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A brain uptake study of [1-^{11}C]hexanoate in the mouse: The effect of hypoxia, starvation and substrate competition

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We evaluated the potential of sodium [1-^{11}C]hexanoate (^{11}C-HA) as a radiopharmaceutical with which to assess oxidative metabolism of the brain by PET. ^{11}C-HA, sodium [1-^{14}C]acetate and [^{3}H]deoxyglucose were simultaneously injected into mice under control, hypoxic and starving conditions. In the control, the brain uptake of ^{11}C was maximal at 3 min (% ID/g = 2.2–2.5), being twice as high as that of ^{14}C, followed by a gradual clearance. The time-radioactivity curve of ^{11}C was similar to that of ^{14}C. Hypoxia enhanced the brain uptake of ^{3}H, but not of either ^{11}C or ^{14}C. Starvation enhanced the brain uptake of ^{3}H and ^{11}C. The clearance rate of ^{11}C was not significantly affected by either condition. In the control brain at 3 min postinjection of HA, 65% of the total radioactivity was detected as labeled glutamate and glutamine, which was gradually decreased by 47% at 30 min. The brain to blood ratios of ^{11}C-HA at 3 min were significantly reduced by butyrate, hexanoate and octanoate loading but not by that with other monocarboxylic acids or ketone bodies.

Key words: [1-^{11}C]hexanoate, brain, oxidative metabolism, β-oxidation, PET

INTRODUCTION

POSITRON EMISSION TOMOGRAPHY (PET) enables the metabolic rate of glucose in the brain (CMRGlc) to be measured. The energy is produced via a non-oxidative as well as an oxidative process. Blomqvist et al. have found that the total CMRGlc in the human measured by PET is preferentially enhanced compared with oxidative CMRGlc when a finger movement task is performed.[1] In patients with Alzheimer's disease, the ratio of CMRGlc to the metabolic rate of oxygen is significantly smaller than that of normal individuals.[2] This uncoupling of glucose and oxygen consumption can be partially explained by the lower anaerobic glycolysis activity in the patients compared with normal individuals. An autoradiographic study has demonstrated that neuronal activation substantially increases total CMRGlc but modestly increases oxidative glucose metabolism in the rat brain.[3]

Although glucose is thought to be the only fuel in the brain, amino acids and ketone bodies are also used as fuel. These metabolic substrates enter the tricarboxylic acid cycle (TCA cycle) via acetyl-CoA. In the rat given [^{14}C]-glucose, the label in the brain is detected in the form of TCA cycle components and amino acids such as glutamate and glutamine.[4] The amino acid pool and the components of the TCA cycle are in equilibrium. We postulated that the radiolabeled compounds entering the TCA cycle could be used to assess the oxidative process and/or the pool of glutamate and glutamine in the brain (Fig. 1) independent of the total CMRGlc. Labeled acetate may be a theoretically suitable substrate, as supported by metabolic studies of exogenously injected acetate by mean of ^{13}C-NMR spectroscopy,[5,6] and autoradiographic studies with ^{14}C-acetate,[7,8] but because of the low penetration of acetate across the blood-brain barrier, ^{11}C-labeled acetate is not suitable for PET studies.

Compared with acetate, medium-chain fatty acids easily penetrate the blood-brain barrier. The brain uptake indices of C6, C8 and C10 fatty acids are 76, 94 and 88%, respectively, of that of ^{3}H-labeled H_2O, whereas that of acetate is only 14%.[9] We synthesized radiolabeled α-methyl fatty acids and found that the uptake of a C6 form was the highest in the mouse brain.[10,11] Although fatty acids are not an energy source for the brain, the brain

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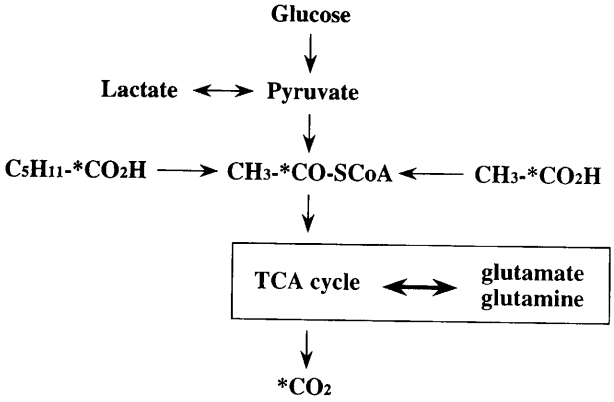

