Effect of Cold Acclimation on Brown Adipose Tissue Fatty Acid Synthesis in Rats Adapted to a High-Protein, Carbohydrate-Free Diet

M.A.F. Moura, N.H. Kawashita,. S.M.R.C. Brito, M.N. Brito, I.C. Kettelhut, and R.H. Migliorini

The effect of cold acclimation on brown adipose tissue (BAT) fatty acid synthesis was investigated in rats adapted to a high-protein, carbohydrate-free diet. At an ambient temperature (25°C), rates of fatty acid synthesis in BAT from rats adapted to the high-protein diet were reduced to 27% of rats fed the balanced diet and increased markedly after cold acclimation (10 days at 4°C), although the increase was smaller than in control rats. BAT weight increase induced by cold acclimation was smaller in rats fed the high-protein diet (30%) than in controls (100%). When expressed per whole tissue, maximal activities of BAT glucose-6-phosphate dehydrogenase, malic enzyme, adenosine triphosphate (ATP)-citrate lyase, and acetyl-coenzyme A carboxylase were markedly reduced in high-protein diet-adapted rats at 25°C and increased after cold acclimation in BAT from the 2 groups. However, when expressed per milligram protein, only acetyl-coenzyme A carboxylase showed an increase in both controls and in rats fed the high-protein diet. G6P-dehydrogenase, malic enzyme, and ATP-citrate lyase increased (per milligram protein) only in rats adapted to the high-protein diet and actually decreased in BAT from cold-acclimated control rats. Initial (before activation) pyruvate dehydrogenase (PDH) complex activity was lower in BAT from rats fed the high-protein diet at 25°C and increased in cold-acclimated rats from the 2 groups. Circulating levels of insulin decreased in the 2 groups after cold acclimation. The data suggest that the cold acclimation-induced increase in BAT lipogenesis in rats adapted to the high-protein diet was due to a restoration of sympathetic activity, which induced both BAT hyperplasia and activation of adipocyte free fatty acid (FFA) synthesis, with an important participation of acetyl-coenzyme A carboxylase and pyruvate dehydrogenase.

ALTHOUGH THE MAIN function of brown adipose tissue (BAT) is to generate heat for thermal protection during cold exposure (nonshivering thermogenesis), it is now well established, at least in small rodents, that BAT also has an important role in adaptive adjustments of overall body energy expenditure, not only to the level of energy intake, but also to the macronutrient composition of the diet (diet-induced thermogenesis).[1,2] We have previously shown[3] that rats adapted to a high-protein, carbohydrate-free diet have a reduced thermogenic capacity, as evidenced by a reduced response of interscapular BAT (IBAT) temperature to norepinephrine infusion and by decreases in IBAT weight, mitochondrial protein, cytochrome oxidase activity, and mitochondrial binding of guanosine diphosphate. These signs of reduced BAT thermogenesis in rats adapted to the high-protein diet were accompanied by a marked reduction in fatty acid synthesis, estimated in vivo with ^3H_2O.[3,4] In agreement with this finding, we have recently shown[4] that the activities of 4 enzymes associated with lipogenesis: glucose-6-phosphate dehydrogenase, malic enzyme, adenosine triphosphate (ATP)-citrate lyase, and acetyl-coenzyme A carboxylase are markedly decreased in IBAT from rats fed the high-protein diet. As previously discussed,[3] the marked reduction in BAT weight and functional state in these rats should be attributed to the composition of their diet and not to a reduced energy intake. Indeed, the difference in energy density between the purified high-protein and control diets was less than 10%, and the rate of body weight gain did not differ in the 2 experimental groups.[3]

It is well known that the activation of BAT thermogenesis induced in normally fed animals by cold exposure is accompanied by increased rates of tissue lipogenesis.[1] The purpose of the present study was to find out if the cold-induced activation of BAT fatty acid synthesis is affected in situations, such as that of rats adapted to the high-protein diet, in which a decreased BAT thermogenic capacity is accompanied by a marked reduction in fatty acid synthesis and in the activities of lipogenic enzymes. To this end, rates of fatty acid synthesis were determined in BAT from rats fed the high-protein or control diet, cold-acclimated (4°C) or kept at ambient temperature. The activities of IBAT G6P-dehydrogenase, malic enzyme, ATP-citrate lyase, acetyl-coenzyme A carboxylase, and of the mitochondrial pyruvate dehydrogenase (PDH) complex, not examined in our previous experiments with rats adapted to the high-protein diet, were also determined.

