Iron Deficiency in the Rat: Effects on Energy Metabolism in Brown Adipose Tissue

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ABSTRACT. Mitochondria were prepared from the brown adipose tissue of control rats and animals made iron deficient by means of a low iron diet. The specific activities of the mitochondrial electron transport system (NADH, succinate and α -glycerophosphate oxidase activities) were markedly and significantly reduced in preparations of brown adipose tissue from the iron-deficient rats as compared with preparations from the control animals. In contrast, concentrations of the cytochrome pigments $a + a_3$, and $c + c_1$ were normal and cytochrome b was slightly reduced (18%) in the mitochondrial preparations from the iron-deficient animals. Treatment of the iron-deficient animals with triiodothyronine significantly increased the amount of brown fat present per kilogram of body weight in both control and iron-deficient rats, but did not significantly affect the specific activities of the mitochondrial electron transport system. (*Pediatr Res* 19: 989-991, 1985)

Abbreviations

T3, triiodothyronine
T4, thyroxine

Dillman *et al.* (1, 2) have shown that cold-induced nonshivering thermogenesis was reduced in iron-deficient rats despite elevated levels of catecholamines. Treatment of the iron-deficient rats with T3, but not T4, appeared to partially correct the defective thermoregulation and suggested that conversion of T4 to T3 was impaired in the animals. More recently, however, studies by Beard *et al.* (3, 4) suggest that anemia may play an important role in the cold sensitivity of iron deficiency in the rat.

Previously studies on nonshivering thermogenesis in cold-acclimated rats have shown that a major proportion of heat production occurs in the brown fat (5), and Himms-Hagen and Desautels (6) have described a mitochondrial defect in the brown fat of the obese (ob/ob) mouse that may explain the reduced cold-induced thermogenesis present in the animals. The present work was, therefore, undertaken to study the effects of iron deficiency and treatment with T3 on mitochondrial function in brown fat since the enzyme systems (mitochondrial electron transport systems) active in energy and heat production contain both heme and nonheme iron components which are essential for enzymatic catalysis.

MATERIALS AND METHODS

Male Sprague-Dawley rats were obtained at 4 wk of age, 1 wk after weaning. Rats to be made iron deficient were given a low iron diet, prepared in our laboratory as described by ICN Nutritional Biochemicals, Cleveland, OH, that contained 10 mg iron/kg. After 1 month on this diet, the hemoglobin concentration of those animals receiving it was determined and studies were performed only on rats with blood Hb concentrations of less than 7 g/100 ml. The control groups were fed the low iron diet during the experimental period, but received weekly intraperitoneal injections of 5 mg of iron in the form of iron dextran (Imferon, Merrell-National Labs, Cincinnati, OH). All animals were allowed food and water *ad libitum*. One group of iron-deficient rats and a group of control rats received daily intraperitoneal injections of 1 μ g of T3 [a pharmacological dose as described by Dillman *et al.* (1, 2)] for 10 days before the experiments were performed.

The rats were sacrificed by cervical dislocation and the interscapular brown adipose tissue was quickly removed and weighed. Mitochondria were prepared from the adipose tissue as described by Skala *et al.* (7), with the exception that a solution of 0.30 M sucrose and 0.02 M Tris (pH 7.5) was used instead of a 0.25 M solution of sucrose.

Difference spectra (dithionite reduced minus oxidized) of the cytochromes of the mitochondrial respiratory chain were determined by means of a sensitive wavelength-scanning Aminco DW-2 spectrophotometer at 25° C and the concentrations of the cytochromes were calculated by use of the following millimolar extinction coefficients: cytochrome $a + a_3$ (605-630 nm), 24.0 (8); cytochrome $c + c_1$ (550-540 nm), 19.1 (9); cytochrome b (562-575 nm), 20.0 (10).

NADH, succinic and α -glycerophosphate oxidase activities were determined polarographically in the mitochondrial preparations at 25° C with a Clark oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH) fitted to a closed glass thermostated chamber of 1.6 ml capacity (Gilson Instrument Co., Madison, WI). Before assay, the preparations of mitochondria were homogenized at top speed for 5 min in a motor driven Teflon-glass homogenizer maintained at 5° C to ensure breakage of the mitochondria in order to obtain maximum enzymatic activities. Assay mixtures (pH 7.5) contained 40 μ mol of substrate (NADH, succinate or α -glycerophosphate), 0.2 ml of 0.2 M potassium phosphate, and 0.02 ml of a 1% solution of cytochrome c in a final volume of 1.6 ml. In control experiments it was determined that all activities (with added cytochrome c present) were completely inhibited by addition of 23 μ g of antimycin A.

Protein was determined by the method of Lowry *et al.* (11). Hb was determined as cyanmethemoglobin with standards employed for calibration (Hycel, Inc., Houston, TX). Statistical analyses were performed by standard methods of one-way analysis of variance for two independent samples as described by

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Snedecor and Cochran (12). NADH, cytochrome *c* type VI, α -glycerophosphate, Tris, and succinate were obtained from Sigma Chemical Co, St. Louis, MO. Antimycin A was a gift from Ayerst Laboratories, New York, NY.

