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The effect of changing dietary fat and carbohydrate on the enzymes of amino acid metabolism

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Changes in the activities of alanine aminotransferase (Ala-AT), aspartate aminotransferase (Asp-AT), glutamate dehydrogenase (GDH), serine dehydratase (SDH), and branched chain amino acid dehydrogenase were monitored in weanling and adult male rats fed diets differing in fat content: 11% (low), 22% (medium), and 42% (high) of total energy from fat. The carbohydrate and fiber contents were adjusted to maintain the caloric density of the diets. The results showed that changing the total dietary fat and carbohydrate altered the activities of enzymes predominantly in liver and white muscle. This effect was dependent on the age of the animal. In adult animals, high fat diets decreased liver Ala-AT and SDH activity and increased GDH activity. Muscle Ala-AT activity was lowered in adults fed a high fat diet. In weanlings, high fat diets lowered liver Ala-AT and Asp-AT activities as well as muscle Ala-AT activity. Animals were fasted overnight to determine the effect of a high fat diet on fasting enzyme levels. In adults, fasting eliminated the decrease in muscle Ala-AT activity seen in high fat-fed animals and decreased liver Ala-AT and SDH activity. In weanlings, fasting increased liver GDH activity and white muscle Ala-AT and Asp-AT activities. These results suggest a complex dependence of amino acid utilization that may depend on dietary regime and the age of the animal. (J. Nutr. Biochem. 6:414–421, 1995.)

Keywords: fat; amino acid utilization; fasting; metabolic control

Introduction

Changes in the flux through one metabolic pathway often effect changes in other pathways. This is especially true for the pathways of macronutrient utilization. For example, fatty acid oxidation and glycolysis compete directly in insulin-dependent tissue,[1] inhibition of fatty acid synthesis leads to an inhibition of gluconeogenesis,[2] and amino acid utilization apparently competes with glycolysis in adult animals fed under hypercaloric conditions[3] or in refed starved animals[4] but not in infants.[5,6] These examples show the complexity of the interaction between the metabolic pathways of carbohydrate (CHO), fat, and protein utilization. To date three different potential regulatory mechanisms have been identified to control these interactions: (a) a competition between different substrates for the enzymes of oxidation pathways,[1,3] (b) allosteric regulation of control enzymes,[2] and (c) direct control of enzyme activity through changes in enzyme phosphorylation[6,7] as well as de novo synthesis.[8,9]

The physiological impetus for the utilization of protein as a metabolic fuel is often though to arise solely from a dietary excess of protein above that normally required for de novo protein synthesis. This hypothesis is based on the fact that protein is not stored in the body as a readily available energy source.[3,10,11] Studies with infants[5,6] and adults[3,4] show that this may not be strictly true and that the relative rates of protein utilization also depend on the fuel mixture in the diet. However, the mechanisms underlying the control of protein oxidation are poorly understood. This is due, in part, to the use of whole body nitrogen balance in studying amino acid utilization. Nitrogen balance only measures overall nitrogen excretion and not individual metabolic events. To overcome this problem, we measured the activities of the enzymes involved in amino acid metabolism with particular emphasis on those that catalyze anapleurotic

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reactions. These measurements allowed us to assess indirectly the relative importance of the various amino acid metabolic pathways as a function of the source of energy (fat or CHO) under conditions of isocaloric intake in young and adult animals. The results show that the activities of the enzymes of amino acid catabolism vary with changing amounts of fat and CHO in diets of constant protein content.

Methods and materials

Animals and protocol

Weanling (48.9 ± 2.3 g, mean ± SD) and 10-week-old (417 ± 12 g) male Sprague–Dawley rats were obtained from Charles River Co. (Quebec). Animals were randomly assigned to individual wire-bottom stainless steel cages and maintained on a 12 hr light/dark cycle. The room temperature was 22± 1°C, and the humidity was held between 45% and 55%. Rats were given free access to water and food for 4 weeks. The diet compositions are listed in *Table 1*. Weanling rats gained 46.0 ± 3.7 g, 54.2 ± 3.8 g, 57.4 ± 5.2 g, and 48.0 ± 3.1 g (n = 36) during the 4 successive weeks of the experiment. Over the same time period, adult rats gained 24.6 ± 23.8 g, 45.6 ± 12.0 g, 35.1 ± 11.1 g, and 23.1 ± 5.8 g (n = 30), respectively. No significant differences in weight gain or body weight were observed between dietary groups. After 4 weeks, rats from each dietary group were split into two groups, and one group was fasted overnight (17 hr). The following morning all animals were killed by exsanguination while under 3% Halothane/O_2 anesthesia. Blood was withdrawn from the abdominal aorta, and tissues were rapidly dissected and frozen in liquid nitrogen. White muscle fibers of the m. quadriceps femoris, the m. semimembranosus, and the m. semitendinosus muscles were visually separated from red muscle fibers. The liver was freeze-clamped in liquid nitrogen. Tissues were stored at −80°C until use. This procedure allows accurate measurements of the in vivo activity of phosphorylated enzymes (S. Brooks, unpublished data).

