

# Body temperature and thyroid hormone metabolism of copper-deficient rats<sup>\*†</sup>

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*The hypothesis that the effects of copper deficiency on body temperature are mediated via the thyroid hormone system was examined in 24 male Sprague–Dawley rats, weighing 240 to 250 g, matched by weight and fed ad libitum diets containing adequate (78.7  $\mu\text{mol/kg}$ ), marginal (31.5  $\mu\text{mol/kg}$ ), or deficient (<7.9  $\mu\text{mol/kg}$ ) amounts of copper for 35 days. Anemia was not found in the copper-restricted groups. Plasma cholesterol concentrations increased ( $P < 0.05$ ) and plasma copper, ceruloplasmin, and liver copper concentrations decreased ( $P < 0.05$ ) in the rats fed the copper-restricted diet as compared with the copper-adequate diet. Cytochrome *c* oxidase and glutathione peroxidase activities in liver and brown adipose tissue were decreased ( $P < 0.05$ ) in the copper-deprived rats. Body temperatures at 24°C were less ( $P < 0.05$ ) in the severely copper-deficient as compared with the moderately copper-deficient and copper-adequate animals. Plasma thyroxine concentrations were decreased ( $P < 0.05$ ) in severely copper-deficient as compared with moderately copper-deficient and copper-adequate rats. Plasma tri-iodothyronine concentrations were reduced ( $P < 0.05$ ) and plasma thyroid-stimulating hormone concentrations were elevated ( $P < 0.05$ ) in both groups of copper-restricted rats relative to the copper-adequate rats. Thyroxine 5'-monodeiodinase activities in liver and brown adipose tissue were decreased ( $P < 0.05$ ) in the copper-restricted animals. Hepatic tri-iodothyronine receptors were increased ( $P < 0.05$ ) by dietary copper restriction. Body fatness was greater ( $P < 0.05$ ) in copper-restricted as compared with copper-adequate rats. These findings indicate that copper deficiency without anemia decreases tissue copper and selenium status and is associated with impaired thyroid hormone metabolism and mild hypothermia in rats at maintained at 24°C. (J. Nutr. Biochem. 6:445–451, 1995.)*

**Keywords:** copper; body temperature; thyroxine; triiodothyronine; monodeiodinase; T<sub>3</sub> receptors; rat

## Introduction

The thyroid hormone system is important in maintaining normothermia in mammals.<sup>1</sup> The production of the biologically active hormone, triiodothyronine (T<sub>3</sub>), from the pre-

cursor, thyroxine (T<sub>4</sub>), is regulated by the enzyme thyroxine 5'-monodeiodinase.<sup>2</sup> There are two major forms of this enzyme: type I 5'-monodeiodinase, which is located principally in the liver and kidney, and type II 5'-monodeiodinase, which is found in the brown adipose tissue (BAT) and the central nervous system.<sup>2</sup> Many factors can affect thyroid hormone metabolism and function. These include genetic, environmental, physiological, and nutritional influences.<sup>3,4</sup> While considerable attention has been given to constitutive and environmental factors that can affect the thyroid hormone system, only recently has the role of nutritional factors, with an emphasis on micronutrients, been explored.

There is increasing experimental evidence that suboptimal intake of some mineral elements can influence the thermogenic capacity of mammals.<sup>5</sup> For example, iron defi-

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Received July 20, 1994; revised January 6, 1995; accepted March 15, 1995.

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ciency decreases plasma thyroid hormone concentrations and results in hypothermia at 24°C and during acute cold exposure in male and female rats.<sup>6-9</sup> Similarly, zinc deficiency impairs thyroid hormone metabolism and causes hypothermia at room temperature and during cold exposure.<sup>10</sup>

Copper (Cu) nutriture also has been reported to influence thyroid hormone status and thermogenesis. Among infants and children with a genetic impairment in Cu absorption, a condition termed Menkes' Kinky Hairy Syndrome, hypothermia at room temperature was a characteristic feature.<sup>11,12</sup> Anemia was not present in any of the infants and children with this metabolic perturbation. However, the activity of cytochrome c oxidase, a Cu-containing enzyme, in the liver and skeletal muscle was decreased.

