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Protein Metabolism in Weanling Rats with Hypothalamic Obesity[1] (39164)

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Weanling rats with lesions of the ventromedial hypothalamic nuclei exhibit normal weight gain and food intake (1). However, such rats (VMN) grow more slowly in length than normal (1), and their carcasses contain increased amounts of lipid and decreased amounts of protein (2, 3).

Other workers have shown that VMN rats conserve less nitrogen than normal (3), and we have demonstrated an increase in urea formation and alanine conversion to glucose in VMN rats (4).

These observations suggest that metabolism in VMN rats may be characterized by a decrease in amino acid incorporation into protein, an increase in protein breakdown, or a combination of the two. In order to investigate these possibilities, we have undertaken a study of amino acid metabolism in an attempt to delineate and define the changes in protein metabolism present in VMN rats.

Methods. Weanling male rats were obtained from Sprague–Dawley at 50–70 g body wt, individually caged, and given tap water and a Purina rat chow diet *ad libitum*. Five days after arrival, the rats were divided into lesioned (VMN) and sham-lesioned (control) groups, and lesions or sham lesions were produced by previously described methods (5). Two weeks later ^{14}C-labeled α-amino isobutyric acid without carrier (1 μCi/100 g rat wt) was injected intraperitoneally followed 30 min later by [^{3}H]inulin without carrier (3 μCi/100 g rat wt). Thirty minutes after the second injection, the rats were sacrificed. Tissue fragments weighing approximately 100 mg were dissolved in 1 ml of 1 *n* NaOH, and plasma (0.1 ml) was mixed with Soluene-100 for determination of radioactivity. ^{3}H and ^{14}C dpm were calculated by the separate addition of ^{3}H and ^{14}C external standards to permit calculation of the percentage efficiency of each isotope in each channel.

In a second group of experiments, [U-^{14}C]alanine, lysine, or leucine diluted with varying doses of carrier was injected intraperitoneally (5 μCi/100 g rat wt) into VMN or control rats 2 weeks after lesion placement. The rats were sacrificed by decapitation, and fragments of the liver, adipose tissue, and diaphragm were weighed and homogenized. The proteins of these homogenates and of the plasmas were precipitated with TCA, warmed to remove nucleic acids, and then extracted with chloroform–ether–ethanol to remove lipids. The proteins were then redissolved in NaOH for determination of protein content (6) and of radioactivity by scintillation counting.

In a third experiment [^{14}C]sodium carbonate (250 μCi) was injected without carrier intraperitoneally into rats which were then sacrificed 2 or 7 days later. Protein was isolated from plasma, thigh muscle, and decapitated, skinned eviscerated carcass and analyzed as described above. The $T_{1/2}$ for muscle protein synthesis and breakdown, respectively, were calculated from the logarithms of muscle protein specific activity and total radioactivity 2 and 7 days after injection (7).

Results. *[^{3}H]inulin and [^{14}C]AIB distribution (Table I).* The plasmas of the VMN rats contained over 50% greater concentrations of both inulin and AIB than did the plasmas of normal controls. The fat pad and liver content of both inulin and AIB was the same in the VMN as in the control rats,

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TABLE I. DISTRIBUTION OF [^{3}H]INULIN AND [^{14}C]AIB IN CONTROL AND VMN RATS AFTER INTRAPERITONEAL INJECTION.

	VMN (9)[a]	Control (4)	$P<$
[^{3}H]inulin			
Dpm/ml plasma	297,000 ± 26,000	188,000 ± 15,000	0.05
Dpm/g tissue			
Fat pad	540,000 ± 47,300	620,000 ± 68,700	NS[b]
Liver	226,000 ± 35,500	181,000 ± 55,100	NS
Diaphragm	651,000 ± 46,000	387,000 ± 46,200	0.01
Dpm per g tissue/dpm per ml plasma			
Fat pad	1.92 ± 0.23	3.27 ± 0.10	0.01
Liver	0.81 ± 0.14	0.98 ± 0.31	NS
Diaphragm	2.35 ± 0.33	2.14 ± 0.39	NS
[^{14}C]AIB			
Dpm/ml plasma	30,000 ± 1,500	18,000 ± 2,500	0.01
Dpm/g tissue			
Fat pad	16,500 ± 1,760	24,400 ± 1,640	NS
Liver	71,500 ± 13,300	35,400 ± 5,460	NS
Diaphragm	74,200 ± 5,260	45,600 ± 5,960	0.01
Dpm per mg tissue/dpm per ml plasma			
Fat pad	0.55 ± 0.06	1.31 ± 0.10	0.001
Liver	2.31 ± 0.31	1.93 ± 0.08	NS
Diaphragm	2.47 ± 0.13	2.50 ± 0.05	NS

[a] Number of animals per treatment group.
[b] Statistically not significant ($P > 0.05$).

whereas the VMN rat diaphragms contained more inulin and AIB than did the control rat diaphragms. In order to correct for differences in the vascular (and presumably extracellular) radioactivity in the tissues, the results were calculated and expressed as the ratio of tissue to plasma radioactivity. Analyzed in this fashion, the data indicate that the cells of VMN livers and diaphragms took up AIB normally whereas the fat pads of VMN rats took up only 40% as much AIB as did the control rat fat pads.

