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Effect of dietary fat on whole body fatty acid synthesis in weanling rats

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The effect of dietary fat on body composition, whole body lipogenesis, and enzyme activity was measured in rats over the first 16 weeks post-weaning. Rats were fed either a low fat (5% w/w fat) or high fat (20% w/w fat) diet for the first 4 weeks. After this time all rats were fed the low fat diet. The results showed no significant effect of diet on the rate of fat synthesis over the first 8 weeks of the experiment. However, the activities of the enzymes of fatty acid synthesis [glucose 6-phosphate dehydrogenase, malic enzyme, adenosine triphosphate-citrate lyase, acetyl-coenzyme A carboxylase (ACCX), fatty acid synthetase] were dependent on the age and dietary status of the animals. The exact pattern depended on the specific enzyme and the tissue source. No significant differences in pyruvate dehydrogenase (PDH) activity were observed. Mathematical analysis of the enzyme activities suggested that ACCX and PDH were the most likely sites of fat synthesis regulation. In addition, an examination of body composition and overall weight retention showed that the "weight increasing" effect of a high fat diet could be completely reversed by subsequent feeding of a low fat diet. However, the reversal required an additional 12 weeks. Interestingly, at this time the rats switched from a high fat to a low fat diet had a lower body weight and lower body fat content than rats fed a low fat diet throughout the course of the experiment. (J. Nutr. Biochem. 10:291–298, 1999)

Keywords: metabolism; dietary fat; fatty acid synthesis; weanling rats; metabolic control

Introduction

The suckling/weaning transition in the rat is accompanied by marked dietary changes. Suckling rats eat a very high fat–low carbohydrate diet (mother's milk has approximately 31% fat on a dry weight basis) and this is relatively quickly replaced by a low fat–high carbohydrate diet at weaning (approximately 5% fat on a dry weight basis). Many biochemical events are associated with weaning and reflect the change in diet fat content. These changes include alterations in the levels of circulating hormones,[1] increases in the enzymes of fatty acid synthesis,[2–5] and decreases in the enzymes of gluconeogenesis.[4–6] For the enzymes of fatty acid synthesis, the changes result from increased dietary fat directly altering gene transcription. Other changes are associated with altered hormone levels.[4–7]

An increased need for fat during development is suggested by studies that measured the incorporation of [U-^{14}C]glucose and [1-^{14}C]acetate into fat extracts of liver slices[8,9] and by [U-^{14}C]glucose incorporation into adipose tissue preparations.[10] The rates of fat synthesis are initially high, but fall dramatically 70 to 80 days after birth,[8,10] which suggests that the requirement for fat is highest during the early growth period. Enzyme activities also follow this pattern.[2–5,8–10] Although previous studies have examined the relationship between high and low fat diets and enzyme activity in weanlings, the relationship between whole body fatty acid synthesis rates and dietary fat during weaning has not been well established. We began the current study after a previous investigation into the effect of dietary fat on enzyme activity in weanling and older rats suggested that rates of fat synthesis may be high even in young high-fat fed animals.[11] This conclusion was based on an assumed proportional relationship between the activities of malic enzyme (ME), adenosine triphosphate-citrate lyase (ATP-CL), and fatty acid synthetase (FAS) and the rate of fatty acid synthesis in vivo.[12] Because the regulation of fatty acid synthesis depends on a number of factors, including dietary fat, total FAS enzyme activity, and the phosphorylation states of acetyl-coenzyme A carboxylase (ACCX) and pyruvate dehydrogenase (PDH),[13] it was important to mea-

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Table 1 Composition and calculated energy density of purified diets used in the present study

Ingredient*	Amount per 100 g diet	
	High fat	Low fat
Casein, ANRC	20.0 g	20.0 g
Carbohydrate		
Cornstarch	6.1 g	39.9 g
Sucrose	29.0 g	29.0 g
Total	35.1 g	68.9 g
Fat		
Lard	6.0 g	1.5 g
Corn oil	14.0 g	3.5 g
Total	20.0 g	5.0 g
Alphacel	19.7 g	1.0 g
Mineral mix-AIN76	3.5 g	3.5 g
Vitamin mix-AIN76A	1.0 g	1.0 g
Choline bitartrate	0.4 g	0.4 g
DL-methionine	0.3 g	0.3 g
Total energy	511 kcal	436 kcal
Metabolize energy (ME)†	408 kcal	400 kcal
% of ME from fat	20%	12%
% of ME from carbohydrate	34%	69%

