats but greater than the AM rats, which showed no significant accumulation during this time period (Table 1).

3.2. Metabolism

The changes in body weight and energy during the refeeding phase suggested a change in the animal's metabolic efficiency since the food consumption was similar between groups during this time period. It is possible to follow changes in metabolism by following changes in the net energy value for maintenance (XE), defined as [34]

$$XE = IME + E_f - 1.35 \times EF - 2.25 \times ELBM \quad (4)$$

where IME is the ingested metabolizable energy, E_f is the loss in body fat energy (if it occurs), EF is the increase in body fat energy and ELBM is the increase in the lean body mass energy. The IME was calculated by assuming that the metabolizable energy of the rat chow was similar to that of semi-purified diets containing similar amounts of cellulose [35]. Body fat and LBM energy were calculated using the previously determined factors of 10.26 and 3.78, respectively [35]. Table 2 presents the absolute and relative changes in XE as a function of energy restriction and refeeding. The values at the far right of Table 2 have been normalized per kg average LBM for the measured period. The results demonstrate a significantly lower XE value during the energy restriction phase (compared to AM rats) and during the ORF-2RF period (compared to WM). No difference between XE values was observed during the period 2RF-7RF when compared to WM rats.

3.3. Fatty acid oxidation and synthesis

The biochemical mechanisms for the observed changes in fat accumulation and in metabolic rates during the refeeding period were examined indirectly. Since loss and re-gain of body fat was a major difference between ER, AM and WM groups, we measured fat oxidation and *de novo* fat synthesis over the time course of the experiment. Fig. 2 presents the data from these measurements. Fatty acid oxidation, as measured by $^{14}CO_2$ release after gavage feeding [U-^{14}C]palmitic acid, was significantly lower (0.4 or 0.5 times that of the AM or WM rats, respectively) in ER rats as measured 2 days after refeeding had begun (2RF). No other significant differences were noted. No significant differences in *de novo* fatty acid synthesis were observed (Fig. 2).

3.4. Enzyme activity measurements

Fig. 3 shows the activities of the enzymes of the fatty acid synthesis pathway from liver: glucose 6-phosphate dehydrogenase, malic enzyme, ATP-citrate lyase and fatty acid synthetase. Energy restriction was associated with a significantly lower ATP-citrate lyase activity and refeeding was associated with significantly higher glucose 6-phosphate dehydrogenase, ATP-citrate lyase and fatty acid synthetase activities when compared to AM and WM animals. At day 7RF, glucose 6-phosphate dehydrogenase and ATP-citrate lyase activities were still significantly higher than the corresponding WM and AM rat activities.

Liver enzymes associated with amino acid utilization

Table 2
Food intake, net energy for maintenance (XE) and total energy expenditure (TEE)

Period	Group	Total DE intake (kcal)	Estimated ME intake (kcal)	Total body fat gain (kcal)	Total lean body mass gain (kcal)	E_f (kcal)	TEE (kcal/d)	XE (kcal/d)	TEE[1] (kcal/d per kg LBM)	XE[1] (kcal/d per kg LBM)
Energy Restriction	ER	671 ± 0	633 ± 0	0	17.4 ± 14.3	45.9 ± 30.1	52.2 ± 2.3^{a}	49.2 ± 3.4^{a}	872 ± 39^{a}	822 ± 57^{a}
	AM	1564 ± 79	1475 ± 74	77.8 ± 30.5	101.6 ± 8.8	0	113.5 ± 6.0^{b}	87.7 ± 7.0^{b,d,e}	1598 ± 85^{b}	1235 ± 99^{b}
ORF-2RF	ER	220 ± 12	207 ± 25	33.8 ± 23.6	15.4 ± 14.6	0	103.6 ± 13.2^{b}	63.2 ± 26.5^{a,c}	1613 ± 205^{b}	985 ± 412^{a,b}
	WM	222 ± 8	209 ± 16	1.1 ± 23.8	1.0 ± 7.9	0	104.5 ± 8.3^{b}	102.7 ± 20.2^{d}	1812 ± 143^{c}	1780 ± 351^{d}
2RF-7RF	ER	579 ± 26	546 ± 25	42.6 ± 26.4	50.8 ± 17.6	0	109.2 ± 5.3^{b}	74.8 ± 11.9^{b,c}	1496 ± 72^{b}	1024 ± 164^{a,b}
	WM	556 ± 17	524 ± 16	52.8 ± 23.1	62.6 ± 11.1	0	104.9 ± 3.3^{b}	62.4 ± 8.7^{a,c}	1586 ± 50^{b}	943 ± 131^{a,b}
ORF-7RF	AM	863 ± 49	814 ± 46	21.4 ± 38.1	34.1 ± 18.9	0	116.3 ± 7.0^{b}	101.2 ± 11.9^{a}	1307 ± 79^{d}	1137 ± 133^{a,b}

