Amino acid metabolism in human subcutaneous adipose tissue in vivo

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(Received 28 September/5 December 1990; accepted 20 December 1990)

SUMMARY

1. Arteriovenous differences for alanine, glutamate and glutamine were measured across subcutaneous adipose tissue and forearm muscle in normal subjects.
2. After an overnight fast, adipose tissue showed net production of alanine and glutamine and uptake of glutamate in each of 11 subjects.
3. In seven subjects, adipose tissue blood flow was measured and the measurements were continued for 6 h after eating a mixed meal. The pattern of amino acid metabolism across the adipose tissue was remarkably little disturbed after the meal, except for a short period of apparent uptake of alanine as the concentration of that amino acid rose.
4. The pattern of amino acid metabolism across adipose tissue was qualitatively similar to that across the forearm, although it differed quantitatively in that glutamate uptake was more prominent (compared with glutamine release) in the adipose tissue.
5. The rates of alanine and glutamine release observed suggest that adipose tissue may play a substantial role in the whole-body production of these amino acids.

Key words: adipose tissue, alanine, amino acids, forearm metabolism, glutamate, glutamine.

INTRODUCTION

Although adipose tissue was at one time regarded as a rather inert repository for excess energy, it is now recognized to have a dynamic pattern of metabolism, playing a major role in regulating the flow of lipid energy in the body on both a minute-to-minute and a longer term basis. It has never been clear, however, whether adipose tissue plays a quantitatively significant role in other aspects of metabolism in the body. Glucose uptake by adipose tissue is insulin-sensitive in vitro [1], but adipose tissue probably plays a very minor role in the whole-body disposal of a glucose load [2, 3]. Similarly, although adipocytes consistently produce lactate in vitro [2], the contribution of adipose tissue to the total amount of lactate released by peripheral tissues in vivo is probably small [3].

Adipose tissue has long been recognized to have a characteristic pattern of amino acid metabolism, but its role in whole-body amino acid metabolism is again not clear. Amino acids may act as substrates for lipogenesis [4, 5]. Branched-chain amino acids may be oxidized, and their nitrogen transferred to form other amino acids for release [6, 7]. Snell & Duff [6], for instance, showed release of alanine from rat epididymal fat pads, and suggested that this might make a significant contribution to whole-body alanine production. Tischler & Goldberg [7] have shown release of both alanine and glutamine by rat adipose tissue. In contrast, isolated rat adipocytes have been shown to utilize exogenous glutamine [8] at rates which, if extrapolated to the whole body, appear physiologically significant.

In man, alanine and glutamine are quantitatively the most important amino acids released by muscle both before and after ingestion of a protein or mixed meal or administration of glucose [9–11]. Glutamate, in contrast, is consistently taken up by muscle, where it may enter the citric acid cycle as 2-oxoglutarate or act as a precursor for glutamine synthesis. Whether similar exchanges occur in adipose tissue in vivo is unknown. We have therefore investigated the quantitative exchange of these three metabolically important amino acids across human adipose tissue in vivo, before and after ingestion of a mixed meal. The exchange was assessed by measurement of arteriovenous differences across the subcutaneous adipose depot together with measurement of blood flow. For comparison, the simultaneous flux of these amino acids was measured across the forearm.

Other measurements made in the same studies, together with the measurements of blood flow, are reported elsewhere [12].
METHODS

Seven normal subjects (four female, three male) were studied (age range 28-42 years, body mass index 19-29 kg/m²) after an overnight fast, having previously consumed their usual weight-maintaining diet. Their body fat content, estimated from skinfold thicknesses as described by Durnin & Womersley [13], was 10-19 kg; their muscle mass, estimated as 40% body weight, was 19-29 kg. (These values were only used for approximate whole-body extrapolations.) Cannulae were introduced, as described previously [3,12], into a vein draining a hand which was then warmed to provide arterialized blood, retrogradely into an antecubital vein draining forearm muscle, and into a vein draining the subcutaneous adipose tissue of the anterior abdominal wall. A cuff was inflated to 200 mmHg around the wrist to exclude hand blood flow before taking samples from the antecubital vein. Forearm blood flow was measured by strain-gauge plethysmography [14] and adipose tissue blood flow by clearance of 133Xe [15] immediately after taking each sample. An additional four normal subjects (one male, three female) aged 21-42 years, had arterialized blood samples and adipose venous blood samples only taken after overnight fast; blood flow was not measured.