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

Ingredient	Amount per 100 g of diet		
	High fat (g)	Intermediate (g)	Low fat (g)
Casein, ANRC	20.0	20.0	20.0
CHO			
cornstarch	6.1	28.6	39.9
sucrose	29.0	29.0	29.0
(total)	(35.1)	(57.6)	(68.9)
Fat			
lard	6.0	3.0	1.5
corn oil	14.0	7.0	3.5
(total)	(20.0)	(10.0)	(5.0)
Wood cellulose	19.7	7.2	1.0
Mineral mix-AIN76	3.5	3.5	3.5
Vitamin mix-AIN76A	1.0	1.0	1.0
Choline bitartrate	0.4	0.4	0.4
DL-methionine	0.3	0.3	0.3
Total energy	1,817 kJ	1,758 kJ	1,729 kJ
% of energy from fat	42.3%	21.9%	11.1%
% of energy from CHO	32.3%	54.8%	66.7%

Mineral-mix-AIN76, vitamin mix-AIN76A, and wood cellulose (Alphacel) were obtained from Harlan Teklad (Madison, WI, USA). Choline bitrartrate and DL-methionine were from Sigma CHemical Co. (St. Louis, MO, USA). Calculations of energy used values of 4.1 kcal/g (casein and methionine), 4.0 kcal/g (sucrose, cornstarch, vitamin mix, and choline bitartrate), 9.2 kcal/g (corn oil and lard), 0.5 kcal/g (minerals mix based on 12% sucrose), and 1 kcal/g (wood cellulose).[33]

Enzyme assays

All chemicals were purchased from either Boehringer-Mannheim Co. (Montreal, Quebec, Canada) or Sigma Chemical Co. (St. Louis, MO, USA). Enzyme activities were determined in crude homogenates prepared by grinding frozen tissue 1:4 in 50 mmol/L Imidazole, 5 mmol/L EDTA, 5 mmol/L EGTA, 100 mmol/L NaF, and 30 mmol/L β-mercaptoethanol (pH 7.0). This buffer prevents changes in enzyme phosphorylation by inhibiting phosphatase action (NaF) and by chelating free magnesium and calcium ions. Homogenates were centrifuged for 15 min in an Eppendorf centrifuge (12,000g) at 4°C, and the supernatant was removed and stored on ice until assay. Enzyme activities were measured at 25°C using an Abbott VP discrete analyzer with a 340/380 nm filter (Abbott Laboratories Ltd., Mississauga, Ontario, Canada) with a microplate reader with a 340 nm filter (Dynatech Labs, Chantilly, VA USA) or with a Cary 219 spectrophotometer (at 22°C). Kinetic rates were obtained from the microplate data using a program written especially for this purpose.[12] When measuring the rate with the Abbott VP analyzer, the NADH concentration was set to 75 μmol/L. When measured with the microplate reader, the NADH concentration was set to 150 μmol/L. Concentrations of oxidized nicotinamide coenzymes are as given in the original methodology. Alanine aminotransferase (Ala-AT) was measured according to Horder and Rej,[13] aspartate aminotransferase (Asp-AT) was measured according to Rej and Horder,[14] glutamate dehydrogenase (GDH) was assayed as described by Schmidt and Schmidt,[15] and serine dehydratase (SDH) was determined according to Suda and Nakagawa.[16] Aldolase was measured in 50 mmol/L Imidazole (pH 7.0), 2 mmol/L $MgCl_2$, 0.2 mmol/L fructose 2,6-bisphosphate plus 1 U triosephosphate isomerase, and 2 U α-glycerophosphate dehydrogenase. Lactate dehydrogenase was measured in 50 mmol/L Imidazole (pH 7.0), 2 mmol/L $MgCl_2$, and 2 mmol/L pyruvate. Branched chain amino acid dehydrogenase was assayed according to Paxton and Harris[17] in a Cary 219 spectrophotometer at 22°C.