MATERIAL AND METHODS

Animals and Treatment

Two types of purified diets, previously described in detail,[3] were used in this study. One of the diets (high protein) contained 70% casein, no carbohydrate, and 8% corn oil, while the other (control) contained 17% casein, 66% carbohydrate, and 8% corn oil. The 2 diets were approximately isocaloric and contained equal amounts of vitamins and minerals. For cold acclimation, male Wistar rats weighing 90 to 110 g were initially housed, 2 per cage, in suspended, wire-bottom cages in a room kept at 25°C with a 12:12-hour light-dark cycle and were adapted to either the high-protein or the control diet, with water ad libitum, for a period of 12 days. After this period, the animals were housed individually in a cold room (4°C), with an identical illumination cycle, for another 10 days, with free access to the same diet of the initial period. Because rates of body weight gain decreased somewhat during the period of cold exposure and to obtain animals of similar body

From the Department of Biochemistry, School of Medicine, University of São Paulo, São Paulo, Brazil.

Submitted February 27, 2001; accepted April 26, 2001.

Supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP No. 97/0974-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq No. 510923/93-3).

Address reprint requests to R.H. Migliorini, MD, Department of Biochemistry, School of Medicine, 14049-900 Ribeirão Preto, Sao Paulo, Brazil.

0026-0495/01/5012-0035$35.00/0

doi:10.1053/meta.2001.27197

weight (190 to 210 g) to use in the experiments, adaptation of the nonacclimated groups of rats (male, 90 to 110 g) to the high-protein or control diet was started 4 to 5 days after the cold-acclimated groups and maintained at 25°C for 17 to 18 days.

In Vivo Fatty Acid Synthesis and IBAT Lipid Content

Rats were injected intraperitoneally with ^3H_2O (5 mCi in 0.75 mL saline) and killed by cervical dislocation 60 minutes after label injection. The IBAT was rapidly removed, carefully cleaned free of adhering fat and muscle, and weighed. IBAT total lipids were extracted with 2:1 chloroform-methanol by the procedure of Folch et al.[5] ^3H_2O was removed from the lower phase (predominantly chloroform) by washing 3 times with an upper phase mixture.[5] After each shaking, the tubes were briefly centrifuged to sharpen the phase boundary, and the upper phase was aspirated and discarded. The chloroform phase was evaporated to dryness under N_2, and triacylglycerols were hydrolyzed with ethanolic potassium hydroxide (KOH) for 1 hour at 70°C. After extraction of nonsaponifiable lipids and acidification with 6 N H_2SO_4, the ^3H-labeled fatty acids were extracted with petroleum ether, and the extract was evaporated to dryness in a scintillation vial and dissolved in toluene-2,5-diphenyloxazole (PPO) for counting in a LS Beckman spectrometer (Beckman Instruments, Palo Alto, CA). The specific radioactivity of body water was determined directly in aliquots of plasma (10 μL of a 1/20 dilution) dissolved in toluene-triton-PPO. Rates of fatty synthesis were calculated following the assumptions of Windmueller and Spaeth.[6]