After a rest period of at least 30 min, blood samples were taken from all three sites as near simultaneously as possible. Three sets of basal blood samples were taken; mean results from these are presented below. Then, between 0 and 20 min, the subjects ate a mixed meal as described previously [12]. The meal had an energy value of 3.1 MJ, of which 41% came from fat and 47% from carbohydrate. Further samples were taken (with respect to the time of starting the meal) at 30, 60, 90 and 120 min, then at hourly intervals until 6 h.

Blood samples (250 μl) were immediately deproteinized in 500 μl of sulphosalicylic acid (3.5%, w/v). (Blood and acid were weighed.) After centrifugation, the supernatant was frozen at -70°C, before estimation of alanine, glutamate and glutamine concentrations by enzymic methods which were adapted for a Cobas Bio centrifugal analyser (Roche Diagnostics, Welwyn Garden City, Herts, U.K.) [11].

Tissue uptake or release of amino acids (expressed as nmol min⁻¹ 100 ml⁻¹ tissue) was calculated as the product of arteriovenous difference (μmol/l) and blood flow (ml min⁻¹ 100 ml⁻¹ tissue). Areas under curves were calculated by using a trapezoidal method. Extrapolations to whole-body rates of production and utilization (which must be treated with caution as discussed further below) were based on the estimates of fat and muscle mass for each individual. (The amount of body fat as estimated from skinfold thicknesses is not necessarily equal to the amount of adipose tissue, but again these values were only used for approximate extrapolations.) All results are presented as means ± SEM for n = 7, unless otherwise stated.

RESULTS

Fasting state

In all 11 subjects studied, alanine and glutamine concentrations in the blood draining adipose tissue were higher than in arterialized blood, whereas the reverse was true for glutamate (P<0.001 for each amino acid by Sign test). Further analysis refers only to the seven subjects in whom blood flow was measured, and from whom antecubital samples were taken.

Concentrations of both alanine and glutamine were higher in forearm venous and adipose venous blood than in arterialized blood in all subjects (Table 1). For glutamate, the concentration was higher in arterialized blood than in venous blood from either site; concentrations in forearm venous and adipose venous blood were similar. The ratio of glutamine release to glutamate uptake was different for the two tissues. Mean values for the ratio were: adipose tissue, 2.7±1.0; forearm, 6.1±2.4 (P<0.05 by Wilcoxon’s signed-rank test).

Extrapolations to the whole-body (assuming adipose tissue and muscle masses all to behave like the depots studied here) showed that in the overnight-fasted state, the potential contribution of adipose tissue was not insignificant in comparison with that of muscle, particularly in the case of glutamate uptake (Table 1).

Responses to ingestion of a meal

After the meal, alanine concentrations in arterialized blood (Fig. 1) rose from a mean baseline value of 248±16 μmol/l to a peak of 414±25 μmol/l at 90 min

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Table 1. Amino acid concentrations and fluxes in seven normal subjects

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Forearm</th>
<th>Adipose</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>248±16</td>
<td>271±18</td>
<td>0.92</td>
</tr>
<tr>
<td>Glutamine</td>
<td>509±21</td>
<td>538±20</td>
<td>0.97</td>
</tr>
<tr>
<td>Glutamate</td>
<td>167±11</td>
<td>152±11</td>
<td>1.07</td>
</tr>
</tbody>
</table>

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After an overnight fast

Blood concn. (μmol/l)

- Arterialized
- Forearm venous
- Adipose venous

Tissue flux (nmol min⁻¹ 100 ml⁻¹ tissue)

- Forearm
- Adipose

Estimated whole-body tissue flux (μmol/min)

- Muscle
- Adipose

Post-prandial average tissue flux (nmol min⁻¹ 100 ml⁻¹ tissue)

- Forearm
- Adipose
Amino acid metabolism in adipose tissue

There was a transient switch to apparent alanine uptake in both forearm and adipose tissue, although this may represent an artefact of the non-steady state. For the last 4 h of the study, both tissues were again releasing alanine. The area under the curve for alanine release [nmol min⁻¹ 100 ml⁻¹ tissue × time (min)] was calculated from 0 min to 360 min, as this measure is less susceptible to non-steady state effects (Table 1). For both tissues, the areas were significantly greater than zero (P < 0.05 by Wilcoxon's signed-rank test) showing consistent release averaged over the whole of the post-prandial period. Averaged rates of production were very similar to those in the baseline period (Table 1).

