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Amino acid and glucose uptake by rat brown adipose tissue

Effect of cold-exposure and acclimation

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The net uptake/release of glucose, lactate and amino acids from the bloodstream by the interscapular brown adipose tissue of control, cold-exposed and cold-acclimated rats was estimated by measurement of arterio-venous differences in their concentrations. In the control animals amino acids contributed little to the overall energetic needs of the tissue; glucose uptake was more than compensated by lactate efflux. Cold-exposure resulted in an enhancement of amino acid utilization and of glucose uptake, with high lactate efflux. There was a net glycine and proline efflux that partly compensated the positive nitrogen balance of the tissue; amino acids accounted for about one-third of the energy supplied by glucose to the tissue. Cold-acclimation resulted in a very high increase in glucose uptake, with a parallel decrease in lactate efflux and amino acid consumption. Branched-chain amino acids, however, were more actively utilized. This was related with a much higher alanine efflux, in addition to that of glycine and proline. It is suggested that most of the glucose used during cold-exposure is returned to the bloodstream as lactate under conditions of active lipid utilization, amino acids contributing their skeletons largely in anaplerotic pathways. On the other hand, cold-acclimation resulted in an important enhancement of glucose utilization, with lowered amino acid oxidation. Amino acids are thus used as metabolic substrates by the brown adipose tissue of rats under conditions of relatively scarce substrate availability, but mainly as anaplerotic substrates, in parallel to glucose. Cold-acclimation results in a shift of the main substrates used in thermogenesis from lipid to glucose, with a much lower need for amino acids.

INTRODUCTION

Brown adipose tissue is widely acknowledged as a main site for non-shivering thermogenesis in the mammal (Foster, 1984). This tissue is able to oxidize substrates for the generation of heat by means of selective uncoupling of ATP synthesis from the respiratory chain (Nicholls, 1979). In rats, cold-acclimation results in hypertrophy of the tissue masses and an increase in the blood flow through the tissue, as well as in higher mitochondrial protein content (Himms-Hagen, 1986). Cold-exposure or -acclimation dramatically increases the heat production of the tissue (Foster & Frydman, 1979), thus helping the animal to maintain its thermal homoeostasis by compensating for its heat loss to the environment.

The acute exposure to cold temperatures drives an otherwise unprepared organism to cope with a crippling situation by decreasing heat losses and, mainly, through increased thermogenesis. This is done by using most substrates available to fuel the heat-production pathways; as brown adipose tissue plays a key role in this process (Foster & Frydman, 1978), a large proportion of energetic substrates available would be oxidized by it. The chronic exposure or acclimation to low temperatures results in adaptive changes that prevent the excessive draining of valuable substrates for thermogenesis; the enlarged brown-adipose-tissue masses (Sundin & Cannon, 1980) have larger fat depots and are geared to use more energetic substrates obtained through increased food consumption (Portet, 1981).

Brown adipose tissue is known to metabolize lipoproteins via a very active lipoprotein lipase (Carneheim *et al.*, 1984). The tissue also uses glucose as substrate, as observed in studies *in vitro* (Czech *et al.*, 1974) and *in vivo* (Ferré *et al.*, 1986). Brown adipose tissue has a powerful glycolytic capability (Cooney & Newsholme, 1982) as well as significant activities of enzymes involved in amino acid metabolism (López-Soriano & Alemany, 1986). The influence of cold greatly alters the tissue amino acid pools and enzymes (López-Soriano & Alemany, 1987), thus suggesting that amino acids can be used as alternative substrates for thermogenesis.

We intended here to ascertain, using an approach involving arterio-venous differences *in vivo*, the actual uptake/release of amino acids and glucose by interscapular brown adipose tissue (IBAT), as a means to determine their contribution to the adaptation of the tissue to the cold. The utilization of glucose in significant proportions as thermogenic substrate has been controverted (Ma & Foster, 1986; Wilson *et al.*, 1987), and thus it was intended to evaluate the contribution of this substrate to the energetic budget of brown adipose tissue.