Other methods

Protein concentrations were measured by homogenizing tissues in 0.5 N NaOH containing 5% SDS followed by heating in sealed tubes at 30°C for 2 hr. Homogenates were then assayed for total protein content with an Abbott VP discrete analyzer using the QuickStart T PROT kit (Biuret method). This procedure has been shown to give accurate protein measurements.[18]

Statistics

All values are reported as mean ± SD. Unless otherwise indicated, all statistical analyses were performed using the Student–Newman–Kuels test after type I ANOVA (two factor) analysis.[19] 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 before statistical analysis. Differences were not considered significant if the P value was greater than 0.05.

Results

The liver protein concentration depended on the metabolic state of the animal and on the dietary regime. In fasted animals, differences were most prominent in weanlings fed

low and medium fat diets: fasted protein concentrations were 40% higher in fed rats. Dietary effects were also noted. Livers from high fat-red weanlings had significantly higher protein concentrations than livers from weanlings fed low and medium fat diets (*Table 2*). In adults, small differences between fasted and fed liver protein concentrations were observed in medium fat-fed animals (*Table 2*). Because protein concentrations changed as a result of fasting and changing dietary compositions, it was important to determine a basis for comparison of enzyme activities. This was accomplished by comparing the activities of aldolase (ALD) and lactate dehydrogenase (LDH), two enzymes that are not altered by fasting or diet.[8,9] The results of the present study showed that LDH and ALD were not significantly affected by diet or fasting when the results were expressed as units per gram of wet weight: ALD activity was 1.48 ± 0.19 (young) and 1.69 ± 0.38 (adult) and LDH activity was 132.2 ± 13.4 (young) and 126.7 ± 23.7 (adult) U/g of wet weight (U/gww) with $n = 36$ (young) and $n = 30$ (adult). The units of U/gww were, therefore, used as the basis of comparison. The protein concentrations of kidney, heart, and white muscle were unchanged by an overnight fast or by dietary regime.

The effects of changing the fat and CHO content of the diet as well as the effects of fasting on GDH, Ala-AT, Asp-AT, SDH, and BCAADH (liver only) activities are presented in *Table 3* and *Figures 1–4*. Individual changes are as described below.

Liver

Diet had a significant effect on Ala-AT activity in young animals. In high fat-fed rats, Ala-AT activity was 36% lower (nonfasted) or 26% lower (fasted) than the value in low and medium fat-fed animals (*Figure 1*). Fasting increased Ala-AT activity under all conditions in both young and adult animals to give an activity that was approximately 25% higher than the nonfasted value (the difference was not statistically significant in adults fed high diets). The Ala-AT activity of adult animals was approximately 2.1 times that of young animals as determined form pooled data.

Increasing dietary fat decreased the total Asp-AT activity in young animals so that rats fed a high fat diet had approximately 30% less activity than those maintained on the low fat diet (*Figure 1*). The intermediate diet produced a result midway between the two extremes. In adult animals, fasting significantly increased Asp-AT activity by about 30% under all dietary manipulations. Diet had no effect on Asp-AT levels in adult rat liver.

Fasting significantly increased GDH activity in both young and adult rats under all dietary conditions (*Figure 1*). Glutamate dehydrogenase activity was also affected by diet; in young fasted animals, increasing fat content increased GDH activity. This effect was not observed in nonfasted animals. In adults, increasing fat content increased GDH activity in both fasted and nonfasted animals. Age also had a significant effect on GDH with younger animals having nearly twice as much GDH activity as older animals. In contrast to Ala-AT, Asp-AT, and GDH, fasting had no effect on SDH activity levels in either young or adult animals. In adult, but not young rats, increasing the fat content of the diet decreased SDH activity. Overall, adult liver had a significantly higher SDH activity: SDH in adults was 4.1 times that found in young rats. Neither dietary regime nor fasting had any effect on liver BCAADH activity as determined from changes in observed activity (*Table 3*).