Fig. 1 A hypothetical model for the metabolism of labeled hexanoate and acetate in the brain. The asterisk represents the radio-label.

mitochondria can oxidize fatty acids.[12] The β-oxidation process of the long-chain fatty acids may play a role in regulating lipid metabolism in the brain.[13,14] On the other hand, immediately after injecting rat with radiolabeled butyrate and octanoate, their major metabolites in the brain were glutamate and glutamine.[15,16] This suggested that short- and medium-chain fatty acids are degraded by β-oxidation and that the generated acetyl-CoA enters the TCA cycle. In view of the high extraction and metabolic trapping of octanoate in the brain as forms of glutamate and glutamine, Rowly and Collins considered that labeled octanoate could be applied as a functional marker of brain activity.[17]

From these findings, we considered the following. A carboxyl-^{11}C-labeled medium-chain fatty acid is taken up by the brain and immediately oxidized by β-oxidation. The generated [1-^{11}C]acetyl-CoA enters the TCA cycle, so that the clearance of the ^{11}C measured by PET probably reflects the oxidative process and/or the amino acid pool in the brain. In a preliminary mouse study, we confirmed that the brain uptake of sodium [1-^{11}C]hexanoate (^{11}C-HA) was twice as high as that of [1-^{14}C]acetate (^{14}C-AA) and that the clearance rates of both the ^{11}C and ^{14}C from the brain were lower than those from the heart.[18]

In this study, we examined the uptake of ^{11}C-HA in the mouse brain under control, hypoxic and starvation conditions, then compared the results with those of ^{14}C-AA and [^{3}H]deoxyglucose (^{3}H-DG). According to published reports, anaerobic glycolysis is enhanced in rats exposed to hypoxia (10% oxygen concentration).[19] This condition reveals whether or not the clearance rate of the ^{11}C-HA in the brain is dependent on the oxidative metabolism. During starvation, the major fuel source in the brain is shifted from glucose to ketone bodies.[20,24] A change in substrate demand could affect the metabolism of the ^{11}C-HA. Finally, the transport system of the ^{11}C-HA across the blood-brain barrier was investigated by competition with short- and medium-chain fatty acids and other monocarboxylic acids including ketone bodies.

MATERIALS AND METHODS

Radiochemicals

Sodium [1-^{14}C]acetate (^{14}C-AA, specific activity of 2.11 GBq/mmol) and ^{3}H-deoxy-D-glucose (^{3}H-DG, specific activity of 392 GBq/mmol) were purchased from Amersham International plc (England) and sodium [1-^{14}C]hexanoate (^{14}C-HA, specific activity of 2.04 GBq/mmol) was from American Radiolabeled Chemicals Inc. (St. Louis, MO). Sodium [1-^{11}C]hexanoate (^{11}C-HA) was prepared as described.[18,25]

Biodistribution studies

Three groups of eight-week-old male ddY mice were studied. The first group of mice were allowed free access to food and water (control). The second group were given the same diet but exposed to 10% O_2/N_2 for two hours before injection of the tracers.[18] The tissue distribution study described below was performed under 10% O_2/N_2 conditions (hypoxia). The third group were given only water for 3 days before the experiment (starvation), in which the mice lost 17% of the body weight compared with the controls.

A mixture of ^{11}C-HA (4.0 MBq), ^{3}H-DG (1.5 kBq) and ^{14}C-AA (1.5 kBq) was intravenously injected into each group of mice. They were killed by cervical dislocation at 1, 3, 5, 10, 15, 30 and 60 min post injection. Blood was sampled by heart puncture with a heparinized syringe, and centrifuged to obtain plasma. The brain and other organs were dissected and divided into two portions. Cerebral cortex (about 50 to 100 mg) and 20 μl of plasma were solubilized with Soluene-350 (Packard) and ^{3}H and ^{14}C were measured in a liquid scintillation counter after the ^{11}C radioactivity decayed. The rest of the tissues and plasma were counted for ^{11}C in a gamma counter and weighed. The tissue uptake of radioactivity was expressed as the % injected dose per g of tissue.