*Mineral mix-AIN76, vitamin mix-AIN76A, and Alphacel were obtained from Harlan Teklad (Madison, WI, USA). Choline bitartrate and DL-methionine were from Sigma Chemical Co. (St. Louis, MO, USA).
† Metabolizable energy was calculated by taking into account the energy digestibility and energy losses to urine of similar diets.[28] In addition a partial digestible energy value of 0.41 was used for Alphacel.[38]

sure fatty acid synthesis directly in weanlings to determine whether it paralleled changes in enzyme activity.

An additional goal of the present study was to determine potential long-term effects of dietary fat on body fat stores and enzyme activity patterns. These concerns arose from literature reports that young animals are not as susceptible to the obesity-generating effects of dietary fat as older animals.[14–16] To follow possible biochemical adaptations to diets with differing amounts of dietary fat as a function of age in growing rats, animals fed high fat diets were switched to control diets after 4 weeks of age. Biochemical parameters and fatty acid synthesis rates were followed for an additional 12 weeks after the switch to obtain a fairly complete picture of the fat synthesis patterns in growing rats.

Materials and methods

Animals and protocol

Weanling (46.7 ± 1.9 g, $n = 124$) male Sprague-Dawley rats were obtained from Charles River Co. (St. Constant, PQ, Canada). At 21 days of age, animals were weaned onto Purina rat chow pellets (5075, Ralston Purina Canada Inc., Strathroy, ON, Canada). On the same day, rats were transported to our facility with Gel Pack transportation medium (Purina, Ralston, NC USA). Animals were randomly assigned to individual wire-bottom stainless steel cages and maintained on a 12-hour light/dark cycle. The room temperature was 22 ± 1°C and the humidity was regulated to between 45% and 55%. Rats were given free access to water and fed either a low fat (Group I) or a high fat (Group II) diet for 4 weeks (*Table 1*). After this time animals previously fed a high fat diet were switched to the low fat diet (Group III) and all animals were fed for another 12 weeks.

Euthanasia and measurement of de novo fatty acid synthesis

All animals were injected intraperitoneally with 4 mCi ^3H_2O (Amersham, Mississauga, ON, Canada) in 0.3 mL water. Animals were euthanized 1 hour later by a 1-minute exposure to O_2 followed by a 3-minute exposure to CO_2. Injection with ^3H_2O enabled the measurement of ^3H incorporation into fatty acids synthesized de novo.[17,18] Blood was withdrawn from the heart (for measurement of ^3H_2O specific activity) and the liver was rapidly dissected and frozen in liquid nitrogen.

Enzyme assays

All chemicals were obtained from either Boehringer-Mannheim Co. (Montreal, PQ, Canada) or Sigma Chemical Co. (St. Louis, MO USA) 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 (12,000xg). 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 USA). Kinetic analysis was performed with third party software.[19] Glucose 6-phosphate dehydrogenase (G6PDH), ME, ATP-CL, and FAS were measured according to Brooks and Lampi.[20] PDH was measured according to Brooks and Storey.[21] Activation time courses (performed at room temperature) were followed to ensure maximal activation. ACCX activity was measured by following $H^{14}CO_3$ (Amersham) incorporation into fat according to Tanabe et al.[22] Total activity was obtained by adding 5 mM $MgCl_2$:citrate as suggested by Thampy and Wakil.[23] Magnesium was added to maintain a constant Mg^{2+}:ATP ratio in the sample.[24]

Other methods and statistics

Carcasses were blended with an equal weight of water in a Waring blender until homogeneous in appearance. Samples were further processed with a Brinkman homogenizer to ensure homogeneity. Protein concentrations were measured according to Brooks et al.[25] Body fat was measured after chloroform-methanol extraction.[26] Water content was measured after freeze-drying. Samples were corrected for added water. All statistical analyses were performed using the Tukey's HSD test after analysis of variance (StatSoft, Inc., 1997, STATISTICA for Windows, Tulsa, OK USA). All values are reported as means ± SD. 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.[27] This procedure always normalized the data. Differences were not considered significant if the probability was greater than 0.05.

Results

Body weight and composition

Body weight and body compositional data of animals are presented in *Figure 1*. Body weights of Group II animals were significantly higher when measured at 3 and 4 weeks.

