Distribution of α-Aminoisobutyric Acid in Tissues of Lean and Genetically Obese Mice (42883)

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Abstract. **Distribution of tracer amounts of the nonmetabolizable neutral amino acid α-[1-^{14}C]aminoisobutyric acid (AIB) between blood and several tissues was measured in lean and *ob/ob* mice over an 8-hr period. As AIB was injected on the basis of body weight and as *ob/ob* mice have a relatively low blood volume, absolute concentrations of AIB in blood and tissues were almost always higher in the obese than the lean mice. However, the ratio of AIB concentration in the tissues to that in the blood was clearly higher in skeletal muscle, diaphragm, and brain, and possibly higher in liver of the lean than of the obese animals. Ratios in heart were similar. The results suggest that lean and genetically obese mice differ in their capacity to transport amino acids between blood and various tissues.** [P.S.E.B.M. 1989, Vol 191]

Lean and genetically obese mice differ in many of their behavioral and metabolic characteristics (1). For example, weight gain of lean mice fed a threonine-imbalanced diet (a threonine-limiting, 8% casein diet containing 6% of a mixture of all indispensable amino acids but threonine) was depressed, whereas obese mice grew equally well when they were fed either the imbalanced or control diet (2). Concentrations of many free amino acids in muscle and liver from the obese mice were low, as were the ratios of the tissue concentrations to those in blood (2). These results suggested that there might be a defect in transport of amino acids from blood into various tissues and organs in the genetically obese mouse. In order to extend these observations, we have compared in intact lean and obese mice the distribution between the blood and other tissues of subcutaneously injected α-aminoisobutyric acid (AIB). AIB is a nonmetabolizable, small neutral amino acid that has been widely used as a model in transport studies (3) both *in vivo* and *in vitro*. Although it is not a specific model for any individual class of neutral amino acids (e.g., the A, ASC, and L systems), its use can provide meaningful information about their transport.

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Materials and Methods

Twenty lean (+/*ob* or +/+) and 20 obese *ob/ob* male mice (C57BL/6J-*ob*) were obtained from The Jackson Laboratory, Bar Harbor, Maine. They were housed individually in metal cages with wire mesh bottoms and kept in a room maintained at about 24°C. The mice, aged 46 ± 3 days, were fed *ad libitum* for 7 to 10 days an 18% casein diet prepared in agar gel form (4). The diet also contained (in g) vitamin mix (4), 0.5; mineral mix (4), 5.0; corn oil, 5.0; choline chloride, 0.2; and equal amounts of glucose monohydrate and cornstarch to make 100 g. The obese mice weighed an average of 32.7 ± 0.7 g at the start of the feeding period and 37.7 ± 0.3 g at the end of the experiment; corresponding weights for the lean mice were 22.8 ± 0.5 and 24.4 ± 0.3 g, respectively.

On the day of the experiment the mice were injected subcutaneously with α-[1-^{14}C]AIB having a specific radioactivity of 53.2 mCi/mmol (0.5 μCi/30-g mouse in a volume of 0.24 ml of saline). Only tracer amounts of AIB (approximately 7–13 nmol/mouse) were used in order to avoid disturbing tissue amino acid pools. Food was available during the experimental periods. At 0.5, 1.5, 4, and 8 hr after the injection, mice were lightly anesthetized with ether before they were decapitated. Blood was collected and brain, heart, liver, diaphragm, and skeletal muscle from the hind legs were taken, rinsed with saline, and blotted; the heart was flushed with saline to remove blood remaining in the major vessels. After the tissues were weighed, portions of all but blood and diaphragm were dried to a constant

weight in a vacuum oven in order to determine tissue water content. Water content of the diaphragm was taken to be the same as in skeletal muscle. Other portions of the tissues were homogenized in 3% sulfosalicylic acid to precipitate tissue proteins (5). Blood was mixed with an appropriate volume of 7.5% sulfosalicylic acid to make the final acid concentration in the supernatant similar to that for the other tissues. After centrifugation of the homogenates, radioactivity was determined in each supernatant with a LKB 1217 Rackbeta scintillation counter. Data for one lean mouse were lost. Results were expressed as dpm/ml blood or as the distribution ratio (dpm per ml tissue water/dpm per ml blood) for the other tissues. These ratios, especially after early linear uptake of AIB into tissues, represent transport via both influx and efflux. Results were based on whole blood measurements because of reports suggesting the importance of erythrocytes in interorgan transport (6–8).