^{14}C-labeled amino acid incorporation into protein (Table II). When [^{14}C]alanine was injected intraperitoneally, its incorporation into the proteins of liver, fat pad, diaphragm, and plasma rose with the amount of alanine injected. The liver and plasma protein radioactivity after 2 hr was greater in the VMN than in the control rats at all concentrations of injected alanine. Neither diaphragm nor adipose tissue proteins showed consistent changes.

When [^{14}C]leucine was injected, incorporation into the proteins of plasma, liver, fat pad, and diaphragm was higher in the VMN rats than in the controls at all concentrations.

Compared to control rats, VMN rats incorporated more lysine into plasma proteins at all concentrations and into liver proteins at all but the highest dose level administered. The incorporation into the proteins of diaphragm and adipose tissue was in general the same in VMN and control rats.

[^{14}C]carbonate incorporation and retention in protein. Two days after the injection of $Na_2{}^{14}CO_3$, the radioactivity in plasma, muscle, and eviscerated carcass proteins was greater in VMN than in control rats. After 7 days, the differences persisted, but they were of much smaller magnitude and in the carcass were not statistically significant (Table III). These findings indicate that protein synthesis and breakdown were both accelerated in the VMN rats. In leg muscle the $T_{1/2}$ for protein synthesis and breakdown, respectively, were 5.3 and 6.2 days in VMN rats compared to 9.2 and 14.4 days, respectively, in the normals.

Discussion. In the present experiments, the incorporation of all amino acids tested

TABLE II. INCORPORATION OF ^{14}C-LABELED AMINO ACIDS INTO PROTEIN OF NORMAL AND VMN RATS SACRIFICED 2 HOURS AFTER INTRAPERITONEAL INJECTION OF 5 μCi/100 GRAMS.

μmoles injected per 100 g	10			30			100		
	VMN	Control	$P<$	VMN	Control	$P<$	VMN	Control	$P<$
Alanine									
No. of animals	4	3		5	4		4	4	
Liver	0.335 ± 0.017[a]	0.168 ± 0.011	0.001	1.03 ± 0.057	0.541 ± 0.036	0.001	3.07 ± 0.167	1.27 ± 0.049	0.001
Diaphragm	0.067 ± 0.012	0.115 ± 0.004	0.02	0.441 ± 0.064	0.260 ± 0.004	0.05	0.887 ± 0.036	0.739 ± 0.048	NS[b]
Fat pad	0.132 ± 0.005	0.819 ± 0.175	0.02	1.02 ± 0.056	1.60 ± 0.230	NS	2.87 ± 0.350	2.12 ± 0.048	NS
Plasma	0.525 ± 0.039	0.240 ± 0.028	0.01	1.34 ± 0.144	0.832 ± 0.092	0.05	4.15 ± 0.321	2.08 ± 0.240	0.01
Leucine									
No. of animals	6	2		5	3		6	4	
Liver	1.24 ± 0.06	0.11 ± 0.00	0.001	2.06 ± 0.08	0.25 ± 0.08	0.001	2.69 ± 0.11	1.31 ± 0.54	0.01
Diaphragm	0.57 ± 0.04	0.02 ± 0.05	0.001	1.00 ± 0.04	0.07 ± 0.02	0.001	1.16 ± 0.01	0.42 ± 0.17	0.001
Fat pad	1.04 ± 0.04	0.04 ± 0.00	0.001	1.51 ± 0.08	0.09 ± 0.03	0.001	1.98 ± 0.16	1.09 ± 0.52	NS
Plasma	2.06 ± 0.11	0.12 ± 0.01	0.001	2.56 ± 0.54	0.89 ± 0.59	NS	3.45 ± 0.16	1.81 ± 0.74	0.05
Lysine									
No. of animals	5	4		5	4		5	4	
Liver	0.58 ± 0.03[c]	0.33 ± 0.04	0.001	1.3 ± 0.10	0.93 ± 0.03	0.05	3.1 ± 0.10	2.9 ± 0.11	NS
Diaphragm	0.28 ± 0.04	0.21 ± 0.02	NS	0.58 ± 0.05	0.51 ± 0.02	NS	1.2 ± 0.04	1.3 ± 0.17	NS
Fat pad	0.37 ± 0.01	0.40 ± 0.05	NS	0.94 ± 0.07	1.0 ± 0.07	NS	1.9 ± 0.07	2.3 ± 0.14	0.02
Plasma	0.64 ± 0.06	0.35 ± 0.02	0.01	2.0 ± 0.27	1.2 ± 0.04	0.05	7.5 ± 0.51	4.5 ± 0.41	0.01