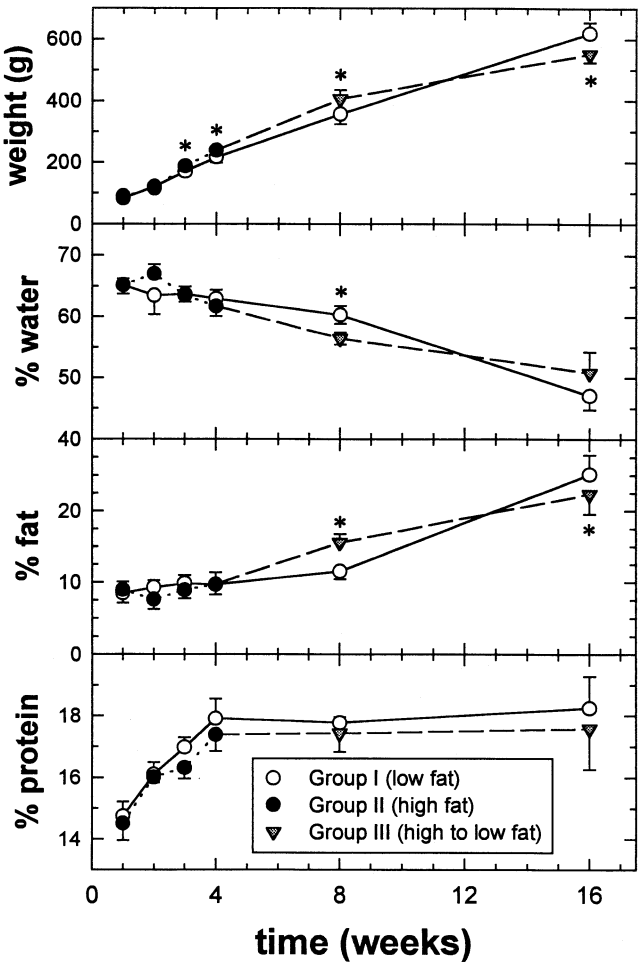

Figure 1 Body weight and body composition for Group I (low fat, open circles), Group II (high fat, black-filled circles), and Group III (high to low fat, grey-filled triangles) rats plotted as a function of time. The symbols marked with an asterisk (*) were significantly different from the corresponding diet group at the same time point. Values are means ± SD for $n = 5$ rats except for week 16, Group I ($n = 4$) and week 16, Group III ($n = 3$).

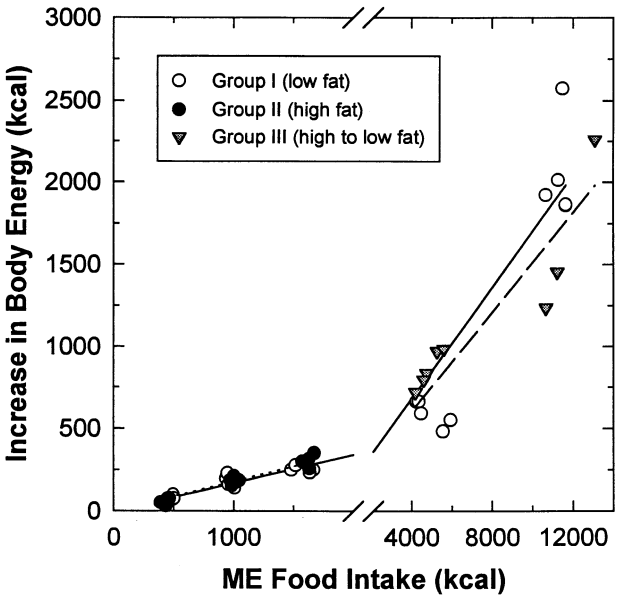

Figure 2 Increase in body energy as a function of cumulative food metabolizable energy intake. Body energy was estimated by: body energy = 10.9 × fat + 4.2 × protein, where the values 10.9 and 4.2 represent the kilocalories of energy per gram of fat and protein, respectively.[28] Absolute increases in body fat and body protein were estimated from body compositional changes in rats sacrificed in successive time periods (weeks 1, 2, 3, 4, 8, and 16). Metabolizable energy food intake was calculated using the values in *Table 1*. Open circles, Group I (low fat); black-filled circles, Group II (high fat); grey-filled triangles, Group III (high to low fat).