Another set of control mice were intravenously given ^{11}C-HA (4.0 MBq) together with carrier HA (1 mmol/kg body weight), and the mice were killed at the times indicated above. A mixture of ^{11}C-HA (4.0 MBq) and one of the following monocarboxylic compounds (1 mmol/kg body weight): sodium acetate, propionate, butyrate, hexanoate, octanoate, pyruvate, lactate, acetoacetate and β-hydroxybutyrate, was injected into the control mice, and they were killed 3 min later. The tissue distribution of the radioactivity was measured as described above.

Metabolism

HA metabolites in brain tissue were analyzed in control mice given a mixture of ^{11}C-HA (4.0 MBq) and ^{14}C-HA (370 kBq). At 3, 15 and 30 min after the injection, the brain was removed and divided into the two hemispheres. Part of the brain was weighed and ^{11}C was counted. The other was weighed and homogenized in 0.2 M $HClO_4$ before counting ^{11}C. The difference between the two

Table 1 Concentrations of glucose and nonesterified free fatty acids in the plasma of three groups of mice

	Glucose (μmol/ml)	NEFA (μmol Eq/ml)
Control	12.14 ± 0.86 (14)	1.45 ± 0.40 (28)
Anoxia	11.23 ± 1.34 (28)	1.42 ± 0.41 (28)
Three-day fasting	6.03 ± 0.86 (14)*	1.94 ± 0.55 (14)*

NEFA: nonesterified free fatty acids
Data represent means ± S.D. (number of mice)
*$p < 0.001$ (Student's t-test, compared to control)

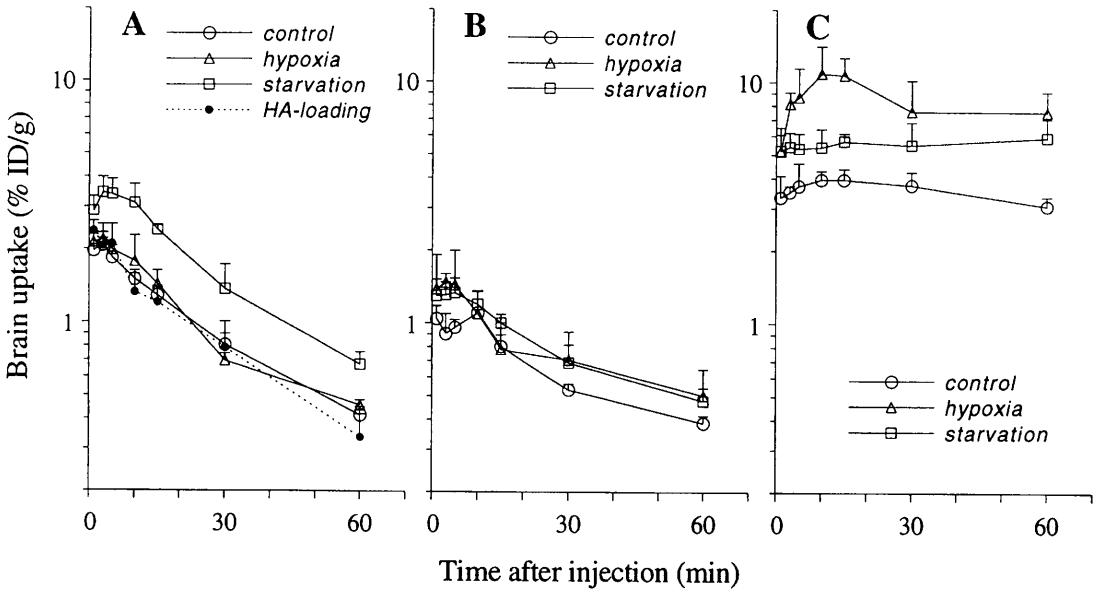

Fig. 2 Time-radioactivity curves of the brain after an i.v. injection of sodium [1-^{11}C]hexanoate (A), sodium [1-^{14}C]acetate (B) and [^{3}H]deoxyglucose (C) into three groups of mice, the control (open circles), hypoxic (open triangles) and starving (open squares) groups. Solid circles in the left figure represent the brain uptake of sodium [1-^{11}C]hexanoate with carrier (1 mmol/kg). The level of the radioactivity is expressed as the % injected dose/g tissue.