MATERIALS AND METHODS

Animals and experimental procedure

Male Wistar rats weighing initially 100–110 g were used. The rats were maintained in a light- (on from 08:00 to 20:00 h), temperature- (21–22 °C) and humi-

Abbreviation used: IBAT, interscapular brown adipose tissue.

dity- (75–85%) controlled animal room, in individual polypropylene-bottomed cages; wood shavings were used as bedding material. They were fed *ad libitum* with type A04 pellets (from Panlab, Barcelona, Spain) [composition: metabolizable carbohydrate, 59%; lipid, 3%; protein, 17%] and tap water. Cold-exposed rats were those left in a cold environment (4 °C) for 4 h; cold-acclimated rats were left for 30 days at 4 °C; controls were maintained under the basal conditions outlined. All rats were killed at the same age (8 weeks), when their weights were 278±7, 269±9 and 219±5 g respectively for controls, cold-exposed and cold-acclimated rats (means±S.E.M. for 12 animals).

Five rats of each group were anaesthetized intraperitoneally with sodium pentobarbital (50 mg/kg body wt.) and then used to determine the blood flow through the IBAT mass by using radioactive microspheres, by the procedure outlined by Foster & Frydman (1978) with the modifications introduced by Jones & Williamson (1984). ^{46}Sc-labelled microspheres (from New England Nuclear, Dreieich, Germany) were used. A polyethylene catheter (internal diameter 0.28 mm) was introduced into the femoral artery. Labelled microspheres (about 300000; approx. 7.5 μCi) were injected into the left ventricle of the heart by direct cardiac puncture over a 10 s period. A reference blood sample was drawn from the femoral artery at a rate of 0.6 ml/min from 5 s before until 1 min after completion of the injection. IBAT was immediately dissected, weighed and counted for radioactivity. Rates of blood flow were calculated as described by Jones & Williamson (1984).

Seven rats of each group were also anaesthetized intraperitoneally with sodium pentobarbital (50 mg/kg body wt.); with the rat in the prone position, the interscapular skin was dissected to expose the mass of IBAT, then the mass was moved slightly so as to expose the Sulzer vein, which was cut; the free-flowing venous blood was directly collected for up to 1 min in heparinized capillary tubes (Fernández *et al.*, 1987). Then the rat was turned to the supine position, and arterial blood was extracted from the exposed lower aorta with a heparinized plastic syringe. Immediately after death, the IBAT weight was estimated.

Sample processing

The packed cell volumes for each animal's venous and arterial blood were determined. The presence of trapped plasma was investigated by measuring the proportion of [U-^{14}C]sucrose added to samples of blood and then trapped in the packed cell pellet after centrifugation under conditions analogous to those used for the samples. A mean volume of trapped plasma of 1% of the packed-cell volume was found, and this value was used to correct the experimental values for packed-cell volume.

Samples of both plasma (immediately obtained by centrifugation of the blood samples) and blood were deproteinized with trichloroacetic acid (final concn. 50 g/l). The clear supernatants were used for measurement of individual amino acids, by using a Rank–Hilger amino acid autoanalyser with fluorimetric detection by the *o*-phthalaldialdehyde reaction (Lee & Drescher, 1978). The integrated peaks were corrected with internal norleucine standards, and the recovery and degree of reaction factors were estimated with standards interspersed between the samples. Blood and plasma glucose were measured by an enzymic method (Bergmeyer *et al.*, 1974). Plasma lactate was determined as described by Passonneau (1974).

The actual uptake of each amino acid by the tissue was calculated from the total blood content of the given amino acid per unit of blood volume in the arterial side minus that in the venous side. This value was corrected by the blood flow, taking into account the IBAT size. These calculations gave individual retention data for each amino acid (and glucose) of each rat. The final data were used for the calculation of the means and S.E.M. for each group. Statistical significance of the differences between groups and against zero were estimated by Student's *t* test.