Measurement of Enzyme Activities

Glucose-6-phosphate dehydrogenase, malic enzyme, and ATP-citrate lyase activites were determined in 100,000 g supernatants obtained from IBAT homogenates.[4] G6P-dehydrogenase was assayed as described by Lee,[7] and malic enzyme was assayed by the method of Ochoa,[8] with the modifications proposed by Hsu and Lardy.[9] ATP-citrate lyase was assayed as described by Srere.[10] For determination of acetyl-coenzyme A carboxylase activity, IBAT was homogenized in 50 mmol/L phosphate potassium buffer, pH 7.3, containing 2 mmol/L EDTA, 4 mmol/L reduced glutathione, and albumin (10 mg/mL). The assay was performed as described by Halestrap and Denton[11] by measuring the incorporation of [^{14}C] bicarbonate (3 μCi) into acid-stable material after incubation of 1,500 g supernatants of whole homogenate with citrate. For determination of pyruvate dehydrogenase, mitochondria were prepared from a pool of IBAT (≈1.5 g) in a buffer containing interconversion inhibitors (10 mmol/L dichloroacetate and 50 mmol/L sodium fluoride [NaF]), which maintains the PDH complex in the in vivo state of activation.[12] The mitochondria sediment obtained was suspended in a small volume of isolation buffer and divided into 2 portions. One portion, used for determination of the initial enzyme activity, was pelleted by centrifugation and immediately frozen in liquid nitrogen. The other portion of mitochondria was washed free of NaF by dilution followed by centrifugation and, after incubation for 30 minutes at 30°C in a medium containing carbonylcyanide *m*-chlorophenylhydrazone, an uncoupler of oxidative phosphorylation, was pelleted by centrifugation, frozen in liquid nitrogen, and used for determination of total PDH activity. Mitochondria were extracted for assay of enzyme and protein concentration by alternate thawing and freezing (liquid nitrogen, 3 times) with extraction buffer.[13] PDH-complex activity was determined spectrophotometrically following nicotinamide adenine dinucleotide $(NAD)^+$ reduction in a assay mixture containing oxamate, an inhibitor of lactate dehydrogenase.[14]

Other Methods of Chemical Analysis

For the gravimetric determination of IBAT fat content, tissue lipids were extracted by the procedure of Folch et al.[5] Protein concentration was determined as described by Lowry et al.[15] Plasma insulin was determined by radioimmunoassay using a commercial kit from Amersham (Little Chalfont, UK).

Statistical Methods

Differences between groups were analyzed using analysis of variance (ANOVA) with $P < .05$ as the criterion of significance.

RESULTS

Daily measurements of body temperature showed that, despite the diet-induced reduced thermogenic capacity, body temperature of rats adapted to the high-protein diet was not affected by cold acclimation, remaining within the range of rats fed the control diet (37.2°C to 37.5°C) until the end of the acclimation period. The data in Table 1 show that at ambient temperature BAT weight was smaller in rats fed the high-protein diet than in controls, and that the difference increased in cold-acclimated rats, as the increase in BAT weight induced by cold acclimation was greater in control rats (100%) than in high-protein diet-adapted rats (30%). BAT protein content increased 2.5 times in cold-acclimated rats of the 2 groups, remaining 50% lower in rats fed the high-protein diet in the 2 experimental conditions. Tissue total lipid content was about 30% lower in high-protein diet-adapted rats at ambient temperature and increased after cold acclimation in the 2 groups, but the increase was more marked in BAT from control rats (Table 1). Plasma insulin levels were lower in rats fed the high-protein diet than in controls at ambient temperature and decreased significantly after cold acclimation in the 2 experimental groups (Table 1).

In Vivo Fatty Acid Synthesis

As shown in Fig 1, rates of fatty acid synthesis from ^3H_2O in BAT from rats adapted to the high-protein diet at 25°C were reduced to about 27% of rats fed the balanced diet and increased approximately 3 times in the 2 groups after cold acclimation. However, in absolute terms, the cold-induced increase in total fatty acid synthesis was lower in rats fed the high-protein diet, whose rates at 4°C remained lower than those of controls at 25°C.

Table 1. Weight, Protein, and Lipid Content of IBAT and Plasma Insulin Levels in Cold-Acclimated Rats Adapted to a High-Protein, Carbohydrate-Free (HP) or Control Diet

	Room Temperature (25°C)		Cold-Acclimated (4°C)	
	Control	HP Diet	Control	HP Diet
IBAT weight (mg)	291 ± 13	238 ± 11*	605 ± 21†	312 ± 11*†
Protein (mg/IBAT)	22 ± 1.0	11 ± 0.8*	54 ± 2.8†	27 ± 1.3†
Lipid (mg/IBAT)	103 ± 7	67 ± 5*	221 ± 8†	89 ± 9†
Plasma insulin (μmol/mL)	49 ± 5	30 ± 4*	23 ± 3†	15 ± 1†

NOTE. Values are means ± SEM of 8 rats.
*$P < .05$ *v* control.
†$P < .05$ *v* 25°C.