In order to determine whether the distribution of AIB in the tissues of each group of mice showed a consistent pattern over time, Tukey's test for transformable nonadditivity was performed for each tissue (9); an appropriate transformation was found for those showing transformable nonadditivity (muscle, heart, and brain). The data, transformed where necessary, were then subjected to analysis of variance by the general linear models procedure (10).

Results

Time of sampling did not have any apparent affect on tissue water content; therefore, values for the four time periods were averaged for each of four tissues within the two groups (Table I). Water content of livers from the obese mice was only 74% of that in the lean animals. Water content of skeletal muscle was slightly but significantly lower in the obese than in the lean animals (96% of the lean control value). There were no apparent differences between the two groups in water content of brain and heart.

After subcutaneous injection of AIB, radioactivity appeared rapidly in the blood and other tissues (Fig. 1). The line in Figure 1A is broken to show that the time at which the highest level of radioactivity occurred in the blood was uncertain. The highest value was observed at the first time point (30 min after injection), thereafter the amount of AIB in the blood decreased continuously. The absolute amounts of AIB detected in the blood during the 8-hr study were consistently greater in the obese than the lean mice ($F = 160.5$, $P < 0.001$).

There was, during the 8-hr experiment, a continuing gradual increase in the mean distribution ratio for AIB with time for each of the examined tissues. At each time interval the ratio in skeletal muscle was consistently and significantly lower in the obese than in the lean mice ($F = 68.8$, $P < 0.001$); by the 8-hr interval the distribution ratio for AIB in muscle was almost twice as high in the lean as in the obese animals (Fig. 1B). Accumulation of AIB in diaphragm was also lower in the obese mice (Fig. 1C), except for the 30-min interval, when the value for one obese mouse was almost twice the mean of the remaining four values; the distribution ratios were always clearly lower in the obese than in the lean mice ($F = 53.4$, $P < 0.001$). At 8 hr the ratio for the obese was less than 70% of that for the lean mice. The distribution ratios for AIB in the diaphragms also increased differently over time in the two groups of mice ($F = 7.21$, $P < 0.001$). The two groups did not differ significantly in AIB accumulation in cardiac muscle groups (Fig. 1D), although the mean distribution ratios were lower in the obese mice at all but the 30-min interval. For both groups of mice the distribution ratios were approximately twice as high in the heart as in either the diaphragm or skeletal muscle.

There was also a significant difference between the two groups in accumulation of AIB in brain (Fig. 1E), with the distribution ratio for the obese being 75% of that of the lean animals after 8 hr ($F = 26.4$, $P < 0.001$). Ratios in liver (Fig. 1F) were, overall, only slightly higher in the lean than the obese mice ($F = 5.73$, $P < 0.023$).

Table I. Water Content of Tissues from Lean and *ob/ob* Mice[a]

	Water (%)	
	Lean	Obese
Brain	78.1 ± 0.3	78.2 ± 0.3
Heart	73.9 ± 0.6	73.4 ± 0.6
Liver	67.8 ± 0.7	50.2 ± 0.7*
Skeletal muscle	74.5 ± 0.8	71.6 ± 0.6**

[a] Values are the mean ± SEM for 19 lean and 20 obese mice.
* For differences between control and obese, $P < 0.001$; ** $P < 0.01$ (Student's t test).

Discussion

These results confirm earlier reports that water content is low in liver and muscle in *ob/ob* mice (11). Also, in agreement with other reports (12–14), liver weight was high and muscle and brain weights were low in the obese animals (not shown). As injections of [^{14}C]AIB were based on body weight, the larger amount of radioactivity in blood taken from the obese mice is in agreement with observations that, in relation to body weight, blood water content and blood volume of these animals are much lower than in the lean controls (15).

As corrections were not made for blood remaining in the organs after decapitation and exsanguination, a varying fraction of the radioactivity in the tissues will be due to trapped blood. Based on the blood content of rat muscle and brain (about 3%; 16, 17), a maximum of 7 and 12%, respectively, of the radioactivity in these