RESULTS

Table 1 shows the IBAT weights and blood flows of controls, cold-exposed and cold-acclimated rats. Cold-exposure induced a significant decrease in IBAT weight, and cold-acclimation resulted in a very large increase in IBAT size. These differences were maintained even when the IBAT weight was corrected by the animal mass.

The blood flow across the IBAT practically tripled in cold-exposed and -acclimated rats compared with controls. When the data were corrected by the size of the IBAT, the differences versus controls were maintained, but cold-exposed rats then had more than twice the blood flow of controls; cold-acclimated animals had 5 times more blood across the IBAT per minute than did controls.

Table 2 shows the plasma and blood concentrations of amino acids, lactate and glucose found in the arterial and venous blood of controls, cold-exposed and cold-acclimated rats. The differences between blood and plasma concentrations of cold-influenced animals were

Table 1. IBAT weight and blood flow in control rats and in rats subjected to cold-exposure and cold-acclimation

Controls were maintained at 21–22 °C; cold-exposed rats were kept at 4 °C for 4 h; cold-acclimated rats were kept at 4 °C for 30 days. All values are means±S.E.M. for five different animals. Statistical significance of the differences versus controls: *$P < 0.05$.

Parameter and units	Control	Cold-exposed	Cold-acclimated
IBAT wt. (mg)	369±13	311±17*	620±38*
Blood flow			
(μl/s per g of IBAT)	6.33±3.00	20.5±1.8*	20.2±0.8*
(μl/s)	2.33±1.00	6.33±0.17*	12.5±1.2*

Table 2. Amino acid, lactate and glucose concentrations in the aortic and Sulzer-vein blood and plasma

The treatments applied to the experimental groups are the same as described in Table 1. All values are means ± S.E.M. for seven different animals, and are expressed in μmol/l, except for glucose, expressed in mmol/l; nd, not determined. Key: P, plasma; B, blood. Statistical significance ($P < 0.05$) of the differences versus controls*, and arterial versus venous†.

		Control		Cold-exposed		Cold-acclimated	
	Fraction	Arterial	Venous	Arterial	Venous	Arterial	Venous
Alanine	P	538±40	593±44	405±39*	395±37*	446±20 †	643±29
	B	610±58	635±40	565±54	544±31	599±57	728±81
Glutamate	P	117±15	86±15	96±9	93±13	74±15	78±6
	B	324±26	312±10	365±33	349±12*	299±22	266±23
Glutamine	P	1099±125	1067±106	923±134	930±21	797±104	834±87
	B	684±61	650±61	927±91*	883±106	747±56	666±60
Aspartate	P	32±2	36±4	29±2	30±1	27±4	27±1*
	B	38±5	47±3	43±4	40±2	32±3	35±4*
Asparagine	P	41±3	52±5	35±4	44±1	31±4	33±3*
	B	80±8	76±8	73±7	73±11	61±8	58±8
Serine	P	202±14	240±24	189±9	193±8	159±14	186±12
	B	276±24	288±14	302±38	293±24	265±23	256±36
Glycine	P	304±32	315±32	284±28	289±28	240±22	279±12
	B	326±20	336±13	374±37	378±25	306±14	332±19
Proline	P	188±24	155±17	158±24	165±28	171±17	180±15
	B	185±14	222±12	169±10	207±17	190±23	233±34
Leucine	P	195±27	183±25	209±13	198±14	172±15	161±13
	B	226±25	228±27	244±22	233±26	192±11	185±9
Isoleucine	P	111±15	104±13	123±8	114±7	96±9	89±7
	B	117±13	119±14	129±12	116±14	96±6	88±6
Valine	P	239±29	219±26	236±15	216±10	216±17	209±20
	B	241±28	233±27	247±18	243±24	223±10	210±13
Threonine	P	209±14	215±11	173±15	189±10	137±8	158±7*
	B	268±20	279±15	294±34	276±22	216±17	210±21*
Histidine	P	104±6	97±6	98±3	103±2	86±3	91±3
	B	106±7	107±7	119±8	116±7	98±6	96±3
Methionine	P	58±3	62±3	57±4	57±5	57±5	64±4
	B	nd	nd	nd	nd	nd	nd
Phenylalanine	P	76±6	75±4	83±5	80±4	59±5	59±4*
	B	92±7	96±7	102±6	102±9	72±2*	73±3*
Tyrosine	P	68±6	73±7	68±4	67±4	46±4	47±4*
	B	84±8	86±8	88±7	86±9	58±4*	61±5*
Tryptophan	P	89±4	70±6	70±4*	69±6	68±4	73±5
	B	24±2	26±2	29±1	32±3	26±2	28±2
Taurine	P	78±11 †	153±24	71±8 †	143±15	80±7 †	241±38
	B	246±22 †	330±14	285±20	258±18*	265±16	349±41
Lactate	P	1.15±0.22	1.82±0.32	1.33±0.32	1.90±0.63	2.62±0.41*	2.73±0.43*
Glucose	P	8.07±0.27	7.22±0.43	8.52±0.54	7.61±0.53	8.54±0.45 †	6.42±0.53
	B	5.82±0.31	5.67±0.36	5.67±0.24	5.18±0.34	5.84±0.35 †	4.56±0.26*