Kidney

Both fasting and diet altered Ala-AT activity in the kidney of young animals but the effect was not large (*Figure 2*). Alanine aminotransferase activity was slightly elevated in the high-fat dietary group as compared with the low fat group (10% higher) and the medium fat group (20% higher). In fasting animals, Ala-AT activity was 10% lower in the high-fat group and 10% higher in the medium fat group. In adult animals, fasting significantly lowered the Ala-AT activity in animals fed the medium fat diet (30%

Table 2 Liver composition of young and adult animals

Component	Diet	Young		Adult	
		Fed	Fasted	Fed	Fasted
Water	Low	65.9 ± 2.5*	64.2 ± 2.1	63.6 ± 3.5	65.9 ± 2.0
	Medium	66.4 ± 1.8	67.5 ± 0.9†	66.5 ± 0.9	66.6 ± 1.7
	High	66.9 ± 0.5	68.1 ± 0.6‡	66.0 ± 0.3	66.9 ± 0.9
Protein	Low	20.7 ± 2.5	28.5 ± 2.0§	23.8 ± 2.0	25.4 ± 1.7
	Medium	20.2 ± 1.0	27.5 ± 1.1[a]	21.9 ± 1.2	25.4 ± 0.9[a]
	High	24.6 ± 1.4[b,c]	26.8 ± 1.6[a]	23.1 ± 1.8	24.5 ± 1.1
CHO	Low	4.96 ± 0.41	0.37 ± 0.08[a]	4.65 ± 0.76	1.21 ± 0.59[a]
	Medium	5.63 ± 1.11	0.33 ± 0.09[a]	4.50 ± 0.55	1.23 ± 0.25[a]
	High	4.98 ± 0.38	0.28 ± 0.04[a]	4.69 ± 0.32	0.89 ± 0.49[a]
Fat	All‖	3.94 ± 1.79	4.67 ± 1.49	5.28 ± 1.78	6.02 ± 1.71

Values are means ± SD for $n = 6$ (young) or $n = 5$ (adult) animals.
*Values are in g/gww × 100%.
†Significantly different from low diet.
‡Significantly different from medium diet.
§Significantly different from corresponding fed diet.
‖Values from all three diets were pooled since no statistically significant differences were observed ($n = 18$, young; $n = 15$, adult).

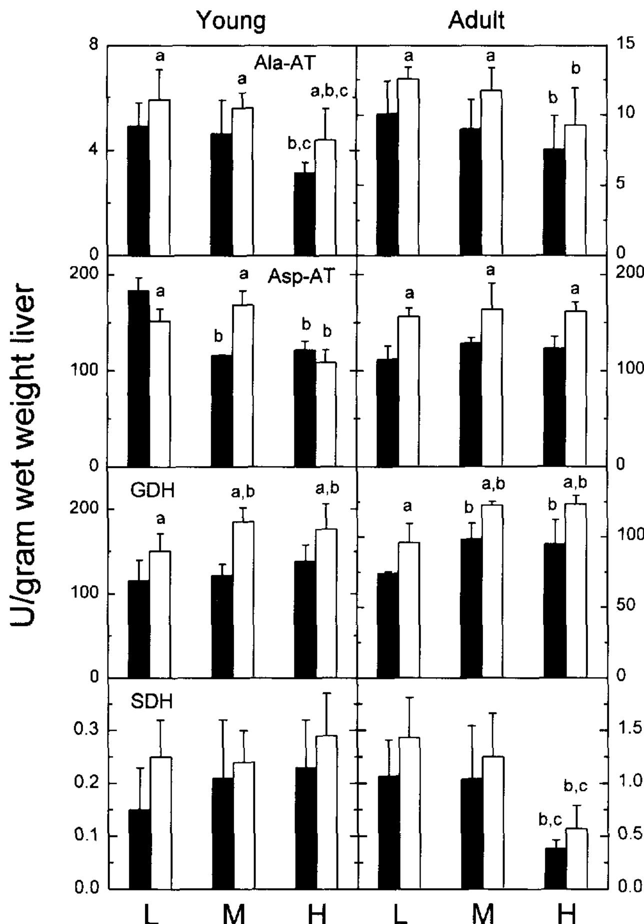

Figure 1 Effect of diet and fasting on liver Ala-AT, Asp-AT, GDH, and SDH activity. Values are means ± SD for n = 6 (young) or n = 5 (adult) different animals for L (low fat-fed), M (medium fat-fed), or H (high fat-fed) rats. Open bars represent animals that had been fasted for 17 hr, and solid bars represent animals that were not fasted. Graphs on the left are for weanling animals and values on the right are for adult animals. International units of enzyme activity (U) per gram of wet weight liver are reported for low, medium, and high fat diets as defined in *Table 1*. Significance is reported as follows: (a) significantly different from corresponding value for fed animals, (b) significantly different from corresponding value for low fat diet, (c) significantly different from corresponding value for medium fat diet. The pooled Ala-AT, GDH, and SDH activities from young and adult animals were significantly different as determined by a two factor ANOVA analysis.