[1] XE and TEE values in units of kcal/d were divided by the average LBM of the animals over the period in question to obtain kcal/d/kg LBM. Values in the same column with different superscripts are significantly different at the $P < 0.05$ level as determined by the LSD test. Standard errors for TEE and XE were estimated from formulas. Values are means ± SD for $N = 6$ rats.

Changes in body fat and lean body mass over the indicated time period were used to calculate total energy expenditure (TEE = IME + E_f) and net energy value for maintenance (XE; see text).

were also investigated over the time course of the experiment (Table 3). Significantly higher alanine aminotransferase, aspartate aminotransferase and glutamate dehydrogenase activities were observed at the end of the energy restriction period. In the case of aspartate aminotransferase, the high activity was still observed 2 days after the start of the refeeding period. Glycolytic enzymes also showed some changes. Pyruvate kinase activity was significantly lower in ER rats at the end of the energy restriction period and significantly higher in ER rats 2 days after the resumption of *ad lib* feeding. Pyruvate dehydrogenase activity was significantly higher at the end of the energy restriction period.

No changes were observed in other enzymes of amino acid utilization: serine dehydratase activity was 1.26 ± 0.7 U/gww ($N = 48$ pooled observations) and branched-chain amino acid dehydrogenase was 0.51 ± 0.14 U/gww ($N = 48$). Gluconeogenic enzyme activities remained constant throughout the time course of the experiment: phosphoenolpyruvate carboxykinase was 0.37 ± 0.11 U/gww ($N = 48$) and fructose 1,6-bisphosphatase was 2.25 ± 0.35 U/gww ($N = 48$). The glycolytic enzymes also were unchanged during the experiment: glucokinase (0.46 ± 0.13 U/gww, $N = 48$), phosphofructokinase ($K_M = 2.49 \pm 0.3$ mM, Hill coefficient = 2.3 ± 0.2, $V_{max} = 1.72 \pm 0.15$ U/gww, $N = 48$) and PK kinetic parameters ($K_M = 0.82 \pm 0.13$ mM, Hill coefficient = 1.2 ± 0.1) did not vary. Liver concentrations of fructose 2,6-bisphosphate, a potent allosteric potentiator of phosphofructokinase did not change (2.64 ± 1.04 nmol/gww, $N = 48$).