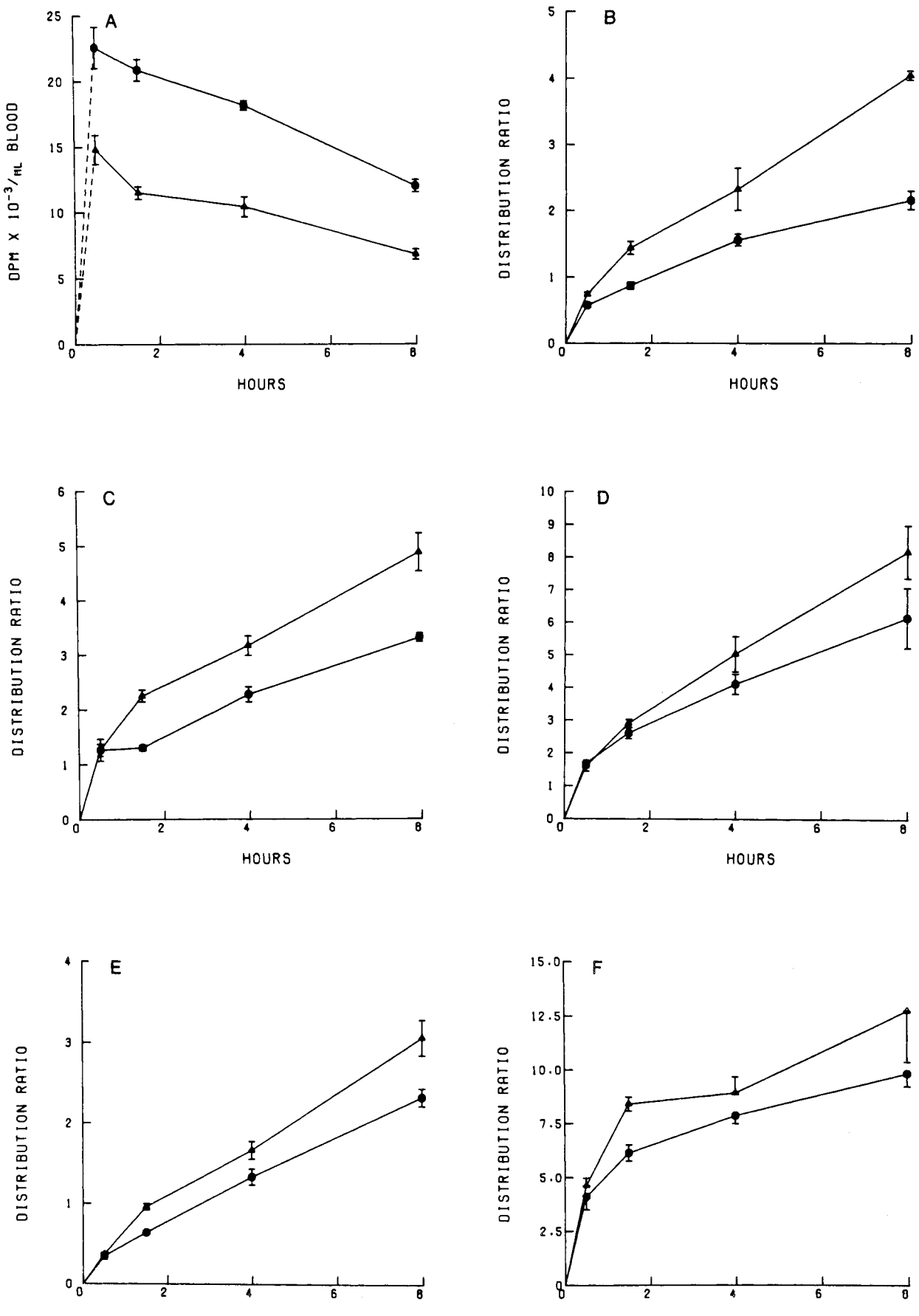

Figure 1. (A) Changes with time in [^{14}C]AIB content of blood of lean and obese mice. (B–F) Distribution ratios for AIB (dpm per ml tissue water/ dpm per ml blood): (B) skeletal muscle; (C) diaphragm; (D) heart; (E) brain; (F) liver. ▲, lean; ●, obese. Values are the means for five mice per group at each point, except four lean mice at 0.5 hr. Bars show SEM; bars falling within symbols are not shown. The dashed lines indicate the possibility that the highest amounts of AIB in the blood may have been reached before the 0.5-hr interval.

organs in the mice could be due to residual blood at the 30-min interval; the contamination would have been less than 2% at 8 hr. Liver normally contains a large proportion of blood (25–30% by weight), which decreases significantly even after moderate blood loss (18); a maximum of about 6–12% of observed radioactivity would be due to trapped blood at the 0.5-hr interval. By 8 hr blood would seldom have contributed more than 4% of total radioactivity. These considerations suggest that the differences in AIB accumulation in livers of the lean and *ob/ob* mice are not especially meaningful.