lower than nonfasted). *Table 3* shows that diet had no effect on Asp-AT, GDH, and SDH activities. Fasting young animals, however, had a lower Asp-AT activity (8% lower than nonfasted) and a higher SDH activity (40% higher than nonfasted). Fasting had no effect on the enzyme activity of adults. A comparison between enzyme activities in adult and young animals shows that young animals had significantly higher levels of Asp-AT (30% higher in young), GDH (10% higher in young), and SDH (50% higher in young).

Heart

The effect of fasting and dietary regime on heart enzymes is presented in *Figure 3* and *Table 3*. In young animals, Asp-AT activity in the high fat dietary group was significantly lower than that of the low fat and medium fat dietary groups. On the other hand, a small but statistically significant increase in SDH activity was observed in animals fed medium and high fat diets. Fasting significantly increased Asp-AT activity (20% higher) and decreased SDH activity (10% lower) in rats maintained on a medium fat diet. Adult animal enzyme levels were not altered by any experimental treatment. Because Ala-AT and GDH activities did not change as a function of diet, the results from the three diet groups were pooled and are presented in *Table 3*. These data show that Ala-AT and GDH activities were also unaffected by fasting. Young animals had significantly less Ala-AT (25% lower) and significantly more SDH (60% higher) than did adult animals.

Muscle

Increasing fat concentrations caused a progressive decrease in the total activity of Ala-AT in nonfasted animals (*Figure 4*). This effect was more pronounced in adults where the activity in the high fat group was 25% lower than that of the low fat group. In young animals, the activity in the high fat group was 9% lower than that of the low fat group. Fasting also had a significant effect on Ala-AT activity but the magnitude and direction of this effect depended on the age of the animal and the dietary condition. In young animals fed a high fat diet, fasting caused a significant increase in activity (30% higher), whereas in adult animals fed a low fat diet, fasting significantly decreased Ala-AT activity (27% lower) as compared with fed animals. A significant effect of diet and fasting was also noted for Asp-AT. In young animals fed low and medium fat diets, fasting increased Asp-AT activity to values that were 80% and 60% higher, respectively. No effect was noted in Asp-AT activity in young animals fed a high fat diet or in adults under any experimental condition. Neither diet nor fasting had any effect on the activity of GDH or SDH in muscle. A comparison of the total enzyme activity in young and adult animals showed that the GDH activity of young animals was 30% higher than that of adults.

Discussion

Excess dietary amino acids can either be converted into other body stores such as glucose[20] or oxidized to CO_2.[11] The relative distribution of amino acids between these two pathways is influenced by several factors including total protein consumption (with high protein diets promoting protein oxidation),[3,11] the amount of glucose in the diet,[3,4] and the rate of fatty acid oxidation.[2] Numerous mechanisms have been derived to explain the interaction of these liver metabolic pathways, including a competition for free coenzyme A,[3] reversible inhibition of pyruvate dehydrogenase by cellular metabolite concentrations,[20] and regulation of enzyme phosphorylation[6,7] and synthesis[21-26] through changes in plasma glucagon and insulin.

In the present study, we focused on examining the role of changing total enzyme activity in the control of amino acid utilization. The total activities of three "control" enzymes (BCAADH,[6,7] SDH,[22] and GDH[27]) and two "equilibrium"

Table 3 Enzymes of amino acid metabolism that did not vary with a change in diet composition

Tissue	Enzyme	Young Fed	Young Fasted	Adult Fed	Adult Fasted
Liver	BCAADH	0.092 ± 0.068*	0.129 ± 0.076	0.207 ± 0.152	0.265 ± 0.103
Kidney	AspAT†	81.6 ± 5.8	74.7 ± 5.3‡	62.5 ± 3.5	59.3 ± 6.1
	GDH[b]	72.8 ± 6.7	69.9 ± 6.2	64.2 ± 5.2	62.6 ± 7.8
	SDH[b]	0.068 ± .012	0.096 ± 0.009[a]	0.060 ± 0.008	0.050 ± .011‡
Heart	Ala-AT†	3.58 ± 0.45	3.56 ± 0.37	4.73 ± 0.90	4.82 ± 0.56
	GDH	8.45 ± 1.32	8.60 ± 1.29	8.85 ± 1.02	8.72 ± 0.96
Muscle	GDH†	1.37 ± 0.20	1.45 ± 0.19	1.11 ± 0.10	1.04 ± 0.07
	SDH	0.292 ± 0.082	0.242 ± 0.082	0.231 ± 0.061	0.244 ± 0.067