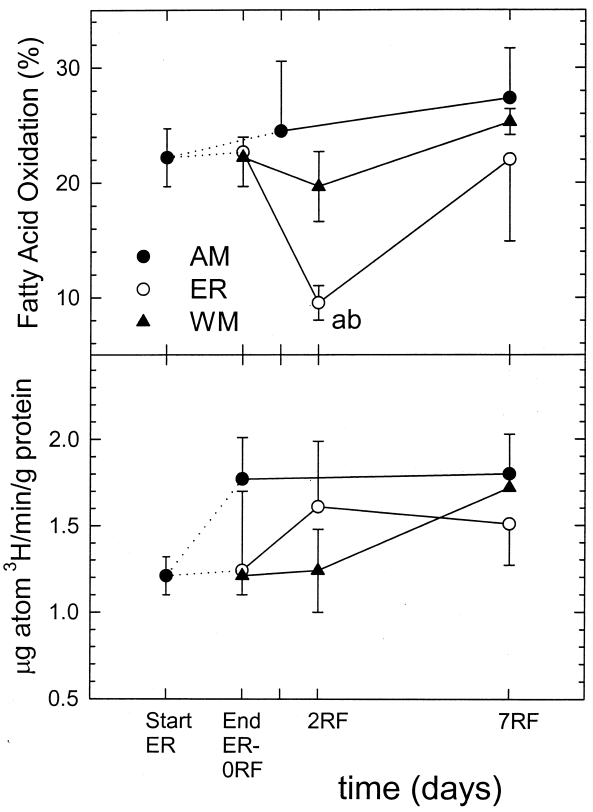

Fig. 2. Rates of fatty acid oxidation (top) and fatty acid synthesis (bottom). For fatty acid oxidation, rats were gavage fed 1.5 μCi of uniformly labelled palmitic acid in 0.5 mL/100 g body weight of corn oil and expired $^{14}CO_2$ was collected for 24 hours. The percentage of radioactivity recovered in the expired air is reported. For fatty acid synthesis, rats were injected i.p. with 4 mCi $^{3}H_2O$ in 0.3 ml water. Fat was extracted from the carcasses and measured for radioactivity. The rates are expressed as μg atom ^{3}H incorporated/min/g protein. Values are means ± SD for $N = 6$ rats. The letter 'a' denotes a significant difference from AM-ORF animals and WM-ORF rats at the $P < 0.05$ level.

4. Discussion

The primary goal of this study was to examine the metabolic changes responsible for the rapid fat accumulation following a period of energy restriction. Fat metabolism is a key point of interest since others have shown that

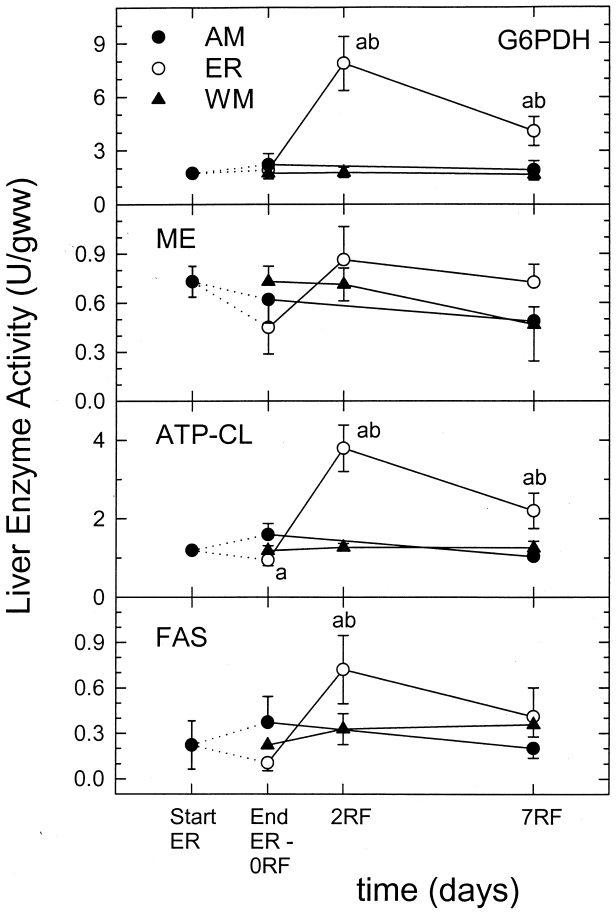

Fig. 3. Maximal activities of the enzymes of the fatty acid synthesis pathway: glucose 6-phosphate dehydrogenase (top), malic enzyme (second from the top), ATP-citrate lyase (third from the top) and fatty acid synthetase (bottom). Values are means ± SD for $N = 6$ livers. (a) Significantly different from AM rats at the $P < 0.05$ level (ER-2RF rats were compared to AM-ORF rats). (b) Significantly different from WM rats at the $P < 0.05$ level.

weight regain is a function of the amount of fat in the diet [11] as well as the type of dietary fat [14]. In fasted-refed animals, increases in the amount of ^{14}C-glucose incorporated into fat [36] as well as changes in the lipogenesis and fatty acid esterification rates to reduce the rate of futile cycling [37] demonstrated the importance of these processes in promoting fat accumulation during refeeding. These changes are most likely the result of the rise in plasma insulin levels that occur during refeeding [38] and may be mediated by changes in enzyme phosphorylation [24].