%ID/g values was assessed as the ^{11}C-CO_2/bicarbonate fraction in the brain tissue. The labeled metabolites, HA, glutamate and glutamine were analyzed by monitoring the ^{14}C radioactivity. The homogenate was centrifuged at 6,000 *g* for 2 min at 2°C. The supernatant was analyzed by HPLC. ^{14}C-HA was analyzed on an Aminex Fermentation Monitoring column (Bio-Lad) as described,[17,25] and ^{14}C-glutamate and ^{14}C-glutamine were measured on a YMC pack ODS-AQ (YMC Co., Ltd.) by the method of Kuge et al.[16]

Glucose and non-esterified fatty acid concentration

The concentrations of glucose and non-esterified fatty acids (NEFA) in the plasma were measured in a Clinical Chemistry Analyzer (CL-760, Shimadzu) and with the NEFA C-test Wako kit (acyl-CoA synthetase and acyl-CoA oxidase, Wako Chemical Industries Ltd., Tokyo), respectively.

RESULTS

Table 1 summarizes the concentrations of glucose and NEFA in the plasma of each group of mice. Starvation significantly decreased the concentration of glucose to 50% of the control, and increased that of NEFA to 134% of the control. Hypoxic conditions did not affect the concentrations of glucose and NEFA.

Figure 2 shows the time-radioactivity curves of the three tracers in the brain under each experimental condition. In the control, the level of ^{11}C-radioactivity increased during the first 3 min after the injection, and then exponentially decreased. The hypoxia did not affect the level of ^{11}C in the brain. Starvation enhanced the brain uptake and the maximal uptake seemed to shift to slightly later, and was accompanied by the delayed clearance of ^{11}C in the plasma. Under this condition the level of ^{11}C decreased exponentially after 10 min. HA carrier loading decreased the level of the ^{11}C immediately after the injection (Fig. 2). No significant difference was evident in the exponential clearance rates of ^{11}C under these conditions. Although

Table 2 Labeled metabolites in the brain

	Percentages		
	3 min	15 min	30 min
^{11}C-CO_2/bicarbonate	8.7%	ND	ND
^{11}C-Hexanoate	< 0.1%	ND	ND
^{14}C-Glutamate	37.5 ± 9.9	27.2 ± 7.0	25.9 ± 9.1
^{14}C-Glutamine	27.5 ± 1.4	23.2 ± 10.0	21.2 ± 7.8

Data represent means ± S.D. (n = 4), ND: not detected

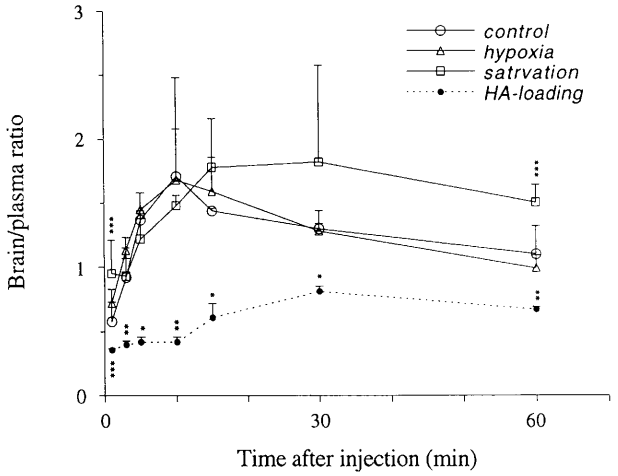

Fig. 3 Brain to plasma radioactivity ratios after an i.v. injection of sodium [1-^{11}C]hexanoate into four groups of mice: the control (open circles), hypoxic (open triangles), starving (open squares) and carrier hexanoate (1 mmol/kg) loaded (solid circles) groups.

the brain uptake of the ^{14}C-AA was half that of the ^{11}C-HA, the time-radioactivity curves of ^{14}C were similar to those of ^{11}C. No significant difference was found in the three time-radioactivity curves of ^{14}C. The brain uptake of ^{3}H-DG was enhanced both by hypoxia and starvation, probably because of anaerobic glycolysis[19] and the lower concentration of plasma glucose.