Blood concentrations of both glutamine and glutamate (Fig. 1) were relatively steady after the meal. Again, in each case the areas under the curves (for release or uptake respectively, from 0 to 360 min) were consistently different from zero (P < 0.05 by Wilcoxon's signed-rank test). Rates of glutamine release were steady throughout for both tissues. There was a tendency (not significant) for glutamate uptake by both tissues to increase after the meal (e.g. the mean adipose tissue uptake rate at 60 min was 133 ± 60 nmol min⁻¹ 100 ml⁻¹ tissue).

DISCUSSION

Although there has been controversy, as outlined above, about the handling of glutamine by adipose tissue in vitro, these results show that adipose tissue in vivo is a consistent net exporter of both alanine and glutamine. It should be remembered that results obtained from measurement of arteriovenous differences reflect net exchange across a tissue, and the possibility of simultaneous uptake and release of amino acids cannot be excluded. Although the tissue studied by this technique includes a proportion of skin, the metabolic contribution of skin appears to be small [3]. The possible role of cells other than adipocytes in the exchanges we observed is considered below.

The metabolic pattern of the adipose tissue, in terms of these three amino acids, was qualitatively similar to that of the forearm, although the relative rates of glutamate uptake and of alanine or glutamine release were different. It is unlikely, for anatomical reasons as well as from the composition of the blood, that there is significant contamination of the adipose tissue venous blood by muscle drainage [3]. We therefore conclude that human subcutaneous adipose tissue in vivo is a net exporter of both alanine and glutamine, and a consumer of glutamate.

In whole-body terms, the contribution of adipose tissue appears to be significant. Extrapolations from individual adipose tissue or muscle beds to the whole body can only be taken as approximate because of regional differences in metabolism and blood flow (see references cited in [12]). These extrapolations, however, suggest that after an overnight fast adipose tissue contributes about one-third as much as muscle to whole-body alanine and glutamine production, and more than one-half as much as muscle to glutamate uptake. Recent measurements of muscle blood flow by the xenon technique suggest that plethysmography, as used in these studies, may overestimate forearm muscle blood flow (A. Kurphad & M. Elia, unpublished work). In this case, the role of adipose tissue
relative to that of muscle would be even greater than estimated here.

After the meal, although blood alanine concentrations rose, there was no indication that this reflected increased release from either muscle or adipose tissue. In fact there was a period of apparent alanine uptake by both tissues, as noted in other work [11]. The calculations based on areas under the flux–time curves for the post-prandial period showed average rates of release very similar to those during the baseline period. Although average rates of amino acid release and uptake across the tissues changed little after the meal, the role of adipose tissue relative to muscle in glutamate uptake became somewhat greater.

Since the nitrogen leaving adipose tissue in the form of alanine (1 mol per mol) plus glutamine (2 mol per mol) is greater than the nitrogen entering in the form of glutamate (1 mol per mol), it appears that there is another source of nitrogen; for example, the catabolism of branched-chain amino acids as shown in vitro [6, 7] or the catabolism of proteins. Similar arguments apply to the carbon skeletons of alanine and glutamine, which may be derived from other amino acids or from glucose.

Adipose tissue in vivo is not a pure preparation of adipocytes. The contribution of skin is inseparable, albeit small. Several other cell types in adipose tissue may be involved in amino acid exchange, including fibroblasts, vascular (endothelial) cells, macrophages, erythrocytes, and leucocytes. However, since several of these cell types, including skin, possess a high glutaminase activity, and/or have been shown to use glutamine avidly in vitro, it seems unlikely that they could be responsible at least for the glutamine production [16–18]. No production of alanine is seen on storage of blood for several minutes [19], so again it seems unlikely that the changes could be caused by the metabolic activity of blood cells during passage through the tissue.

In conclusion, we find human adipose tissue in vivo to have a pattern of amino acid metabolism which is qualitatively similar to that of skeletal muscle. Rates of alanine and glutamine release and of glutamate uptake by adipose tissue are not insignificant compared with those of skeletal muscle.

ACKNOWLEDGMENTS

We thank S. M. Humphreys and M. J. Kirk for technical assistance. The Sheikh Rashid Diabetes Unit is supported by the Oxford Diabetes Trust. S.W.C. held an MRC Training Fellowship.

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