Table 3. Uptake/release of amino acids, glucose and lactate by the IBAT of control, cold-exposed and cold-acclimated rats

The values are means ± S.E.M. for each amino acid, glucose or lactate and group, calculated from the data of Tables 1 and 2, and are presented as pmol (amino acids) or nmol (glucose and lactate) taken up (+) or released (−)/s and referred to the whole IBAT mass. Statistical difference from zero: * $P < 0.05$.

	Control	Cold-exposed	Cold-acclimated
Alanine	−67±75	+69±251	−1684±529*
Glutamate	+19±54	+82±142	+377±177
Glutamine	+97±82	+306±266	+956±363*
Aspartate	−23±10	+15±18	−22±33
Asparagine	+13±27	+2±46	+47±76
Serine	−31±32	+40±147	+149±250
Glycine	−29±44	−61±122	−325±164
Proline	−89±16*	−234±90*	−531±248
Leucine	−7±18	+62±47	+64±86
Isoleucine	−7±12	+77±25	+94±38*
Valine	+16±14	+6±67	+141±119
Threonine	−26±30	+99±124	+83±23
Histidine	−3±5	+19±24	+17±80
Methionine	−1±2	+10±4	−22±10
Phenylalanine	−10±4	−4±34	−18±30
Tyrosine	−4±6	+10±28	−38±33
Tryptophan	−7±7	−24±20	−18±39
Glucose	+0.4±0.3	+3.1±0.4*	+15.0±1.1*
Cell fraction	−0.8±0.4	−0.2±0.2	−0.5±0.5
Plasma fraction	+1.2±0.2*	+3.3±0.6*	+15.5±1.8*
Lactate	−1.6±0.7*	−3.6±2.1	−1.4±5.8

Table 4. Amino acid energy and nitrogen balance of rat IBAT; effect of cold-exposure and cold-acclimation

These values have been calculated from the means presented in Tables 1–3. The ATP equivalence of amino acids and glucose taken up by the whole IBAT has been calculated by considering the standard degradative processes, and are to be taken as indicative values for the potential energy released through their complete oxidation (i.e. the numbers of mol of ATP per mol of substrate used for the calculations are: Ala 15, Gln 26, Glu 26, Asp 17, Asn 17, Ser 15, Gly 3, Pro 31, Leu 37, Ile 39, Val 33, lactate 17 and glucose 36). The data are presented as pmol taken up (+) or released (−) to the bloodstream/s by the whole IBAT of the animals studied. The amino acid nitrogen balance for these amino acids corresponds to the balance in pmol of amino acids (multiplied by a factor of 2 for Gln and Asn) taken up (+) or released (−) by the whole IBAT.