This difference was still apparent 4 weeks after the switch in diets (week 8), so that Group III animals were heavier than those of Group I. However, by week 16, Group III animals weighed less than those in Group I. Differences in body composition were also apparent but only at weeks 8 and 16 (*Figure 1*). Epididymal and perirenal fat deposits increased equally in all groups throughout the experimental period (data not shown).

Differences in body weights and fat content of the Group III animals at weeks 8 and 16 were due to differences in energy retention that may have resulted from changes in metabolism or food intake. To determine the cause of these differences, total food consumption and body energy gains were more closely examined. No statistical differences in the total food consumption per rat were noted before the switch in diet (Group I: 462 ± 13 g vs. Group II: 466 ± 14 g; P = NS), at week 8 (Group I: 825 ± 185 g vs. Group III: 806 ± 126 g), or at week 16 (Group I: 2,382 ± 100 g vs. Group III: 2,482 ± 283 g). A comparison of food energy intake (estimated from the partial digestibility of similar diets; *Table 1*) versus body energy gain calculated from increases in body fat and body protein[28] for the 16 weeks of the experiment revealed no differences between the three groups (*Figure 2*). The slight (but not statistically significant) changes in food intake observed at weeks 8 and 16 are opposite to the observed changes in body energy, suggesting that the changes in body energy are due to changes in energy expenditure.

Measurement of fatty acid synthesis

Total fatty acid synthesis rates and fatty acid synthesis rates relative to body protein are presented in *Figure 3*. As expected, the total animal fatty acid synthesis rate increased with increasing age due mostly to an increase in body weight (*Figure 3*, top). During the first 4 weeks of the study, no differences were observed between groups I and II. At week 8, Group III animals had a slightly higher (not significant) rate of fat synthesis relative to Group I rats. At week 16, the exact opposite was true (statistically significant): Group I rats had higher total rates of fat synthesis than did Group III animals. These differences were due in part to differences in body weights (*Figure 1*) and to small (non-significant) differences in the relative rate of fat synthesis (*Figure 3*, bottom).

The rates of fatty acid synthesis also were calculated as a function of metabolic weight (rate per gram total body