Values are means ± SD for n = 18 (young) and n = 15 (adult) animals.
*Values are in U/g of wet weight.
†Pooled values from young and adult rats were significantly different as determined by a two factor ANOVA analysis.
‡Significantly different from the value for fed animals.

enzymes (Ala-AT and Asp-AT) were followed. Previous work has related changes in Ala-AT, Asp-AT, GDH, and SDH activity to changes in enzyme synthesis[40] and BCAADH to changes in enzyme phosphorylation.[6,7] Control enzymes are designated as such because they catalyze pathway steps that are highly regulated. For example, BCAADH activity is the rate-limiting step of branched-chain (leucine, isoleucine, and valine) amino acid oxidation,[7] and SDH catalyzes the terminal oxidative step of serine and threonine metabolism.[25,26] Glutamate dehydrogenase catalyzes the oxidative deamination of glutamic acid and is closely regulated by metabolite effectors in rat liver.[28,29] The changes in activity of Ala-AT,[23] Asp-AT,[24] GDH, and SDH[21,22,25,26] are due to changes in the total amount of enzyme present, whereas changes in BCAADH are due to changes in the degree of enzyme phosphorylation.[6,7]

The results of the present study show that changes in dietary fat and CHO content exert some influence on the overall activity of key regulatory enzymes of amino acid metabolism. Significant changes occurred in GDH and SDH activity: high fat diets increased GDH activity in young and adult rats and lowered SDH activity in adults.

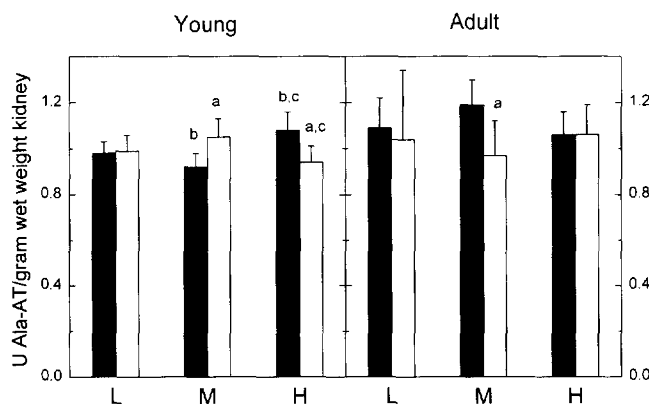

Figure 2 Effect of diet and fasting on kidney Ala-AT. Values are means ± SD for n = 6 (young) and n = 5 (adult) different animals. See *Figure 1* for an explanation of symbols.

Carbon flux through glutamate is especially important since alanine and glutamine account for 60 to 80% of the amino acids released from skeletal muscle[24] and taken up by the liver. Rat liver GDH is thought to be controlled primarily through changes in ADP and GTP levels.[29] However, kinetic analysis has shown that the reaction greatly favors reductive animation by approximately 32-fold[30] so that the total enzyme activity may be limiting during periods of high gluconeogenic activity. This would be especially prominent during the initial stages of starvation that are characterized