Like fasted animals, energy restricted animals also rapidly accumulate fat during the refeeding phase. This has been demonstrated by others [10–14] as has been confirmed in the present work. In agreement with other labs, we have found that fat regain is related to the period of decreased metabolic rate (lower XE; Table 2) that persisted, in our case, up to 2 days after the end of the energy restriction period. Certainly, any discrepancy between ingested energy and XE will affect weight gain. However, our results also demonstrate that a decreased oxidation of dietary fat plays a major role in promoting fat accumulation during refeeding. In order to assess its contribution to fat gain, we calculated the overall fat balance for energy-restricted and refed rats as compared to AM controls. It was necessary to calculate fat balance because we did not have access to a whole body calorimeter. We assumed that total daily fatty acid synthesis rates could be estimated by extrapolating our 1 hour measurements to 24 hours (Table 4). Daily rates of fatty acid oxidation were then calculated. The calculations suggest that the daily fatty acid oxidation rate approaches the rate of fatty acid ingestion in control animals. This rate was apparently unaffected by energy restriction as shown by the calculation of similar rates of fatty acid oxidation in AM and ER animals. This latter conclusion is supported by the actual measured extent of ingested palmitic acid oxidation in AM and ER rats (Fig. 2). The calculated rate of fat oxidation in refed rats, however, was approximately 1 g/d/rat lower than AM or ER rats, in agreement with the lower observed extent of dietary fat oxidation (Fig. 2). The negative value in Table 4 reflects the imprecision in extrapolating a 1 h fatty acid synthesis measurement to a daily rate. It also shows that these calculations are approximations since fatty acid oxidation rates cannot be ≤0 g/d/rat. The imprecision is most likely due to errors in extrapolating fatty acid synthesis rates (the food intake and changes in body fat stores were measured directly) because others have

Table 3
Enzymes that responded to energy restriction and/or refeeding

Enzyme	Pooled N	Pooled activity[a] (U/gww)	Activity in ER rats at d 0RF (U/gww)	Activity in ER rats at d 2RF (U/gww)
Asp-AT	36	17.9 ± 3.9	28.6 ± 5.1	24.6 ± 3.8
Ala-AT	42	138 ± 12	162 ± 20	NS[b]
GDH	42	165 ± 19	219 ± 22	NS
PK (V_{max})	36	23.7 ± 5.9	15.6 ± 2.1	29.6 ± 4.5
PDH	42	1.25 ± 0.22	1.86 ± 0.27	NS

[a] Pooled activity represents the mean of the AM and WM groups plus the activity in the ER animals at the start of the energy restriction period and the activity in the ER rats on d 7RF since these were not significantly different from each other. In addition, the d 2RF ER values were included in the pooled activity for Ala-AT and GDH. Values are means ± SD for indicated numbers of rats (pooled activity) or for $N = 6$ rats (ER d ORF and ER d 2RF activities).
[b] Not significantly different and, therefore, included in the pooled activity.

Table 4
Fat balance in normal, energy restricted and refed rats

Group	g/d/rat			
	Fat intake	Fatty acid synthesis	Body fat changes	Calculated fat oxidation
AM	1.154	0.312	0.554	0.912
ER	0.513	0.300	−0.324	1.136
ER − d 2RF	1.092	0.358	1.584	−0.134

Fat intake was calculated from food intake and assumed 95% absorption. Fatty acid synthesis was estimated from radioactivity incorporation [34] and extrapolated to 24 hours. Body fat changes represent the difference between fat accretion and fat loss obtained from whole body fat analysis. Fatty acid oxidation was calculated as: the difference between fatty acid intake, fatty acid synthesis and changes in body fat.

shown an increase in lipogenesis in liver upon refeeding fasted rats [36] whereas our data does not. The former results were observed after feeding a 1% corn oil diet to promote fatty acid synthesis rates. The diets of the rats in this present experiment contained 4.5% fat by weight and may have lowered the rates of fatty acid synthesis.