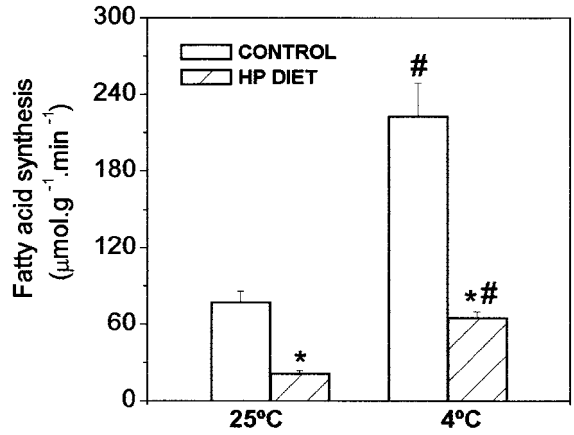

Fig 1. Effect of cold acclimation on fatty acid synthesis in interscapular BAT from rats adapted to a high-protein, carbohydrate-free (HP) or control diet. Data are means ± SEM from 8 to 10 animals. *$P < .05$ *v* controls; #$P < .05$ *v* 25°C.

The results of measurements of enzyme activities are shown in Figs 2 and 3 and are expressed per milligram protein and by whole IBAT. In rats fed the balanced diet, the activities of malic enzyme, G6P-dehydrogenase (Fig 2), and ATP-citrate lyase (Fig 3) expressed per milligram protein, decreased by 25%, 40%, and 25%, respectively, after cold acclimation. In contrast, the activity of acetyl-coenzyme A carboxylase (Fig 3) increased by 40% in cold-acclimated control rats. Confirming previous results,[4] the activities of G6P-dehydrogenase and malic enzyme (Fig 1) and of ATP-citrate lyase and acetyl-coenzyme A carboxylase (Fig 2) were markedly reduced in IBAT from high-protein diet-adapted rats at ambient temperature. Different from control rats, the activities of the 4 enzymes (per milligram protein) increased (by about 200%) after cold acclimation in rats fed the high-protein diet (Figs 2 and 3). On the other hand, because of the cold-induced BAT hypertrophy and increase in protein content, the activities of these enzymes, when expressed per whole gland, increased several times in the 2 groups of rats after cold acclimation, although remaining lower in rats fed the high-protein diet than in controls at the end of the acclimation period (Figs 2 and 3).

The data in Table 2 show that the initial (before activation) PDH-complex activity (expressed per milligram of mitochondrial protein) was reduced in BAT from rats fed the high-protein diet at ambient temperature and increased in both groups of rats after cold acclimation, but remained lower in high-protein diet-adapted rats than in controls. Total PDH activity was also reduced in rats fed the high-protein diet at 25°C and showed a tendency to increase after cold stimulation, but statistical significance was not reached ($P < .10$). Expressed as percent of total activity, initial PDH activity was lower in high-protein diet-adapted rats (25%) than in controls (45%) at ambient temperature and increased to 45% after cold acclimation.

DISCUSSION

The data of the present work show that, as in rats fed the balanced diet, cold acclimation induces in rats adapted to the high-protein diet an increase in BAT total fatty acid synthesis, which, however, is smaller in absolute terms than the increase observed in control rats. As previously discussed,[4] the marked reduction in BAT lipogenesis in high-protein diet-adapted rats (at ambient temperature) is probably due to a combination of neural and hormonal factors. Numerous studies, reviewed by Himms-Hagen,[1] suggest that chemical signs elicited by qualitative and quantitative changes in the diet modulate the activity of sympathetic neurons in the ventromedial and other hypothalamic areas that control BAT thermogenesis. Electrical stimulation of ventromedial hypothalamus markedly increases lipid synthesis in BAT,[16,17] an effect that is almost completely abolished by sympathetic denervation of the tissue.[18] We have recently shown[19] that norepinephrine turnover rate, which is mainly dependent on sympathetic impulse traffic, is greatly reduced in BAT from rats fed the high-protein diet, suggesting that the decrease in fatty acid synthesis may be, in part, due to a hypothalamic-mediated suppression of BAT sympathetic activity. On the other hand, rats fed high-protein diets have low levels of circulating insulin and high levels of glucagon,[20,21]