[a] Nmoles per mg protein. Mean ± SEM.
[b] Statistically not significant ($P > 0.05$).
[c] Lysine results expressed as pmoles per mg protein.

TABLE III. RADIOACTIVITY RECOVERED FROM PROTEIN AFTER INJECTION OF 250 μCi OF [^{14}C]Na_2CO_3 INTO VMN AND CONTROL RATS.

	VMN (dpm/mg protein)	Control (dpm/mg protein)	$P<$
Muscle			
2 days	29 ± 2[a](5)[b]	16 ± 2	0.01
7 days	15 ± 1	11 ± 2	0.05
Plasma			
2 days	1048 ± 122	681 ± 26	0.05
7 days	166 ± 13	97 ± 11	0.02
Carcass			
2 days	48 ± 3	31 ± 3	0.01
7 days	24 ± 1	19 ± 3	NS[c]
	(Dpm/mg tissue)	(Dpm/mg tissue)	
Muscle			
2 days	6.35 ± 0.49	4.40 ± 0.25	0.01
7 days	3.61 ± 0.30	3.46 ± 0.73	NS

[a] Mean ± SEM.
[b] Number of animals per group.
[c] Not statistically significant.

into liver and plasma proteins was greater in VMN than in control rats. The changes in the incorporation into plasma protein probably reflects albumin synthesis by the liver although no plasma protein fractionations were performed. The enhanced incorporation into liver proteins cannot be ascribed to increased transport of the radioactive amino acids into liver cells of the VMN rats since there was no difference in the hepatic uptake of [^{14}C]AIB in the VMN as compared to the control rats. Likewise, any possible differences in amino acid pool size cannot explain these results since the changes in incorporation were apparent over a very large range of amounts of injected amino acids. Therefore, our findings suggest an enhancement of hepatic protein synthesis per se.

With regard to diaphragm and adipose tissue proteins, only the incorporation of leucine was enhanced in the VMN rats, there being no differences between the VMN and control groups with reference to alanine or lysine incorporation. The increased leucine incorporation is probably not due to increased transport into the cells of diaphragm and fat pad since there was no observed increase in [^{14}C]AIB transport into these tissues in VMN rats. In addition, the persistence of these differences over a large dose range of injected leucine make it unlikely that the changes can be attributed to altered pool size in VMN rats. The failure to observe increased incorporation of alanine into muscle and fat pad protein in the VMN rats may be partially explained by postulating an enhanced hepatic utilization of alanine for protein synthesis and gluconeogenesis in these rats. This explanation, although certainly not verified by the present studies, is in accord with previous data showing an increased *in vivo* conversion of [^{14}C]alanine to liver glycogen and plasma glucose (4).

The above evidence for increased protein synthesis in VMN rats is further supported by the data from the [^{14}C]carbonate experiment, which clearly demonstrated that the VMN rat tissues contained increased radioactive proteins 2 days after injection of the [^{14}C]carbonate and that the $T_{1/2}$ for synthesis of the mixed muscle proteins was 5.3 days in the VMN rats and 9.2 days in the controls.

The experiment with [^{14}C]carbonate showed, in addition, that protein catabolism is accelerated in the muscle of VMN rats. We have previously demonstrated an increase in gluconeogenesis from amino acids in VMN rats based on studies of urea production and of alanine incorporation into liver glycogen and blood glucose (4). The present experiments indicate that accelerated protein breakdown in the muscles of VMN rats provides at least part of the amino acid supply necessary to support the increased gluconeogenesis observed in these rats.

Summary. Our findings indicate that protein synthesis is enhanced in weanling rats with hypothalamic obesity, that this enhancement is not dependent on increased amino acid transport into cells, and that muscle protein breakdown is also enhanced in these rats. The mechanisms of these changes are yet to be discovered. Finally, muscle protein has been demonstrated to

be a probable source of the amino acids required to support enhanced gluconeogenesis in these rats.

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