	Parameter	Control	Cold-exposed	Cold-acclimated
(1)	Ala+Glu+Gln+Asp+Asn+Ser energy balance (pmol of ATP/s)	+1129	+12099	+8987
	Amino acid N balance (pmol of N/s)	+119	+814	+824
(2)	Gly+Pro energy balance (pmol of ATP/s)	−2753	−7372	−17405
	Amino acid N balance (pmol of N/s)	−115	−292	−855
(3)	Leu+Ile+Val energy balance (pmol of ATP/s)	+4	+5419	+10790
	Amino acid N balance (pmol of N/s)	+2	+143	+300
(4)	Usable amino acids: [Σ(1+2+3)energy balance] (pmol of ATP/s)	−1626	+10135	+2372
	Amino acid N balance (pmol of N/s)	+6	+665	+269
(5)	Glucose and lactate			
	Glucose energy balance (pmol of ATP/s)	+12600	+92800	+551000
	Lactate energy balance (pmol of ATP/s)	−26500	−61300	−23500
	Net energy balance (pmol of ATP/s)	−13900	+31500	+527500
	Energy ratio [Σ(amino acids)/(glucose+lactate)]	(−)0.117	(+)0.322	(+)0.004

very similar to those of controls, with significant decreases in cold-exposed rats only for arterial-plasma alanine and tryptophan and venous-plasma alanine, as well as increases in arterial-blood and venous-blood glutamine. Cold-acclimation induced decreases in arterial-blood phenylalanine and tyrosine, and decreases in venous-blood aspartate, threonine, phenylalanine, tyrosine and glucose, as well as decreases in venous-plasma aspartate, asparagine, threonine, phenylalanine and tyrosine. Very little change was observed in the concentrations of amino acids and glucose between the arterial and venous blood or plasma, the only statistically significant differences observed being: a venous-plasma increase in alanine in cold-acclimated rats, a venous increase in plasma taurine in all groups tested (which was also significant for control blood), as well as blood and plasma glucose in

cold-acclimated rats, which decreased considerably in the venous side of the IBAT. Lactate concentrations in the plasma of cold-acclimated rats were higher than those of the other two groups. The arterial-blood packed-cell volume values were 41.6 ± 0.8, 42.1 ± 0.6 and 41.4 ± 0.5 for control, cold-exposed and cold-acclimated animals respectively.

Table 3 shows the actual IBAT arteriovenous balance for amino acids, glucose and lactate, calculated from the data in Tables 1 and 2. In controls, the proportion of most amino acids exchanged by the IBAT with the blood was actually small, with practically only glutamine being taken up in some sizeable proportion (with smaller amounts of glutamate, asparagine and valine) and alanine and proline being released (with smaller proportions of aspartate, serine, glycine and threonine). Cold-exposure resulted in a very variable net uptake of glutamine, alanine and glutamate, as well as of leucine, isoleucine and threonine, with only glycine and proline being released to the bloodstream. Cold-acclimated rats showed a different pattern, as now both glutamine and glutamate were taken up, with serine, leucine, isoleucine and valine as well as threonine also being taken up in lower proportions, whereas proline, glycine and especially alanine were liberated into the venous blood.

For glucose, the contribution of the blood-cell and plasma compartments was changed; the proportion of blood glucose carried in the blood cell compartment was a mean 19.0%, 13.0% and 14.3% respectively for controls, cold-exposed and cold-acclimated arterial blood, and 25.6%, 14.9% and 17.5% respectively for the venous side. The blood-cell compartment actually increased its glucose content in passing through the IBAT, the amount of glucose thus incorporated being very similar in all three groups. In controls, the plasma glucose fraction lost practically the same proportion of glucose that appeared in the cells. The net uptake of glucose (that taken up from the plasma minus that released in the cell fraction) increased with cold-exposure to very high values in cold-acclimation.