The brain uptake of ^{11}C-HA was evaluated from the radioactivity ratios of the brain to the plasma (Fig. 3). The ratios increased for the first 10 min, then slowly decreased both under the control and hypoxic conditions. Under starvation, the ratio at 1 min after injection was significantly larger than that for the control, and increased for 15 min, then remained constant. HA carrier loading significantly reduced the ratios compared with the control during the investigated periods.

The labeled metabolites of HA in the brain tissue of the control mice are summarized in Table 2. At 3 min after injecting of the tracers, ^{14}C-HA was scarcely detectable (< 0.1% of total ^{14}C). The major metabolites were glutamate and glutamine, the percentages of which gradually decreased. The ratio of labeled CO_2/bicarbonate was 9% at 3 min but this became undetectable.

Tables 3 and 4 represent the effect of monocarboxylic acids on the initial brain uptake of ^{11}C-HA. With the co-administration of octanoate, the brain uptake of ^{11}C-HA significantly decreased at 3 min after injection. However, because the levels of ^{11}C in the blood increased, the brain to blood ratios significantly decreased upon the co-injection of C4, C6 and C8 fatty acids. The effects of hexanoate and octanoate were comparable. In the heart, liver and muscle, the levels of ^{11}C increased with the increase in ^{11}C-levels in the blood. Only the ratios of liver to blood decreased upon the co-administration of hexanoate and octanoate. On the other hand, pyruvate, lactate or ketone bodies did not affect the brain uptake of ^{11}C-HA or its distribution in other tissues.

DISCUSSION

Glucose is the only metabolic fuel in the brain, but under extreme circumstances such as starvation, the major fuel source is shifted from glucose to the ketone bodies, that is, acetoacetate, β-hydroxybutyrate and acetone.[20–24] In the normal brain, our findings[10,11,18] and those of others[15,16] suggest that medium-chain fatty acids are degraded by β-oxidation followed by the TCA cycle. In this study, we examined the brain uptake of ^{11}C-HA in mice under hypoxia and starvation.

Under hypoxic conditions (10% oxygen), anaerobic glycolysis was enhanced, as shown by the brain uptake of ^{3}H-DG as in the rat,[19] but there was no significant difference between the control and hypoxic groups in the initial uptake or the clearance rates of ^{11}C-HA

After 3 days of starvation, the metabolism of ^{11}C-HA in the whole body was altered in term of delayed blood clearance and enhanced brain uptake. The myocardial uptake was also enhanced (data not shown) as described.[18] Based on the brain-to-blood ratios (Fig. 3), the initial brain uptake of the ^{11}C-HA was significantly enhanced immediately after injection. Although the clearance rate of the ^{11}C-HA was not affected compared with the control (Fig. 2), the two time-ratio curves were different, suggesting a different type of ^{11}C-HA metabolism in the brain tissue.

Immediately after the injection of labeled HA, the unchanged form of the HA in the brain was scarcely detected, and the major metabolites were glutamate and glutamine, as is true of labeled octanoate.[15,16] A ^{13}C-NMR

Table 3 Effect of sodium monocarboxylates on the distribution of radioactivity at 3 min after i.v. injection of [1-^{11}C]hexanoate into mice

	% Injected dose/g tissue Tissue/blood ratio					
	Control	Acetate	Propionate	Butyrate	Hexanoate	Octanoate
Blood	2.32 ± 0.32	2.55 ± 0.09	2.88 ± 0.30***	3.59 ± 0.29*	5.37 ± 0.60*	4.23 ± 0.66*
Brain	2.51 ± 0.31	2.25 ± 0.16	2.70 ± 0.26	2.42 ± 0.07	2.24 ± 0.27	1.85 ± 0.21**
	1.09 ± 0.14	0.88 ± 0.09	0.94 ± 0.10	0.68 ± 0.04*	0.44 ± 0.02*	0.44 ± 0.07*
Heart	2.18 ± 0.29	2.41 ± 0.15	2.31 ± 0.17	4.12 ± 0.59*	4.97 ± 0.47*	4.13 ± 0.62*
	0.95 ± 0.13	0.94 ± 0.05	0.80 ± 0.04	1.15 ± 0.15	0.97 ± 0.06	0.99 ± 0.18
Liver	3.76 ± 0.63	4.12 ± 0.26	5.12 ± 0.30**	5.29 ± 0.60*	6.16 ± 0.51*	5.63 ± 0.44**
	1.62 ± 0.08	1.62 ± 0.13	1.79 ± 0.13	1.48 ± 0.16	1.16 ± 0.11*	1.35 ± 0.22***
Muscle	0.87 ± 0.08	1.05 ± 0.15	0.94 ± 0.04	1.29 ± 0.07*	1.89 ± 0.23*	1.60 ± 0.16*
	0.38 ± 0.04	0.41 ± 0.04	0.33 ± 0.02	0.42 ± 0.02	0.39 ± 0.03	0.38 ± 0.03