Our results suggest that the obese mouse is unable to clear AIB from the blood and to concentrate this amino acid in certain tissues nearly as well as its lean counterpart. Low distribution ratios were observed in most tissues from the obese mice, despite the fact that the average absolute amounts of AIB in blood and tissue water were generally greater in these than in the lean mice (for all tissues and at all times except for skeletal muscle at 8 hr). As the total dose of AIB ranged from 7 to 13 nmol/mouse, tissue AIB concentrations were far below those required to demonstrate saturability of neutral amino acid transport systems in peripheral tissues (for which K_m values are stated to be in the millimolar range; 19). Therefore, low distribution ratios in tissues from the obese mice are most unlikely to reflect saturation of AIB transport as a result of the higher levels of AIB in the blood of the obese than in the lean mice (the highest concentration did not exceed 0.2 n*M*). Although small neutral amino acids compete more effectively than large neutrals for transport of AIB into various tissues, ratios for AIB in the obese mice are also unlikely to reflect differences in blood amino acid profiles as concentrations, especially for the small neutrals, were not clearly different between lean and obese mice fed either low (threonine-supplemented) or high protein diets (2). However, the low ratios could reflect altered rates of AIB efflux in the obese mice.

Although blood AIB concentration fell throughout the 8-hr experiment, tissue AIB concentrations in both groups generally continued to increase during the first 4 hr and then leveled off; the concentration in brain increased even further during the final 4-hr interval. In most cases the time-dependent changes in the distribution ratios resembled those observed for fed rats injected with AIB (20). For each of the mouse tissues the distribution ratios exceeded 1 at some time during the observation period, thereby providing evidence that AIB was actively concentrated in the organs. Absolute dpm in tissue water can be estimated by multiplying the dpm/ml blood (Fig. 1A) by the corresponding ratio shown for the selected tissue. For example, radioactivity in control muscle at 0.5 hr (Fig. 1B) was about 11,000 dpm/ml tissue water (0.75 × 15,000 dpm/ml blood) and about 28,000 dpm/ml at 8 hr (4.0 × 7,000 dpm/ml blood).

The lowered ability of the obese mice to concentrate the nonmetabolizable, small neutral AIB in the tissues is consistent with our earlier observation that the concentrations of natural small neutral amino acids (threonine, serine, alanine, and glycine) were low in skeletal muscle and in liver as well (except alanine); at the same time blood concentrations of small neutral, large neutral, and basic amino acids were such that the ratios of amino acid concentrations in these tissues to those in blood of the obese mice were consistently less than the ratios observed in the control tissues (e.g., 50–60% and 30–85% of the control ratios for muscle and liver, respectively (2)).

Transport of AIB has been studied *in vitro* in soleus muscle taken from mice made obese by treatment with gold thioglucose (21). After incubation of the intact muscle in a medium containing AIB, the ratio of intracellular to extracellular AIB was lower in the obese than in the control mice. Le Marchand-Brustel *et al.* (21) also stated that there was a decrease in AIB transport into soleus muscles taken from genetically obese mice.

Extensive evidence showing that membrane function is altered in the *ob/ob* mouse has been recently reviewed (22). Changes in receptors, enzyme activities, and various aspects of secretion and transport (e.g., reduced glucose transport) are associated with alterations in membrane lipid composition and, as a result, with altered membrane fluidity. Differences in the structure of membranes in the obese mouse may well result in changes in amino acid transport between the blood and tissues. Transport of certain nutrients is associated with sodium and potassium transport, which in turn is regulated by Na^+,K^+-ATPase. The Na^+/K^+ ratio is high in muscle from obese mice (11) whereas ATPase activity is low in muscle (14, 23) as well as in liver or kidney (24–26). Binding of [^{3}H]ouabain (a Na^+,K^+-ATPase inhibitor) which has been used as a measure of Na^+,K^+-ATPase activity (14) is also low in brains of obese mice (25).

The presence of a defect(s) in amino acid transport may assist in explaining several observations concerning protein metabolism in the *ob/ob* mouse. These mice are less efficient than lean mice in converting dietary protein to tissue protein (27). A further study showed that obese mice eat more protein but retain less nitrogen in the carcass (primarily skeletal muscle, skeleton, and fat-free connective and adipose tissues) than do lean controls. Nitrogen retention in the whole body was sometimes low in the obese mice fed low or normal amounts of protein (28). Miller *et al.* (29) stated that the amount of hepatic protein turnover in *ad libitum*-fed obese mice is less than in lean mice with the same dietary intake. Trostler *et al.* (30) reported that the fractional degradation rate of protein in skeletal muscle, in contrast, is greater from obese than from lean mice and the rates of synthesis do not differ.

Our studies support the concept that, in the genet-

ically obese mouse, there are defects in the distribution (influx and/or efflux) of amino acids between blood and various tissues. Diaphragm and skeletal muscle, along with brain, appear to be most severely affected.

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