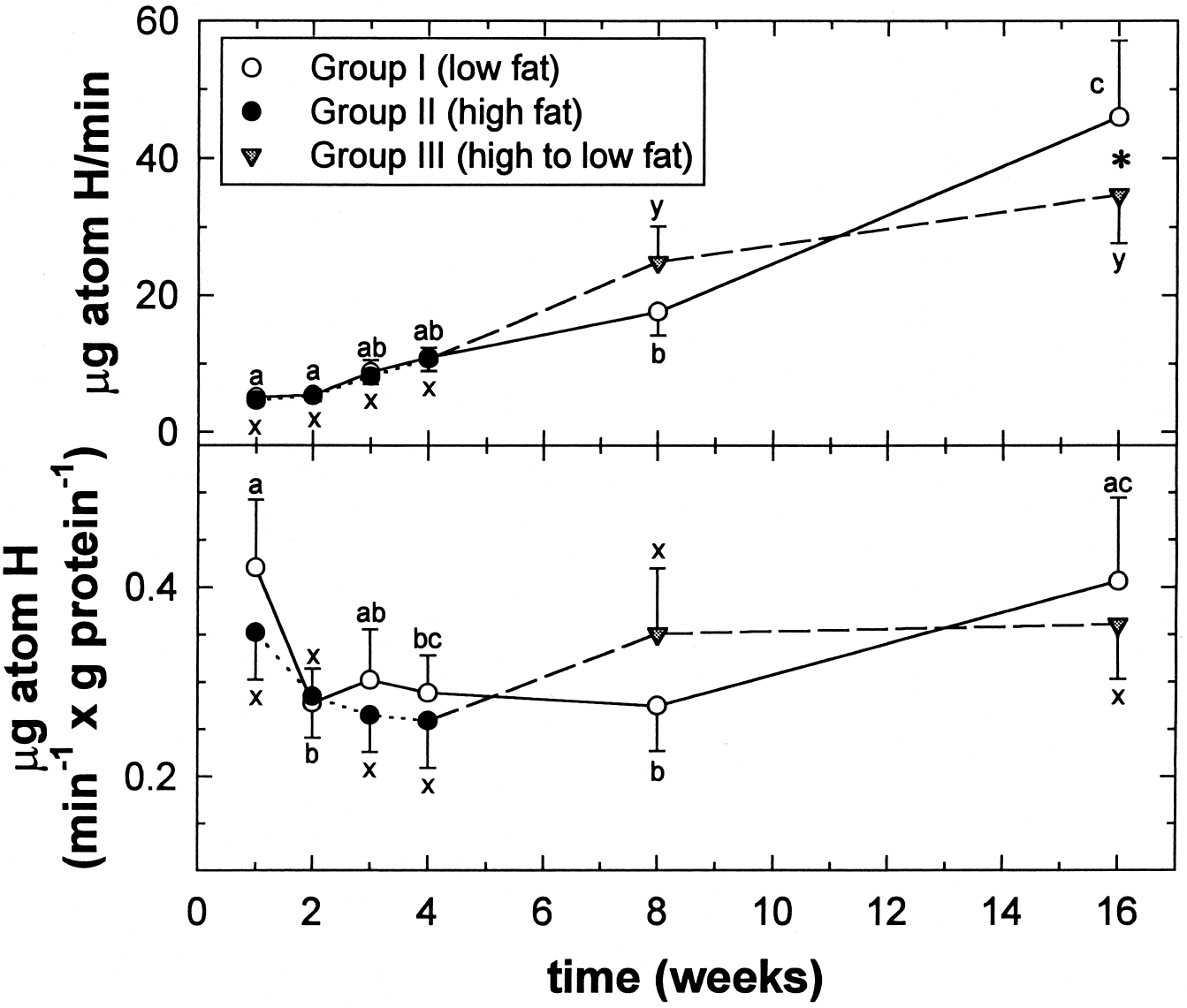

Figure 3 Fatty acid synthesis rates measured as a function of increasing age and diet. *Top:* Data are expressed as micrograms atom hydrogen incorporated into fat per minute (total fat synthesis rate). *Bottom:* Fatty acid synthesis rates were divided by total body protein to estimate fat synthesis rates per gram of metabolic weight. Values marked with different letters (a, b, and c for Group I; x and y for Groups II and III) were statistically different from other values within a diet group. The asterisk (*) denotes a significant difference between the diet groups. Values are means ± SD for $n = 5$ rats except for week 16, Group I ($n = 4$) and week 16, Group III ($n = 3$). Open circles, Group I (low fat); black-filled circles, Group II (high fat); grey-filled triangles, Group III (high to low fat).

protein; *Figure 3*, bottom). Expressing the rates in this fashion revealed a different pattern than that which was seen when total synthesis rates were compared. In Group I rats, rates were initially high (week 1) and dropped to a constant value until week 8, after which they increased to week 1 levels. No significant differences were observed in animals fed high fat diets (groups II and III). However, at weeks 8 through 16, the relative fat synthesis rates paralleled the pattern observed in total fat synthesis and body weight; that is, the rates were slightly higher than control at week 8 and slightly lower than control at week 16. No significant differences were observed between diet groups at any single time point.

Enzyme activity measurements

The enzymes of the fat synthesis pathway were measured in liver and white adipose tissue (WAT; *Figure 4*) over the 16 weeks of the experimental time course. Enzyme activities varied both with age and with dietary condition. In general, enzyme activity was higher in Group I animals than in Group II animals, but this trend was not observed for all enzymes at all time points (note asterisks in *Figure 4* for specific differences). When groups I and III were compared, only ACCX from WAT showed significant differences.

In addition to differences between dietary groups, temporal differences within dietary groups were observed. In