In all three groups studied there was a net, albeit small in controls and cold-acclimated rats, release of lactate into the venous blood. The highest proportion of lactate efflux was observed in the cold-exposed groups.

In Table 4 some of the results presented above are summarized as net balances across the IBAT in the three situations tested. Amino acids have been divided, in terms of their functionality and influx/efflux behaviour, into four groups: (1) non-essential (alanine, glutamate, glutamine, aspartate, asparagine and serine), (2) glycine and proline, (3) branched-chain amino acids (leucine, isoleucine and valine). The other essential amino acids (4) have not been taken in consideration, as their actual movement was very small, except for threonine, and there is no reason to suppose they can be either synthesized or metabolized by the IBAT. Taurine was not again taken into consideration, as it does not seem to be degraded by this tissue (or by any other known), and its synthesis could be obscured by the fact that no reliable data on cysteine were available. In Table 4, the 'amino acid nitrogen' balance across the IBAT was calculated from the mean data in Table 3. The possible equivalence of these amino acids, lactate and glucose in utilizable energy units, by means of their total metabolic oxidation through common known pathways, was calculated, and the results were expressed in pmol of ATP equivalents, to obtain a global idea of their possible energetic weight. The 'net energetic balance' for the above-cited groups of amino acids, lactate and glucose were then calculated and presented in Table 4.

DISCUSSION

The animal model used implies the use of anaesthesia, as in all studies *in vivo* carried out through the estimations of arteriovenous differences in IBAT in concentrations of metabolites (Foster & Frydman, 1978; Thurlby & Trayhurn, 1980; Rothwell & Stock, 1981). This approach implies an alteration of the non-anaesthetized situation, since the blood flow is altered by anaesthesia (James *et al.*, 1986); however, this is at present the only practical approach available to obtain an insight *in vivo* into the actual metabolite balance across IBAT.

The data presented for IBAT weight and blood flow agree with the known effects of cold on these parameters, as a short exposure to cold results in severe decrease in fat depots in the IBAT (López-Soriano & Alemany, 1987), with much increased blood flow and heat production (Desautels & Himms-Hagen, 1979). In cold-acclimation, on the other hand, IBAT function cannot rely on emergency measures such as the utilization of the tissue's own fat reserves, because alternative fuels are then used. The thermogenic capability of the tissue is then enhanced (Sundin & Cannon, 1980), as is its ability to take up energetic substrates, such as glucose (Ferré *et al.*, 1986), lipoproteins and fatty acids (Carneheim *et al.*, 1984).

The potentially high capability of IBAT to metabolize amino acids (López-Soriano & Alemany, 1986) does not seem to be functional under standard conditions. In general, amino acids were used to a considerable extent in a situation of actual emergency, as is cold-exposure.

The utilization of amino acids as energetic substrates poses the problem of nitrogen disposal. This is solved in the kidney by the liberation of NH_4^+ to the urine (Pitts, 1964) and by the liver by the production of urea (Aikawa *et al.*, 1973); other tissues must rely on the liberation of free NH_4^+, as in intestine (Windmueller & Spaeth, 1974), or the synthesis of glutamine as nitrogen vector towards the splanchnic bed (Ruderman, 1975). The IBAT seems to be a net glutamine user and alanine releaser, at least during cold-acclimation, thus being different from both liver and muscle, organs to which its enzymic machinery for amino acid metabolism has been related (López-Soriano & Alemany, 1986). Its ability to liberate alanine agrees with this same function described for white adipose tissue (Snell & Duff, 1977), thus behaving as a typical peripheral organ. However, the ability to take up glutamine stresses the similarities with intestine, an organ that takes up glutamine, releases alanine and produces free NH_4^+ (Brosnan *et al.*, 1983; Windmueller & Spaeth, 1974), which is later detoxified by the liver. No data are available as to the possible production of free NH_4^+ by the IBAT, but the net positive amino acid nitrogen balances observed seem to suggest the possible release of NH_4^+ by the IBAT, which contains active glutaminase (Cooney *et al.*, 1986) and adenylate deaminase (López-Soriano & Alemany, 1986). NH_4^+ is itself a powerful signal to control glycolysis (Abrahams & Younathan, 1971) and Na^+–K^+ ATPases (Robinson, 1970).