Copper deficiency has been reported to impact circulating thyroid hormone concentrations. Weanling Cu-deficient rats had decreased basal T<sub>4</sub> concentrations and blunted pituitary-thyroid axis function suggesting hypothyroidism.<sup>13</sup> In addition, recent evidence indicates that Cu deficiency induces a secondary selenium (Se) deficiency as indicated by reduced tissue selenogluthathione peroxidase activity.<sup>14,15</sup>

The present study was undertaken to examine the hypotheses that Cu deficiency affects body temperature at thermoneutral conditions and that this effect is mediated by thyroid hormone status. We also assessed the roles of type I and II 5'-monodeiodinase activities in modulating the thyroid hormone response to graded Cu deprivation.

## Methods and materials

Male Sprague-Dawley rats weighing approximately 250 g (Harlan Sprague-Dawley, Madison, WI, USA) were sorted by weight into three groups ( $n = 8$ ) and fed one of the following experimental diets. The treatments were Cu-adequate (CuA, 78.7  $\mu\text{mol}$  Cu/kg of diet), Cu-marginal (CuM, 31.5  $\mu\text{mol}$ /kg), and Cu-deficient (CuD, <7.9  $\mu\text{mol}$ /kg) diets. The basal CuD diet was based on 65% carbohydrate (50% sucrose and 15% corn starch), 20% casein, 5% corn oil, 5% cellulflower, 3.5% mineral mix, 1% vitamin mix, 0.3% methionine, and 0.2% choline. The vitamin and mineral mixes were previously described.<sup>16</sup> The Cu content of the CuM and CuA diets was increased by deletion of small amounts of corn starch and concomitant increases in the CuD mineral mix. Diet and distilled-deionized water were provided ad libitum. Animals were housed individually in stainless steel cages in a climate-controlled room maintained at  $24 \pm 1^\circ\text{C}$  (mean  $\pm$  SE) with a 12 hr light:dark cycle (lights on at 0600 hr).

## Body temperature measurements

The animals were fed the experimental diets for 35 days. The animals were picked up and handled daily during the light cycle to habituate them to human contact. During the final 3 days of the study, core body temperatures were determined daily by using a thermistor (YSI model 701, Yellow Springs, OH, USA) calibrated to 0.1°C that was inserted 10 cm into the rectum. The thermistor was left in the rectum until a stable temperature ( $\pm 0.1^\circ\text{C}$ ) was attained. Body temperatures were measured at 4 to 5 hr into the 12-hr light cycle.

After an overnight fast, the animals were injected with pentobarbital (50 mg/kg of body weight). Blood was obtained by cardiac puncture and collected into heparinized and anticoagulant-free plastic syringes. The animals were killed by exsanguination from the heart.

## Biochemical assessment of copper status

Heparinized blood was analyzed for hematocrit and hemoglobin concentration by using a Coulter Counter (model #S-Plus IV, Coulter Electronics, Hialeah, FL, USA). Plasma was separated from heparinized blood by centrifugation and then assayed enzymatically for cholesterol<sup>17</sup> and ceruloplasmin<sup>18</sup> with a Cobas Fara automated analyzer (Roche Diagnostics Systems, Nutley, NJ, USA). Another plasma sample was analyzed for Cu concentration by inductively coupled argon plasma emission spectroscopy.

Liver and BAT were dissected, flushed with ice-cold saline, then weighed. The median lobe of the liver was removed for mineral assays, and the remainder of the liver was frozen at  $-80^\circ\text{C}$  for enzyme analyses. After lyophilization and digestion of tissue specimens with nitric acid and hydrogen peroxide,<sup>19</sup> their trace element contents were determined by using inductively coupled argon plasma emission spectroscopy (model 503, Perkin-Elmer, Norwalk, CT, USA). Parallel analyses of the National Institute of Standards and Technology reference samples (1577a, bovine liver for organs) yielded mineral contents within the specified ranges of accepted values.

## Enzyme analyses

Samples of liver and BAT were homogenized (1:3 and 1:10, wt/vol, respectively) in 0.1 mol potassium phosphate/L, 0.25 mol sucrose/L, 0.1 mmol EDTA/L (pH at 7.0), and 1 mmol HEPES/L, 0.25 mol sucrose/L, and 0.1 mmol EDTA/L (pH at 7.2), respectively. After low-speed centrifugation (1500 g, 4°C for 15 min), aliquots (1 mL for liver and 0.5 mL for BAT) were taken for ultracentrifugation (100,000 g, 4°C for 1 hr). The remainder of the low-speed supernatant of BAT and the pellet of the liver ultracentrifugation (2 mL of 0.1 mol potassium phosphate/L and 0.1 mmol EDTA/L at pH 7.0) was rehomogenized and diluted with 3 mL of 0.1 mol potassium phosphate 0.1 mmol EDTA/L at pH 7.0. All samples for glutathione peroxidase activity were frozen at  $-20^\circ\text{C}$ . Samples for 5'-monodeiodinase activity were stored at  $-80^\circ\text{C}$  until analysis.