Co-injected dose of each substrate was 1 mmol/kg body weight.
Data represent means ± S.D. (n = 4)
*p < 0.001, **p < 0.01, ***p < 0.05 (Student's t-test, compared with the control)

Table 4 Effect of pyruvate, lactate and ketone bodies on the distribution of radioactivity at 3 min after i.v. injection of [1-^{11}C]hexanoate into mice

	% Injected dose/g tissue Tissue/blood ratio				
	Control	Pyruvate	Lactate	Acetoacetate	β-Hydroxybutyrate
Blood	1.93 ± 0.09	2.21 ± 0.25	2.11 ± 0.35	2.21 ± 0.13**	2.29 ± 0.46
Brain	2.25 ± 0.34	2.37 ± 0.13	2.32 ± 0.22	2.56 ± 0.14	2.62 ± 0.47
	1.17 ± 0.14	1.08 ± 0.16	1.13 ± 0.12	1.16 ± 0.04	1.15 ± 0.07
Heart	1.94 ± 0.10	2.26 ± 0.16*	2.35 ± 0.23***	2.88 ± 0.36**	2.60 ± 0.55***
	1.03 ± 0.07	1.05 ± 0.10	1.13 ± 0.11	1.18 ± 0.16	1.13 ± 0.12
Liver	3.46 ± 0.37	3.72 ± 0.40	4.34 ± 0.44***	3.47 ± 0.28	3.66 ± 0.48
	1.80 ± 0.21	1.70 ± 0.29	2.10 ± 0.40	1.57 ± 0.10	1.61 ± 0.13
Muscle	0.81 ± 0.05	0.98 ± 0.20	0.97 ± 0.12	1.17 ± 0.12*	0.99 ± 0.13***
	0.42 ± 0.03	0.45 ± 0.12	0.47 ± 0.05	0.53 ± 0.04	0.44 ± 0.05

Co-injected dose of each substrates was 1 mmol/kg body weight.
Data represent means ± S.D. (n = 4)
*p < 0.001, **p < 0.01, ***p < 0.05 (Student's t-test, compared with the control)

study also showed that the ^{13}C-label of the hexanoate is incorporated into glutamate in the heart.[26] These results mean that the ^{11}C-HA is metabolized by β-oxidation to ^{11}C-acetyl-CoA, some of which is used for *de novo* synthesis of glutamate, glutamine and other compounds.[5,6] Since the components in the TCA cycle and these amino acids are in equilibrium,[4] gradual clearance of the ^{11}C from the brain after the initial 3 to 5 min accumulation reflects the oxidative degradation of ^{11}C-HA via the TCA cycle. The clearance rate of the radioactivity may be determined both by the rate of the TCA cycle and by the size of the amino acid pool.

Oldendorf has suggested the presence of a transport system for the short-chain monocarboxylic acids in the brain.[9] This study showed that immediately after injection of ^{11}C-HA, the brain to blood radioactivity ratios were significantly decreased by a co-injection of butyrate, hexanoate and octanoate but not by that of other monocarboxylic acids including ketone bodies, although the brain uptake of ^{11}C-HA was significantly reduced only by the octanoate. Carrier-mediated transport of ^{11}C-HA across the blood-brain barrier is suggested, but further investigation is required to define the transport system of the medium-chain fatty acids.

The results of this study suggest the β-oxidation of the medium-chain fatty acid ^{11}C-HA in the mouse brain and that the initial uptake of ^{11}C-HA could be a good marker of a substrate demand shift from glucose to non-glucose compounds. Nevertheless, significant changes have not been demonstrated in the clearance rate of ^{11}C-HA under hypoxia or starvation. Probably the two conditions applied were not severe enough to cause changes in oxidative metabolism detectable by the clearance rate, and/or the extremely large amino acid pool moderated the clearance rate.

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