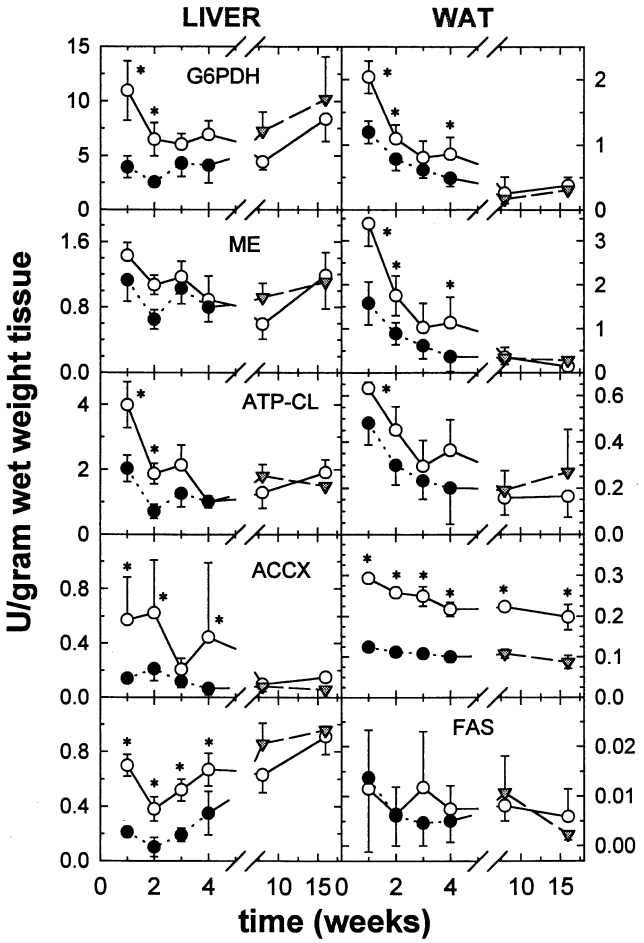

Figure 4 Effect of diet and age on liver (*left*) and white adipose tissue (WAT; *right*) enzymes of the fatty acid synthesis pathway. Maximal activities of glucose 6-phosphate dehydrogenase (G6PDH; *top*), malic enzyme (ME; *second from top*), adenosine triphosphate-citrate lyase (ATP-CL; *middle*), acetyl-coenzyme A carboxylase (ACCX; *second from bottom*), and fatty acid synthetase (FAS; *bottom*) were plotted as a function of increasing age. Diet symbols are: Group I (low fat, open circles), Group II (high fat, black-filled circles), and Group III (high to low fat, grey-filled triangles). The symbols marked with an asterisk (*) were significantly different from the corresponding diet group at the same time point. Values are means ± SD for $n = 5$ rats except for week 16, Group I ($n = 4$) and week 16, Group III ($n = 3$).

the livers of Group I rats, total G6PDH and ATP-CL activities were significantly higher at week 1 than at later times. Also in Group I rats, liver ACCX values remained high until week 8. Liver ME, WAT ACCX, and WAT FAS activities remained constant and liver FAS activity increased over the time course of the experiment. No effect of dietary regime or time was observed on total liver PDH activity (0.92 ± 0.47, $n = 57$ pooled animals) or on the percentage of PDH in the active form (62 ± 22%, $n = 57$ pooled animals).

Discussion

The primary goal of the present study was to determine the rates of whole body fatty acid synthesis in weanling rats fed different amounts of dietary fats. The results show that total fatty acid synthesis increased with age and this was largely independent of the amount of fat in the diet up to 4 weeks of age (*Figure 3*). Increasing total rates of synthesis were an expected consequence of increasing lean body mass in the growing rats. Normalizing the values by dividing by total body protein (an estimate of metabolic body weight) revealed some slight differences between the two diet groups but these were not statistically significant. After 1 week on the respective diets, animals fed low fat diets had rates of fatty acid synthesis that were approximately 20% higher than those in Group II animals (not significant). This modest difference had disappeared by week 2 and no difference was evident for the remaining 3 weeks of the Group I/Group II phase of the experiment.

The measurement of similar fat synthesis rates in animals fed different diets was surprising in light of the differences in enzyme activities (*Figure 4*) and the many studies suggesting that high fat diets suppress fat synthesis via changes in the activity of fatty acid synthesis pathway enzymes.[1–4,7,8,9,12] We sought to further examine this situation by plotting the ratio (R) of total fatty acid synthesis to total enzyme activity. Total enzyme activity was defined as the sum of liver plus WAT (estimated as perirenal + epididymal) activity for G6PDH, ME, ATP-CL, ACCX, and FAS. For PDH, total enzyme activity was defined as the liver activity only. The R value was plotted as a function of age and diet to identify potential control loci (*Figure 5*). In this analysis, similar R values from different diet groups indicate a similar relationship between enzyme activity and fat synthesis rates for both diet groups at a single time point. The absolute value of R has no physical meaning because pathway control is complex and depends on many factors.[39] This may also explain the variation in the R value over the time course of the experiment. The good correspondence between the Group I and Group II R values for ACCX and PDH suggests that control rests primarily with these enzymes or that these enzymes are similarly regulated during early growth. The other enzymes appear not to be similarly regulated (G6PDH, ME, ATP-CL, or FAS) and the discrepancies between groups I and II for these enzymes were confined to the first 3 weeks of the experiment. After this time, the R values were in good agreement (no differences between diet groups). This analysis suggests that changes in some individual enzyme activities, which were observed during the early phase of weaning, may not accurately reflect changes in fatty acid synthesis rates in vivo but that this period is short lived.