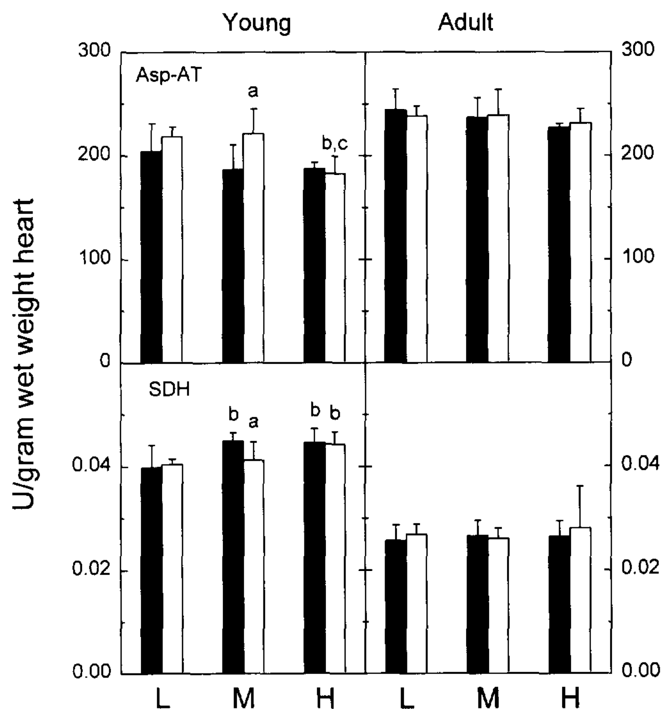

Figure 3 Effect of diet and fasting on heart Asp-AT and SDH activity. Values are means ± SD for n = 6 (young) and n = 5 (adult) different animals. See *Figure 1* for an explanation of symbols. The pooled Asp-AT and SDH activities from young and adult animals were significantly different as determined by a two factor ANOVA analysis.

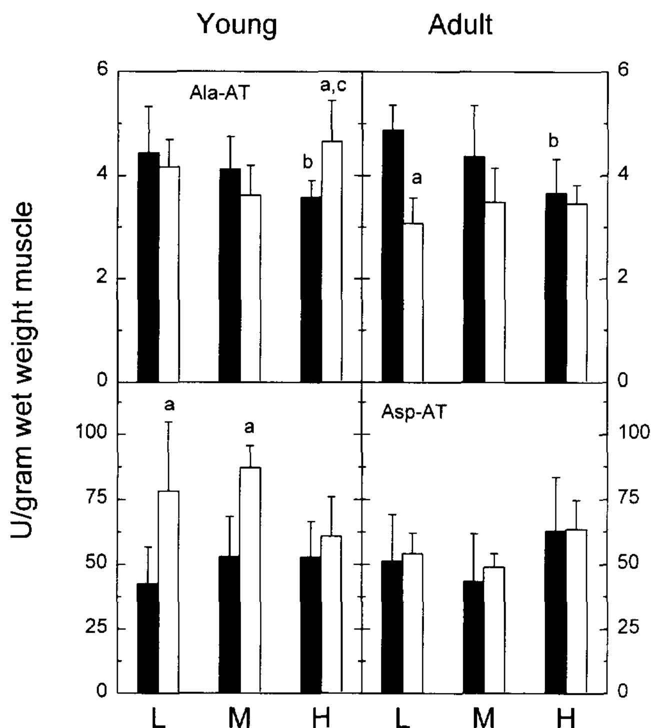

Figure 4 Effect of diet and fasting on muscle Ala-AT and Asp-AT activity. Values are means ± SD for n = 6 (young) and n = 5 (adult) different animals. See *Figure 1* for an explanation of symbols.

by increased gluconeogenesis.[31] The small effect observed at high dietary fat concentrations may reflect the fact that relatively little CHO is available from the diet under these conditions.

The effects of diet on Asp-AT and Ala-AT activity are hard to reconcile with the belief that they catalyze reactions that are at or near equilibrium.[20,31,32] Dietary effects on Ala-AT were observed in liver, kidney, and muscle; effects on Asp-AT activity were seen in liver, heart, and muscle. In general the effect of increasing fat in the diet was to lower activity, whereas an overnight fast increase activity although exceptions to his trend were noted. Calculations of the relative flux through Ala-AT in liver,[20] taking into account the relative differences in the protein turnover of rats and man,[33] suggest that Ala-AT maximal activity may only be 2- to 5-fold higher than the total flux through the enzyme. These calculations provide a rationale for the dietary regulation of Ala-AT.[23] During starvation, the flux through Ala-AT is likely to increase severalfold since L-alanine is the primary amino acid released from muscle during this time.[31] The effect of high fat diets is probably due to the reciprocal relationship between fatty acids and glucose as oxidative substrates.[34] This effect is still observed during starvation suggesting that the decline in insulin that accompanies fasting accelerates the substitution of lipid (from body stores) for CHO.[34]