The amino acid nitrogen balance was very close to zero in the controls, increasing considerably in cold-

exposed rats at the expense of non-essential and branched-chain amino acids, with the counterpart of glycine and proline efflux. In cold-acclimated rats, the trends were maintained, but now a very large proportion of the nitrogen input was returned to the bloodstream in the form of alanine.

Glycine and proline presented a negative balance in all three groups of animals studied, whereas the other non-essential and branched-chain amino acids showed a positive balance. The net balance for all supposedly IBAT-usable amino acids was positive in cold-acclimated rats, but the combined value was much lower than that obtained for cold-exposed rats. Glycine is released by other tissues in post-prandial states (Yamamoto *et al.*, 1974) and is a good candidate for nitrogen disposal, as its energy content is very low, yielding on hepatic oxidation NH_4^+ and CO_2.

The proline efflux is not so easy to explain, as there are no reports linking its synthesis to the inter-organ transfer of amino acid energy or nitrogen. Proline has been often considered to be related only to the degradation of structural proteins, mainly collagen, despite it being a very good substrate for hepatic gluconeogenesis and energy metabolism (Hensgens *et al.*, 1978). The synthesis of proline is very closely related to the availability of glutamate (Johnson & Strecker, 1962). In the IBAT of rats subjected to cold stress, glutamate content increases considerably at the expense of other amino acids (López-Soriano & Alemany, 1987). Glutamine was actually taken up, despite a lowered ability to synthesize glutamine (López-Soriano & Alemany, 1987). Thus the synthesis of proline seems a plausible outlet for the excess glutamate accumulated. In this way, proline would become a clear candidate for nitrogen (and energy) exportation by the IBAT.

In cold-acclimated rats, the build-up of glutamate can help to drive the alanine transaminase reaction towards the synthesis of alanine, helped by the larger availability of pyruvate because of enhanced glycolysis (Cooney & Newsholme, 1982), in its turn fuelled by the large increase in glucose uptake (Ferré *et al.*, 1986). Thus the production of alanine can be a direct consequence of the increase both in glutamate (as nitrogen donor), as well as in C_3 fragments coming from the increased glucose availability in the tissue.

The energetic balance paralleled the data for amino acid nitrogen; however, for a very comparable nitrogen balance, cold-exposed rats extracted more energy from their non-essential amino acids than did the cold-acclimated ones. The latter group released much more energy in the form of alanine, proline and glycine than did the cold-exposed rats.

Branched-chain amino acids were actively used by the IBAT under conditions of cold stimulation. This utilization seems to be related to the activity of the branched-chain amino acid transaminase, which is higher in cold-adapted than in cold-exposed animals (López-Soriano & Alemany, 1987). The values for retention of these amino acids match closely this capability. The oxidation of branched-chain 2-oxo acids is related to fatty acid oxidation (Bender, 1985); thus a progressive adaptation of the IBAT to use fats as fuels, like that encountered in the adaptation to cold (Carneheim *et al.*, 1984), agrees with its more active utilization by the IBAT with cold-acclimation.

The data presented indicate that, in general, amino acids do not seem to be prime substrates for IBAT energy maintenance, even under cold stress; this is in agreement with the mainly plastic function of amino acids in the overall energetic economy of the mammal. Amino acids are used as fuels only when there is scarcity of energetic or gluconeogenic substrates, or when they are in excess as a consequence of dietary manipulation. The behaviour observed for the IBAT under cold-stimulation actually matches this general rule, as amino acids were used only when there was a need for emergency thermogenic substrates.