The mechanisms that underlie the reduced rate of dietary fat oxidation are unknown at this time. It is possible, however, that the explanation may lie in the difference between energy intake and XE. In normally fed rodents, daily rates of fat oxidation appear to be regulated only by protein and carbohydrate intake: fat oxidation is used to fuel the difference in energy balance between total energy expenditure, carbohydrate oxidation and protein oxidation [19,39]. If this is true, a decreased XE combined with an increased energy intake would decrease fat oxidation simply because the energy needs of the refed animals may be met by preferential oxidation of dietary carbohydrate and protein. This means that fat oxidation may appear to be actively inhibited by an unidentified mechanism whereas, in reality, it is reduced to compensate for the relative increase in carbohydrate and protein intake. Measurements of food consumption show that the increase in carbohydrate plus protein energy intake (39 and 43 kcal/d after 2 and 7 days of refeeding, respectively) could more than compensate for the increase in XE observed after 2 and 7 days of refeeding. These calculations represent a crude approximation of the actual energy balance since total energy expenditure was not measured and XE represents only about 60–80% of the total energy expenditure.

The above physiological explanation may explain overall energy balance but does not take into account events that occur at the cellular level that can act to inhibit fatty acid oxidation. A strong candidate for regulating fatty acid synthesis is malonyl-CoA [24] since this metabolite inhibits carnitine palmitoyl transferase-1. Inhibition of carnitine palmitoyl transferase-1 would ultimately reduce the translocation of fatty acids into mitochondria and, consequently reduce fatty acid oxidation. Several different factors may promote the accumulation the precursors of fatty acid synthesis, and in particular, malonyl-CoA. For example, increased malonyl-CoA can result simply by increased carbon flux into the Krebs Cycle either via increased carbohydrate utilization or increased protein transamination during refeeding. Increased malonyl-CoA concentrations can also arise because the high circulating insulin concentrations that accompany refeeding [38] may activate acetyl CoA carboxylase to increase flux to malonyl-CoA. The role of malonyl-CoA remains to be investigated in refed rats.

In addition to fat balance during and after energy restriction, the activities of key metabolic enzymes as well as concentrations of circulating metabolites were measured. These were used to obtain an idea of the metabolic state of the rats at the end of the energy restriction period. They were also used to investigate potential mechanisms for mediating the metabolic depression. The increases in Asp-AT, Ala-AT, and GDH at the end of the energy restriction period were similar to with those observed in overnight fasting rats [30] where protein catabolism is high. However, the lack of changes in circulating glucose and β-hydroxybutyric acid as well as the lack of any decrease in liver glycogen show that the animals could easily maintain carbohydrate reserves on the 50% energy diet and were not, metabolically, similar to starved rats. The changes in Asp-AT, Ala-AT and GDH, coupled with the decreased PK activity are suggestive of a higher gluconeogenic flux, which would be expected in energy restricted rats. However, the lack of an increase in phosphoenolpyruvate carboxykinase (thought to be rate-limiting for gluconeogenesis [40]) and the lack of a change in fructose 2,6-bisphosphate (a sensitive indicator of a shift from glycolysis to gluconeogenesis [41]) argue against any significant increase in gluconeogenesis. These results, therefore, argue against any profound rearrangement in anabolic pathways during energy restriction that could account for a metabolic depression. However, phosphorylation of regulatory enzymes is not the only mechanism whereby metabolism is regulated. Changes in cellular metabolites can also control enzyme (and ultimately pathway) activity. Many metabolites act as inhibitors and activators of regulatory enzymes and could regulate glycolysis, fatty acid oxidation and gluconeogenesis. Metabolites were not measured in the present work. In addition, only liver enzymes were followed in the present study even though fat cell and skeletal muscle metabolism can profoundly influence whole body substrate utilization patterns [42]. Hormonal changes can also regulate energy utilizing pathways [43]. These considerations mean that more detailed experiments are required to determine the precise locus of control for fat metabolism during energy restriction and refeeding.

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