The pattern of control suggested by *Figure 5* is consistent with the known regulation of ACCX[29,30] and PDH[7,40] by reversible phosphorylation via insulin, glucagon, and epinephrine action,[7,29,40] although this normally has been associated with acute rather than long-term control.[13] The present results suggest that long-term control of fat synthesis can be mediated by changes in ACCX activity and that PDH may be regulated in tandem with ACCX in developing rats.

The age-dependent changes in fat synthesis and enzyme activities presented in *Figure 4* are similar to those previously reported but the precise timing of the changes is not as well defined as other studies where rats were bred in-

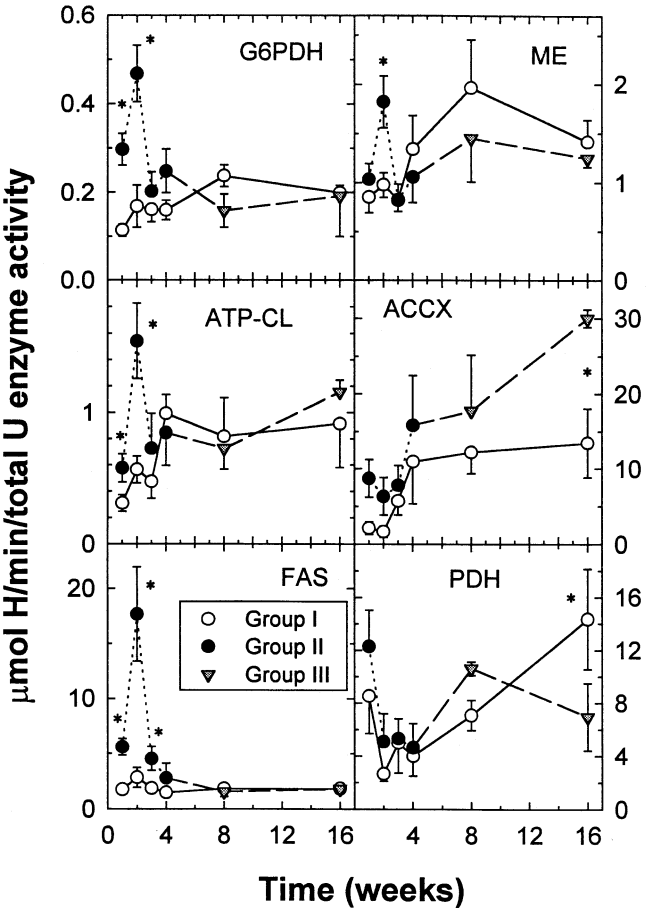

Figure 5 Ratio of total fatty acid synthesis to total liver enzyme activity as a function of diet and animal age. The R value (see text) was calculated for each animal and plotted as a function of age. Values represent means ± SD for $n = 5$ animals except for week 16, Group I ($n = 4$) and week 16, Group III ($n = 3$). The symbols marked with an asterisk (*) were significantly different from the corresponding diet group at the same time point. Open circles, Group I (low fat); black-filled circles, Group II (high fat); grey-filled triangles, Group III (high to low fat). G6PDH, glucose 6-phosphate dehydrogenase; ME, malic enzyme; ATP-CL, adenosine triphosphate-citrate lyase; ACCX, acetyl-coenzyme A carboxylase; FAS, fatty acid synthetase.

house.[8–10] Previous results showed an increase in the rate of fat synthesis and in enzyme activity at weaning and this change persisted until 40 to 50 days after birth.[3,8] Changes in mRNA levels have been shown to persist up to 11 weeks of age.[3] In the present study, the rats were weaned at 21 to 22 days of age and obtained within 1 to 2 days from the breeder. Adding the time on the respective diets means that the decrease in fatty acid synthesis (*Figure 3*) and the activities of the fat synthesis enzymes (*Figure 4*) occurred at approximately 30 days of age. The differences may be due partly to the use of different species (Sprague-Dawley rats were used in the present study vs. Wistar rats in other studies[3,8–10]) or to differences in weaning protocols. Despite these differences in the absolute value of the time course, the relationship between enzyme activity and fatty acid synthesis parallels the results of previous studies.[3,8–10]

