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Hyperammonemia decreases body fat content in rat

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We have developed an animal model of hyperammonemia consisting of feeding rats a diet containing 20% (w/w) ammonium acetate. Ingestion of this diet markedly affects carcass composition, with a 46% reduction in lipid content. The ammonium diet alters levels of several key compounds involved in lipid metabolism. Long-chain acylcarnitine is increased in liver by approx. 60% while free carnitine and acetylcarnitine are unaffected. The hepatic content of acetyl-CoA increases by approx. 50%. The level of ketone bodies in blood increases by 32% but remains unchanged in liver. Our data indicate that hyperammonemia alters lipid metabolism and results in a significant decrease in body lipid content.

Hyperammonemia; Body composition; Lipid metabolism; Carnitine; Acetyl-CoA; (Ketone bodies)

1. INTRODUCTION

We have recently developed an animal model of hyperammonemia consisting of feeding rats a diet containing 20% (w/w) ammonium acetate [1–5]. Since rats fed on this diet gain less weight than those on a standard diet [1,5], it was of interest to ascertain body composition and organ weights in more detail. We show here that in hyperammonemic rats the lipid content is reduced to about one half, suggesting an alteration in lipid metabolism. We have therefore determined the levels of carnitine and derivatives thereof, which play a key role in the regulation of lipid metabolism [6]. We have also measured the hepatic content of acetyl-CoA, the final product of fatty acid oxidation, and the hepatic and blood levels of ketone bodies. Our results indicate that hyperammonemia indeed markedly alters lipid metabolism.

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2. MATERIALS AND METHODS

2.1. *Animals and diets*

Male Wistar rats (150–200 g) were fed the ammonium diet [1] for 6 weeks. Control animals were fed the standard diet ad libitum and another group was pair-fed. Rats were killed by decapitation and livers immediately removed and freeze-clamped.

2.2. *Analysis of carcass composition*

The method used was based on that reported in [7]. First, the water content of the carcass was measured by lyophilization; then, after hydrolysis with HCl, lipids were extracted with chloroform/methanol (2:1) and quantified using a Boehringer Mannheim kit. The nitrogen content of the defatted extract was determined by the Kjeldahl method.

2.3. *Metabolite determinations*

Carnitines were determined as in [8]. Acetyl-CoA was measured fluorimetrically as in [9]. Ketone bodies were determined as described [10], with NADH and NAD+ formed being quantified fluorimetrically.

3. RESULTS AND DISCUSSION

We have recently described an easily reproducible animal model of sustained hyperammonemia consisting of feeding rats an ammonium-containing diet [1–5]. It was previously shown that rats fed ammonia eat less and gain less weight than

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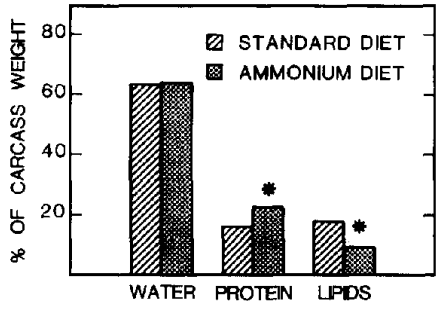

Fig.1. Effect of ingestion of ammonium diet on body composition. Groups of 4 rats were fed standard or ammonium diet ad libitum for 6 weeks. Initial body weight was 189 ± 19 g. Final carcass weights were 305 ± 18 and 235 ± 12 g for controls and ammonium-fed rats, respectively. Water, protein and lipids weights were 194 ± 9, 50 ± 2 and 54 ± 6 g for controls and 150 ± 4, 52 ± 4 and 23 ± 2 g for ammonium-fed rats. All differences were statistically significant ($p \leq 0.001$), except for water content, when expressed as percentage of carcass weight, and for total protein content.

animals on standard diet [5]. We have now studied in more detail the effect of this diet on body composition and organ weight. We show here (fig.1) that ingestion of the ammonium diet produces a dramatic change in body composition. The water content per g carcass remains the same as in controls. However, the lipid content per g wet wt is reduced to about one half and therefore protein content per g increases.