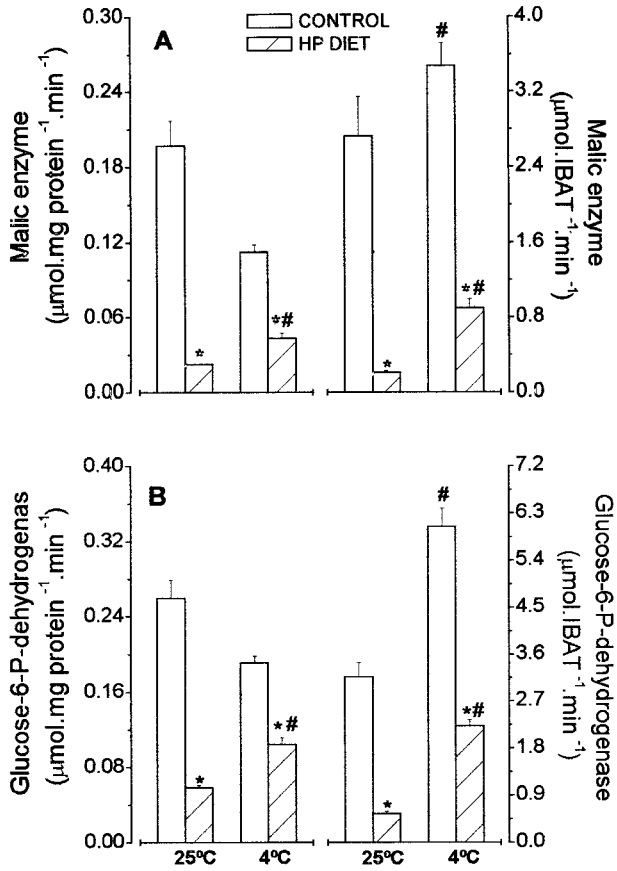

Fig 2. Effect of cold acclimation on the activities of malic enzyme (A) and glucose-6-phosphate dehydrogenase (B) in IBAT from rats adapted to a high-protein, carbohydrate-free (HP) or control diet. Activity per IBAT (right side of the figure) was estimated by multiplying the activity per milligram protein by cytosol protein content in whole tissue. Data are means ± SEM from 6 to 10 animals *$P < .05$ *v* controls; #$P < .05$ *v* 25°C.

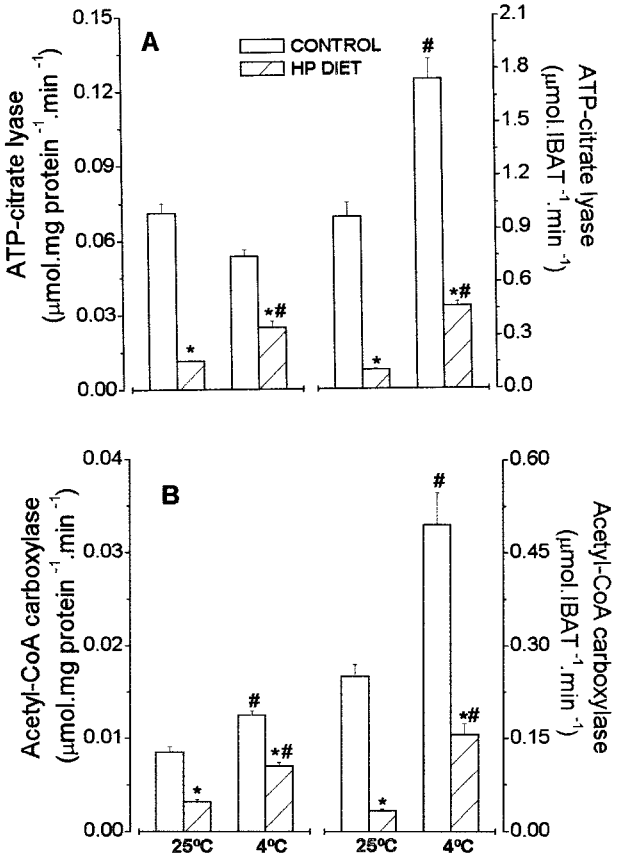

Fig 3. Effect of cold acclimation on the activities of ATP-citrate lyase (A) and acetyl-coenzyme A carboxylase (B) in IBAT from rats adapted to a high-protein, carbohydrate-free (HP) or control diet. Activity per IBAT (right side of the figure) was estimated by multiplying the activity per milligram protein by cytosol (ATP-citrate lyase) or 1,500 g supernatant (acetyl-coenzyme A carboxylase) protein content in whole tissue. Data are means ± SEM from 8 to 10 animals. *$P < .05$ *v* controls; #$P < .05$ *v* 25°C.

and it has been found that insulin stimulates BAT lipogenesis both in vivo and in brown adipocytes in vitro.[22,23] Thus, a low insulin-to-glucagon ratio at the tissue level may also contribute to the reduction in fatty acid synthesis in rats adapted to the high-protein diet.