An additional objective of the present study was to determine potential long-term effects of dietary fat on body fat stores and enzyme activity patterns. Although cafeteria type diets [>20% (w/w) fat, highly palatable diets] readily produce obesity in adult rats, they do not produce the same results in juvenile rats or they do so only after a delay.[14–16] A part of the difference between adults and young rats has been attributed to differences in energy intake, but this contribution is apparently small.[14–16] Calculations of ATP yields from fatty acid versus carbohydrate oxidation suggest that the increased body fat accumulation in animals fed high fat diets may result from differences in the metabolic efficiencies of fat versus CHO oxidation.[31] However, several other factors also may contribute to the increase in body fat. For example, the activities of many enzymes respond to changes in dietary fat and CHO,[8–10] but these changes depend on the type of fat as well as the quantity ingested.[2,12] This means that different high fat diets will have different effects on de novo fatty acid synthesis and on subsequent body fat accumulation.

In the present study, weaning on a high fat diet significantly increased body weight measured at weeks 3 and 4, although this increase was modest (approximately 11%) compared with animals weaned on a low fat diet. This change persisted up to 4 weeks after a switch from a high fat to a low fat diet but was reversed 8 weeks after the switch in dietary fat. Higher body weight was associated with an increased percentage of body fat at week 8 and lower body weight was associated with a decreased percentage of body fat at week 16. The increased body fat represented available energy stores as shown by decreased fat utilization after a 17-hour food restriction period in Group II animals (data not shown). The increase in body fat at week 8 (4 weeks after the switch from high to low fat) may be due partly to an increased rate of fatty acid synthesis observed at week 8. Changes in macronutrient oxidation also may play a role. High fat diets can inhibit glucose uptake[32] and increase the levels of plasma insulin to alter carbohydrate and protein metabolism.[33] On the other hand, changes in fat metabolism also have been associated with high fat diets: rats maintained on high fat diets showed an increased capacity for fatty acid oxidation.[34] In the present experiment, this latter effect appears to have been small because changes in body weight and body fat in Group III animals paralleled the changes in fat synthesis.

In general, the data suggest that fat synthesis is an important biochemical activity of growing animals even when these animals have been supplied with high amounts of dietary fat. This is dramatically illustrated by measurements with 17-hour fasted animals that continue to synthesize fat at rates similar to those of nonfasted animals (data not shown). Using this data, as well as literature values, we have tried to quantify the relative importance of fatty acid synthesis to growing animals. It is possible to estimate the amount of fat required for growing rats from the data of Oppenheimer et al.[35] From their data one can calculate that rats weighing approximately 200 g (approximately 6 weeks of age) require a total of 1.0 g/d of fat (de novo synthesis plus dietary intake, corrected for the absorption coefficient of fat)[36] when maintained on rat chow containing 4.5% fat.[35] This value approximates the dietary fat intake of Group I rats (0.93 g/d) but is much lower than that of Group

II rats (mean of 3.5 g/d, corrected for absorption coefficients).[36] These calculations show why fat synthesis is important in animals fed low fat diets because an appreciable amount of fat must be used for energy metabolism. On the other hand, fat synthesis is not as important energetically to high-fat fed animals because they ingest approximately 3.5 times their metabolic requirement. Because the high-fat fed animals should have an excess of fat in their diet, one can estimate the relative increase in fat oxidation of these animals by estimating fat synthesis rates from ^{3}H incorporation rates.[37] The estimates of fat synthesis are 0.24 g/d (low fat diet) and 0.23 g/d (high fat diet). Therefore, it is possible to calculate that fatty acid oxidation in high-fat fed animals is approximately 3.2-fold higher than that of low-fat fed rates (roughly the ratio of the fat intake).[11] These calculations suggest that young animals are adept at matching fatty acid oxidation to fat ingestion to avoid high weight gains. This conclusion is supported by other studies of the relationship between weight gain and high fat diets: younger animals seem better able to balance energy needs with energy consumption.[14–16]

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