RESULTS AND DISCUSSION

Mitochondrial preparations isolated from brown adipose tissue of control and iron-deficient rats were studied to determine the effects of iron deficiency on the enzymatic activities and the concentrations of the various cytochrome pigments associated with the terminal electron transport systems that generate the energy and heat produced in brown fat. As shown in Table 1, Hb concentrations were markedly reduced in the iron deficient rats and the activities of the electron transport enzymes (NADH, succinate, and α -glycerophosphate oxidases) were markedly (more than 50%) and significantly ($p < 0.01$ for all activities) reduced in the preparations from the iron-deficient animals. However, as described in Table 2, levels of cytochromes $a + a_3$ and $c + c_1$ were unaffected and concentrations of cytochrome *b* were only slightly lowered (18%) in the iron-deficient preparations. These results strongly suggest that the markedly lowered enzymatic activities may be due to a reduction of the nonheme iron-sulfur components of the electron transport systems, since the heme-iron components (cytochromes) were relatively unaffected by iron deficiency. As further shown in Table 1, the average weight of brown adipose tissue was not significantly different in the iron deficient and control animals.

When control and iron-deficient rats were treated with 10 daily injections intraperitoneally of 1 μ g of T3 before the experiments were performed, significant increases (approximately 30%, $p < 0.01$) in the amount of brown fat per kg body weight were found in both groups of animals as shown in Table 1, although the specific activity and activity per gram of brown fat of the enzymes concerned with electron transport were not significantly altered.

In summary, the activities of the mitochondrial electron transport systems of brown adipose tissue were markedly reduced in the iron-deficient rats. The reduced activities may have been due to depletion of the nonheme iron-sulfur components of the enzyme systems since the cytochrome components appeared to be relatively unaffected, as has been previously observed in studies of iron-deficient rat skeletal muscle (13, 14). The lowered activity of the mitochondrial electron transport systems represents a markedly impaired ability of the brown adipose tissue to generate heat and, therefore, may be a factor in the inability of iron-deficient rats to maintain normal cold-induced thermogenesis (1, 2). When iron-deficient rats were treated with daily injections of T3 there was a significant increase in the amount of brown fat present per kilogram of body weight and, therefore, of the capacity per kilogram of the animals to produce heat, even though the specific activities of the mitochondrial electron transport system were not affected. It has been previously shown that brown fat thermogenesis is regulated by norepinephrine and T3 [see review by Nichols (15)] and this is in accord with the published findings by Dillman *et al.* (1, 2) and Beard *et al.* (3) that treatment with T3 increased the ability of iron-deficient rats to adapt to cold temperatures. The findings of Beard *et al.* (3, 4) that transfusing iron-deficient rats restored cold resistance to normal suggests that anemia may be an important factor in the cold sensitivity of iron deficiency. However, Beard *et al.* (3) also found that transfusion allowed a rise in T3 levels suggesting that thyroid hormones may play a role in the improvement in cold resistance produced by transfusion.

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Table 1. Effects of iron deficiency on the activities of mitochondria of brown adipose tissue*

Animals studied	Hb (g/100 ml blood)	Wt per rat (g)	g brown fat per rat	g of brown fat per kg rat wt	Activity of electron transport systems					
					NADH oxidase		Succinic oxidase		α -Glycerophosphate oxidase	
					Specific activity	Activity per g brown fat	Specific activity	Activity per g brown fat	Specific activity	Activity per g brown fat
Control group	15.2 $\pm$ 0.1	294 $\pm$ 6	0.36 $\pm$ 0.02	1.24 $\pm$ 0.06	0.224 $\pm$ 0.019	1.55 $\pm$ 0.14	0.115 $\pm$ 0.020	0.804 $\pm$ 0.138	0.072 $\pm$ 0.006	0.508 $\pm$ 0.052
Iron deficient group	6.3 $\pm$ 0.2	273 $\pm$ 4	0.37 $\pm$ 0.03	1.36 $\pm$ 0.10	0.104 $\pm$ 0.011	0.586 $\pm$ 0.062	0.052 $\pm$ 0.008	0.292 $\pm$ 0.047	0.053 $\pm$ 0.006	0.304 $\pm$ 0.038
Control group treated with T3	15.3 $\pm$ 0.1	271 $\pm$ 8	0.43 $\pm$ 0.01	1.58 $\pm$ 0.04	0.227 $\pm$ 0.020	1.35 $\pm$ 0.14	0.106 $\pm$ 0.017	0.642 $\pm$ 0.131	0.076 $\pm$ 0.006	0.458 $\pm$ 0.049
Iron-deficient group treated with T3	6.1 $\pm$ 0.2	240 $\pm$ 6	0.43 $\pm$ 0.02	1.79 $\pm$ 0.08	0.135 $\pm$ 0.011	0.637 $\pm$ 0.160	0.051 $\pm$ 0.008	0.241 $\pm$ 0.039	0.059 $\pm$ 0.007	0.278 $\pm$ 0.039

* All values represent the averages of data for 13 or more separate experiments $\pm$ SEM. Specific activity is defined as the μ moles of substrate oxidized per minute per milligram mitochondrial protein. Activity per gram tissue is defined as μ moles substrate oxidized per minute per gram wet tissue.

Table 2. *Effects of iron deficiency on the concentrations of cytochrome pigments in mitochondria of brown adipose tissue**

Animals studied	Cytochrome concentration (nmol/mg protein)			Hb (g/100 ml blood)
	Cyt <i>c</i> + <i>c</i> ₁	Cyt <i>b</i>	Cyt <i>a</i> + <i>a</i> ₃	
Control group	0.835 ± 0.038	0.663 ± 0.039	0.429 ± 0.018	13.8 ± 0.1
Iron deficient	0.806 ± 0.025	0.541 ± 0.020	0.413 ± 0.019	5.4 ± 0.1
<i>p</i>	>0.5	0.02	>0.5	<0.001

* All values represent the averages of data for 10 or more separate experiments ± SEM.

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