The activation of BAT thermogenesis and the associated marked increase in tissue fatty acid synthesis induced by cold acclimation in animals fed balanced diets has been attributed mainly to stimulation by cold of BAT sympathetic activity.[1,24] Accordingly, the cold-induced increase in fatty acid synthesis in BAT from rats adapted to the high-protein diet was probably due to a reactivation of tissue sympathetic activity. This contention is supported by our recent finding that exposure of high-protein diet-adapted to cold (4°C) for a short period (8 hours) induces a marked increase in IBAT norepinephrine turnover rate.[19] Confirming results obtained by others in normally fed rats,[25] circulating levels of insulin actually decreased after cold acclimation in both high-protein–adapted and control rats (Table 1). It has been found[26] that increased rates of fatty acid synthesis can be induced by intermitent electrical stimulation of BAT sympathetic fibers, suggesting a direct control of BAT lipogenesis by the sympathetic nervous system (SNS). Although the biochemical mechanism of this direct effect of SNS on BAT lipogenesis is not known, it is interesting to note that it has been shown[27-29] that electrical stimulation of hypothalamus and activation of SNS increases BAT glucose uptake by a mechanism that seems to be independent of insulin, apparently increasing the affinity of GLUT-4 for the hexose without changing their number in the membrane.[30] Specific β-3 adrenergic agonists have also been shown to stimulate glucose uptake by BAT.[31] In addition to activating the cellular metabolic processes that accompany the increased thermogenesis, the SNS has been shown to promote, with mediation by β-1 adrenergic receptors, the differentiation of preadipocytes and hyperplasia and hypertrophia of the tissue.[32,33] Thus, both types of action probably contributed to cause the marked stimulation of lipogenesis by cold in both high-protein diet-adapted and control rats.

BAT fatty acid synthesis stimulation by cold was accompanied in both experimental groups by increased activities of enzymes associated with lipogenesis, either generators of reduced nicotinamide adenine dinucleotide phosphate (NADPH) for lipid synthesis (G6P-dehydrogenase and malic enzyme) or participants of fatty acid synthesis pathway (pyruvate dehydrogenase, ATP-citrate lyase, and acetyl-coenzyme A carboxylase). Interestingly, although the activity of the 4 cytosolic enzymes investigated increased after cold acclimation when estimated per whole tissue, only acetyl-coenzyme A carboxylase showed an increase in specific activity (per milligram protein) in both control and high-protein–fed rats. The specific activity of the other 3 (G6P-dehydrogenase, malic enzyme, and ATP-citrate lyase) increased only in rats adapted to the high-protein diet and actually tended to decrease in BAT from cold-acclimated rats fed the balanced diet (Figs 2 and 3). These findings can be interpreted as indicative that in high-protein diet-adapted rats, which have markedly low levels of enzyme activities, both hyperplasia and activation of cellular metabolism were important for the cold-induced increase in the activity of the cytosolic enzymes examined, whereas in rats fed the control diet, this was true only for acetyl-coenzyme A carboxylase, the increase in the

Table 2. Initial and Total Activity of PDH Complex (nmol · mg protein^{-1} · min^{-1}) in IBAT From Cold-Acclimated Rats Adapted to a High-Protein, Carbohydrate-Free (HP) or Control Diet

	Room Temperature (25°C)		Cold-Acclimiated (4°C)	
	Control	HP Diet	Control	HP Diet
PDH initial activity	63.2 ± 2.4	21.3 ± 1.0*	78.8 ± 3.3†	48.1 ± 3.9*†
Percentage of total activity	45	25	50	45
PDH total activity	139 ± 6.2	82.3 ± 3.8*	155.1 ± 20.6	106.2 ± 13.4

NOTE. Values are means ± SEM of 4 experiments, each with a pool of ~1.5 g tissue.

*$P < .05$ *v* controls.