As shown in table 1, the relative weights of brain, liver and kidney increase significantly, while that of gastrocnemius muscle is unaltered, thus indicating, in agreement with the marked loss of lipids, that the decrease in body weight is due mainly to a striking loss of adipose tissue.

Table 1

Effect of ammonium ingestion on organ weights of rats

Organ	Weight (% of body)	
	Control diet	Ammonium diet
Brain	0.52 ± 0.07	0.61 ± 0.10[a]
Liver	3.31 ± 0.36	3.83 ± 0.56[a]
Kidney	0.60 ± 0.07	0.67 ± 0.05[a]
Gastrocnemius	0.58 ± 0.04	0.57 ± 0.04

[a] $p < 0.02$

Rats were fed standard or ammonium diet ad libitum for 6 weeks. Final body weights were 322 ± 28 and 260 ± 20 g for rats in control and ammonium groups, respectively. Values are means ± SD of 12 rats for each group

Table 2

Effect of ingestion of ammonium diet on carnitine and ketone bodies in blood

	Diet		
	Ad libitum	Pair-fed	Ammonium
Free carnitine	47 ± 5	49 ± 5	31 ± 5[a]
Acetoacetate	59 ± 13	51 ± 15	121 ± 13[a]
β-Hydroxybutyrate	91 ± 10	108 ± 9	77 ± 10[b]

[a] $p \leq 0.001$ vs both ad libitum and pair-fed controls
[b] $p \leq 0.05$ vs ad libitum and $p \leq 0.001$ vs pair-fed controls

Rats were fed ammonium diet for 6 weeks. Controls were fed standard diet ad libitum or pair-fed. Values (in μM) are means ± SD of triplicate samples from 5 rats for each group

We have therefore determined the levels of some of the main metabolites involved in lipid metabolism in rats fed standard or ammonium diet. To discern whether the effects are due to ammonia or a consequence of lower food intake, we have also used pair-fed controls. As is the case for other hyperammonemias, blood carnitine is decreased by 34% in rats fed the ammonium diet (table 2). In liver, while free carnitine and acetylcarnitine are not affected, the long-chain acylcarnitine is increased in hyperammonemic rats by 60–70% (table 3). This indicates increased transport of long-chain fatty acids to mitochondria for oxidation to produce acetyl-CoA. Indeed, the level of acetyl-CoA is also increased by 47% (table 3). These results suggest that in hyperammonemic rats increases in the transport of lipids into mitochondria and in the

Table 3

Effect of ingestion of ammonium diet on carnitine, acetyl-CoA, and ketone bodies in liver

	Diet		
	Ad libitum	Pair-fed	Ammonium
Free carnitine	110 ± 13	102 ± 12	109 ± 8
Acetylcarnitine	19 ± 2	19 ± 3	17 ± 2
Long-chain acylcarnitine	12 ± 1	11 ± 1	19 ± 2[a]
Acetyl-CoA	15 ± 1	14 ± 2	22 ± 1[a]
Acetoacetate	78 ± 19	82 ± 7	93 ± 17
β-Hydroxybutyrate	226 ± 32	263 ± 67	267 ± 29

[a] $p \leq 0.001$ vs both ad libitum and pair-fed controls

Rats were fed ammonium diet for 6 weeks. Controls were fed standard diet ad libitum or pair-fed. Metabolite determinations were carried out as described in section 2. Values (in nmol/g) are means ± SD of duplicate samples from 7 rats for each group

corresponding oxidation occur, leading to higher levels of acetyl-CoA. The greater degree of utilization of lipids is in agreement with the marked decrease in lipid content of the body demonstrated by fig.1. The excess of acetyl-CoA formed could be diverted to ketone bodies. The level of ketone bodies in the liver is not modified (table 3), while in blood shows an increase of 32% (table 2). This suggests that in hyperammonemic rats synthesis and export of ketone bodies by the liver increase. These ketone bodies can be used as an energy source by other tissues, e.g. brain and heart.

In conclusion, we have shown here that ammonium ingestion alters lipid metabolism and results in a significant decrease in body lipid content.

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