Results from previous studies have shown that feeding rats an extremely high fat diet (80% of calories from fat) for as little as 7 weeks resulted in rats with 36% heavier epidydymal fat pads and a 47% increase in adipocyte volume.[35] The results of the present study suggest that changes in the available energy reserves can be altered by feeding rats for only 4 weeks on a diet where 42% of calories were derived from fat. In animals with high fat reserves, the glycerol released by triglyceride lipolysis may act as a glucose precursor during the early phase of starvation reducing amino acid utilization for gluconeogenesis.[36] This nitrogen-sparing effect has been previously observed in cafeteria fed animals by showing a reduced rate of [^{14}C]L-alanine utilization for gluconeogenesis[37] and correlating this with small changes in Ala-AT.[38] This effect, however, was not seen in the Zucker rat and may in fact be reversed in this animal model.[39] The magnitude of this effect should depend directly on the size of the fat reserve. Thus, animals with larger fat reserves could delay amino acid utilization until fatty acid oxidation no longer contributed enough glycerol to maintain blood glucose concentrations. Indirect evidence in support of this hypothesis comes from starvation studies in obese Zucker rats where nitrogen excretion (protein utilization) did not increase up to 80 days after starvation began even though amino acid utilization continued at a reduced rate throughout the starvation period.[40] In this case, presumably, glycerol from triglyceride lipolysis was able to provide enough gluconeogenic carbon to delay the sharp increase in protein utilization seen in control rats. The ability of the obese Zucker rat to delay amino acid utilization may be potentiated by an overall preference for fatty acid over glucose oxidation.[41] These studies agree with the results presented here. Increasing dietary fat reduced (1) Ala-AT activity in the livers of high fat-fed rats, (2) Asp-AT activity in the livers from weanlings, (3) SDH activity in the livers of high fat-fed rats, and (4) the starvation-associated increase in Asp-AT activity in young muscle. Changes in the activities of these enzymes are not necessarily related to their oxidation rate in vivo. However, previous studies have shown that changes in amino acid utilization are associated with changes in Ala-AT,[23] Asp-AT,[24] and SDH.[21,22] Thus, these studies suggest that gluconeogenesis from amino acids may be delayed in high fat-fed animals.

When dietary amino acids are in excess, conditions that promote glycolysis should reduce amino acid utilization because of the relatively high insulin/low glucagon concentrations that should prevail. This conclusion is supported by studies with starving rats where it has been shown that glucose inhibits the whole-body rate of leucine oxidation more effectively than does fat when animals are fed calorie-reduced diets[4,42]; CHO consumption has a protein-sparing effect but fat does not. Some of these experiments[4] were carried out at protein intakes that exceeded N balance maintenance requirements. Their results are, therefore, applicable to the present study where the daily protein consumption was well above maintenance values for growing rats.[43] In general, amino acid oxidation studies[4] measured flux through the branched chain amino acid degradation pathway; leucine oxidation was followed by radioactive tracers. The results of these studies may, consequently, be influenced by BCAADH activity (which is controlled by reversible enzyme phosphorylation)[17] as well as by substrate level competition for free CoA that would reduce the entry of amino acid degradation products into the Krebs Acid Cycle.[3] It is often difficult to determine the nature of the control in the absence of detailed analyses of enzyme rates and concentrations of metabolic intermediates. In the present

study, we measured the relative importance of the various amino acid degradation pathways by following enzyme activities in tissues of rats fed different diets. Our results show that amino acid enzyme activities behave in a fashion exactly opposite to that predicted by amino acid oxidation studies on starving animals; high fat diets apparently have a greater amino acid sparing effect than does CHO. The differences between the present study and those measuring amino acid oxidation directly are probably due to the duration of starvation, and a lengthy period of fat feeding that preceded the fasting period.

At present the mechanism for the fat-mediated reduction in enzyme levels remains unclear. Serine dehydratase, Ala-AT, and Asp-AT activities increase with increasing plasma glucagon (through α-adrenergic stimulation)[40] and decreasing plasma insulin. This response was also apparent in the present paper where, in general, the activities of SDH, Ala-AT, and Asp-AT increased during starvation. It is known that high fat diets (low CHO diet) reduce plasma insulin levels (relative to high CHO diets) since the release of insulin is less stimulated by dietary fat than by dietary CHO. Fats can also stimulate glucagon release.[44] This would argue for an increase in SDH, Ala-AT, and Asp-AT with decreasing dietary CHO but the opposite is observed. The present results, therefore, suggest that another unidentified mechanism is responsible for regulating SDH, Ala-AT, and Asp-AT activities in vivo under the conditions of our experiment.

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