†$P < .05$ *v* 25°C.

activity of BAT G6P-dehydrogenase, malic enzyme, and ATP-citrate lyase in these animals depending solely on the hyperplasic effect of SNS. The present results support the view, derived from hormonal and nutritional studies, that the reaction catalyzed by acetyl-coenzyme A carboxylase, whose maximal activity is much lower than that of the other enzymes, is a rate-limiting step in fatty acid synthesis. The finding that the initial specific activity of the mitochondrial PDH was reduced (both in absolute values and as a percentage of total activity) in rats fed the high-protein diet at ambient temperature, with no change of total enzyme activity, could be due, at least in part, to the low levels of insulin in these animals. Indeed, insulin normally acts to dephosphorylate (and activate) pyruvate dehydrogenase, the phosphorylated (inactive) form of the enzyme increasing in the absence of the hormone.[34,35] However, the increase in cold-acclimated high-protein diet-adapted rats in both initial and total BAT PDH activities was probably due to an activation of cellular metabolism by the SNS, because circulating levels of insulin decreased during cold acclimation.

In summary, despite the greatly reduced levels at ambient temperature, rates of fatty acid synthesis and activities of lipogenic enzymes in BAT from rats adapted to the high-protein diet increased significantly after cold acclimation, although less markedly than in BAT from normally fed controls. This increase in lipogenesis seems to be due to a restoration of BAT sympathetic activity, which induces BAT hyperplasia and activation of fatty acid synthesis, with an important participation of acetyl-coenzyme A carboxylase and pyruvate dehydrogenase.

ACKNOWLEDGMENT

We thank Maria A.R. Garófalo, Neusa M.Z. Resano, Victor D. Galban, and Elza A. Fillipin for technical assistante.

REFERENCES

1. Himms-Hagen J: Brown adipose tissue thermogenesis and obesity. Prog Lipid Res 28:67-115, 1989

2. Rothwell NJ, Stock MJ: A role for brown adipose tissue in diet-induced thermogenesis. Nature 281:31-35, 1979

3. Brito MN, Brito NA, Migliorini RH: Thermogenic capacity of brown adipose tissue is reduced in rats fed a high-protein, carbohydrate-free diet. J Nutr 122:2081-2086, 1992

4. Brito MN, Brito NA, Brito SRC, et al: Brown adipose tissue triacylglycerol synthesis in rats adapted to a high-protein, carbohydrate-free diet. Am J Physiol 276:R1003-1009, 1999

5. Folch J, Lees M, Stanley GA: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1957

6. Windmueller HG, Spaeth AE: Perfusion “in situ” with tritium oxide to measure hepatic lipogenesis and lipid secretion. J Biol Chem 241:2891-2899, 1966

7. Lee CY: Glucose-6-phosphate dehydrogenase from mouse. Methods Enzymol 89:252-260, 1982

8. Ochoa S: Malic enzyme. Methods Enzymol 1:739-753, 1955

9. Hsu RY, Lardy HA: Malic enzyme. Methods Enzymol 13:230-235, 1969

10. Srere PA: The citrate cleavage enzyme. I. Distribution and purification. J Biol Chem 234:2544-2547, 1959

11. Halestrap AP, Denton RM: Insulin and the regulation of adipose tissue acetyl-coenzyme A carboxilase. Biochem J 132:509-517, 1973

12. Caterson ID, Kerbey AL, Cooney GJ, et al: Inactivation of pyruvate dehydrogenase complex in heart muscle mitochondria of gold-thioglucose-induced obese mice is not due to a stable increase in activity of pyruvate dehydrogenase kinase. Biochem J 253:291-294, 1988

13. Denyer GS, Cooney GJ, Storlien LH, et al: Heterogeneity of response to exercise of rat muscle pyruvate dehydrogenase complex. Pflügers Arch 419:115-120, 1991

14. Chretien D, Pourrier M, Bourgeron T, et al: An improved spectrophotometric assay of pyruvate dehydrogenase in lactate dehydrogenase contaminated mitochondrial preparations from human skeletal muscle. Clin Chim Acta 240:129-136, 1995

15. Lowry OH, Rosenbrough NJ, Farr AL, et al: Protein measurement with Folin phenol reagent. J Biol Chem 193:265-275, 1951