The utilization of glucose as an important thermogenic substrate by the IBAT has been postulated (Cooney & Newsholme, 1984; Young *et al.*, 1985). Other studies, using indirect approaches, suggest that glucose cannot be a significant substrate for thermogenesis (Wilson *et al.*, 1987).

The efflux of lactate found in IBAT under the three situations studied suggests that the glucose uptake by the tissue and high glycolytic capability (Cooney & Newsholme, 1982) result in a high availability of C_3 units, which were released in controls in a larger proportion than the glucose used. Under cold-exposure, this lactate release was even higher, probably owing to the inhibition of the glycerol phosphate shunt elicited by excess available cytoplasmic acyl-CoA (Himms-Hagen, 1986) evolved from the adrenergic stimulation of lipogenesis (Shimazu & Takahashi, 1980). Then a cytoplasmic accumulation of NADH would follow, driving the heart-type (Cannon & Nedergaard, 1982) IBAT lactate dehydrogenase to incorporate this excess reducing power into lactate. Then, a large proportion of the C_3 units generated by glycolysis could not be used for energy in the mitochondria, a situation helped by the large flux of acyl-derived acetyl-CoA; the excess C_3 units would then be released as lactate (about 70% of the glucose taken up). This can help to preserve glucose in situations of relative scarcity.

Cold-acclimation, with the IBAT now adapted to a smooth and maintained output of heat from the available substrates (mainly glucose in rats fed on a mainly starchy diet), resulted in a very large consumption of glucose, as both (it is postulated) energy substrate and lipogenic substrate (Cooney & Newsholme, 1984). The triacylglycerol synthesis would maintain lower proportions of acyl-CoA in the cytoplasm, thus preventing the inhibition of the glycerol phosphate shunt. The NADH and NADPH generated in the cytoplasmic handling of glucose would be consumed in lipogenesis, thus driving the reaction of lactate dehydrogenase towards pyruvate. These factors will result in opening widely the avenue for C_3 fragments derived from glucose to enter the mitochondria and to be oxidized as acetyl-CoA. The output of lactate was only 4.5% of the glucose taken up by the tissue. In cold-exposure, the relative availability of pyruvate and glutamate in the cytoplasm resulted in a large proportion of alanine being released by the tissue, equivalent to about 6% of the glucose taken up. This alanine efflux is not comparable with that of other peripheral tissues (Felig, 1973), in the sense that the C_3 skeleton originates probably from glucose, and not glycogen or other amino acids; however, it serves the role of nitrogen carrier towards the splanchnic bed, as in other tissues (Snell, 1979).

Under cold-exposure, the energetic utilization of amino acids became significant, as they contributed

about one-third of the net glucose-derived energy available for the tissue function, a considerable change from the 11.7% of controls, whereas during cold-exposure their contribution was about 0.4%. It has to be borne in mind, however, that their actual energetic significance would be even lower, as in the calculations the implication of lipids has not been considered.

In conclusion, the IBAT can use amino acids as fuel in situations of emergency; however, it can use much of the energy of the amino acids without being overloaded with excess nitrogen. In the adaptation to cold, as well as in controls, the utilization of amino acid energy is much lowered, with a nitrogen balance much more equilibrated. This is accomplished by releasing some amino acids, such as alanine and proline, but using increasing proportions of branched-chain amino acids, their nitrogen being practically the only one that poses some problem of elimination in cold-acclimation situations. Glucose is actively taken up by the IBAT; in cold-exposure, glucose would be returned to the bloodstream in a large proportion as lactate. In cold-acclimation, however, glucose will very probably be used as fuel for thermogenesis, in addition to providing C_3 skeletons for alanine efflux and lipogenesis.

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