16. Shimazu T, Takahashi A: Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue. Nature 284:62-63, 1980

17. Takahashi A, Shimazu T: Hypothalamic regulation of lipid metabolism in the rat: Effect of hypothalamic stimulation on lipogenesis. J Auton Nerv Syst 6:225-235, 1982

18. Minokoshi Y, Saito M, Shimazu T: Sympathetic denervation impairs response of brown adipose tissue to ventromedial hypothalamic stimulation. Am J Physiol 251:R1005-R1008, 1986

19. Brito MN, Brito NA, Garófalo MAR, et al: Sympathetic activity in brown adipose tissue from rats adapted to a high protein, carbohydrate-free diet. J Auton Nerv Syst 69:1-5, 1998

20. Eisenstein AB, Strack I, Gallo-Torres H, et al: Increased glucagon secretion in protein-fed rats: Lack of relationship to plasma amino acids. Am J Physiol 236:E20-E27, 1979

21. Peret J, Foustock S, Chanez M, et al: Plasma glucagon and insulin concentration and hepatic phosphoenolpyruvate carboxykinase and pyruvate kinase activities during and upon adaptation of rats to a high protein diet. J Nutr 111:1173-1184, 1981

22. McCormack JG: The regulation of fatty acid synthesis in brown adipose tissue by insulin. Prog Lipid Res 21:195-223, 1982

23. McCormack JG, Denton RM: Evidence that fatty acid synthesis in the interscapular brown adipose tissue of cold-adapted rats is increased in vivo by insulin by mechanisms involving parallel activation of pyruvate dehydrogenase and acetyl-coenzyme A carboxylase. Biochem J 166:627-630, 1977

24. Trayhurn P: Fatty acid synthesis in vivo in brown adipose tissue, liver and white adipose tissue of the cold-acclimated rat. FEBS Lett 104:13-16, 1979

25. Edwards CI, Howland RJ: Adaptive changes in insulin and glucagon secretion during cold acclimation in the rat. Am J Physiol 250:E669-E676, 1986

26. Minokoshi Y, Saito M, Shimazu T: Sympathetic activation of lipid synthesis in brown adipose tissue in the rat. J Physiol 398:361-370, 1988

27. Cooney GJ, Caterson ID, Newsholme EA: The effect of insulin and noradrenaline on the uptake of 2-(1-^{14}C) deoxyglucose in vivo by brown adipose tissue and other glucose-utilizing tissues of the mouse. FEBS Lett 188:257-261, 1985

28. Shimizu Y, Nikami H, Saito M: Sympathetic activation of glucose utilization in brown adipose tissue in rats. J Biochem (Tokyo) 110:688-692, 1991

29. Takahashi A, Shimazu T, Maruyama Y: Importance of sympathetic nerves for the stimulatory effect of cold exposure on glucose utilization in brown adipose tissue. Jpn J Physiol 42:653-664, 1992

30. Shimizu Y, Satoh S, Yano H, et al: Effect of noradrenaline on the cell surface glucose transporters in cultured brown adipocytes: Novel mechanism for selective activation of GLUT 1 glucose transporters. Biochem J 330:397-403, 1998

31. Liu X, Perusse F, Bukowiecki LJ: Mechanisms of the antidiabetic effects of the beta 3-adrenergic agonist CL-316243 in obese Zucker-ZDF rats. Am J Physiol 274:R1212-R1219, 1998

32. Bukowiecki LJ, Collet AJ, Follea N, et al: Brown adipose tissue hyperplasia: A fundamental mechanism of adaptation to cold and hyperfagia. Am J Physiol 242:E353-E359, 1982

33. Géloën A, Collet AJ, Guay G, et al: β-Adrenergic stimulation of brown adipocyte proliferation. Am J Physiol 254:C175-C182, 1998

34. Cooney GJ, Denyer GS, Jenkins AB, et al: In vivo insulin sensitivity of the pyruvate dehydrogenase complex in tissues of rat. Am J Physiol 265:E102-E107, 1993

35. Stansbie D, Denton RM, Bridges BJ, et al: Regulation of pyruvate dehydrogenase and pyruvate dehydrogenase phosphate phosphatase activity in rat epididymal fat-pads. Effects of starvation, alloxan-diabetes and high-fat diet. Biochem J 154:225-236, 1976