Triton X-100 was added to the homogenate to a final concentration of 0.02% (vol/vol). The homogenate then was assayed for cytochrome c oxidase activity by monitoring the loss of ferrocytochrome c at 550 nm.<sup>15</sup>

Glutathione peroxidase activity of liver and BAT specimens was assayed in thawed homogenates prepared as described earlier by using the method of Paglia and Valentine.<sup>20</sup> The assay used 100  $\mu\text{L}$  of diluted sample (liver, 1:150 and BAT, 1:2, homogenate: phosphate buffer) and followed the oxidation of NADPH utilizing the substrate 0.00495% H<sub>2</sub>O<sub>2</sub>, which is specific for the Se-dependent glutathione peroxidase. Results are expressed as nmol of NADPH oxidized/min/mg of protein for liver and BAT.

Liver iodothyronine 5'-monodeiodinase activity (type I) was determined in microsomal fraction by quantitating the release of <sup>125</sup>I from [<sup>125</sup>I] 5'-L-3,3',5 T<sub>3</sub> or rT<sub>3</sub> (DuPont New England Nuclear, Boston, MA, USA), as described previously.<sup>21</sup> Samples of frozen liver were homogenized as described previously. Aliquots of microsomal fractions (30  $\mu\text{L}$ ) were incubated at 37°C for 10 min with 152  $\mu\text{L}$  of substrate mixture of 0.1 mol potassium phosphate buffer/L containing 0.1 mmol EDTA/L at pH 7.0, 10  $\mu\text{L}$  of 60 mmol dithiothreitol (DTT)/L, 1  $\mu\text{L}$  of 0.154 mmol rT<sub>3</sub>/L in 0.05 mol sodium hydroxide/L containing 1% (wt/vol) bovine serum albumin (BSA), and 7  $\mu\text{L}$  of repurified <sup>125</sup>I-rT<sub>3</sub> placed in a 500  $\mu\text{L}$  centrifuge tube covered with a stream of nitrogen and capped. After a 10-min incubation at 37°C, the reaction was stopped by the addition of 50  $\mu\text{L}$  of ice-cold human serum, 10 mmol propylthiouracil/L (1:1, vol/vol), and 300  $\mu\text{L}$  of 12.5% (wt/vol) ice-cold trichloroacetic acid. The centrifuge tube was capped and centrifuged at 13,000 rpm at 4°C for 10 min. A 450  $\mu\text{L}$

aliquot of the supernatant was taken and placed on a Dowex 50W-2X column equilibrated with 10% (vol/vol) acetic acid. The  $^{125}\text{I}$  was eluted with three 1 mL washes of 10% acetic acid and counted in a Packard Cobra Auto-Gamma counter (Downers Grove, IL, USA). The results are expressed as nmol of  $^{125}\text{I}$  released/min/mg of protein.

Type II 5'-monodeiodinase activity in BAT was determined as described by Leonard et al.,<sup>22</sup> using 2 nmol 5'-[ $^{125}\text{I}$ ]rT<sub>3</sub>/L as substrate in the presence of 1 mmol PTU/L and 20 mmol DTT/L. Although T<sub>4</sub> is a better substrate for type II 5'-monodeiodinase, the physiological responses of the enzyme are equally reflected with either substrate.<sup>22,23</sup> Results are expressed as nmol  $^{125}\text{I}$  released/min/mg of protein. Protein concentrations were determined by using a modified Lowry procedure (Sigma Kit #P5656).

### Thyroid hormones and receptors

Cardiac blood was collected into anticoagulant-free plastic tubes and permitted to clot at room temperature. Serum was obtained by centrifugation. Thyroxine and T<sub>3</sub> concentrations in serum were assayed by using an automated procedure (IMX Diagnostic System, Abbott Laboratories, Abbott Park, IL, USA). Serum thyroid-stimulating hormone (TSH) was determined by using a commercially available radioimmunoassay kit specific for rat TSH (rTSH; Amersham Corp., Arlington Heights, IL, USA). All determinations for each hormone were made in one assay. The inter- and intraassay coefficients of variation in our laboratory are <5% and <4% for T<sub>4</sub> and T<sub>3</sub>, and <8% and <6%, respectively, for rTSH.

Solubilized thyroid hormone receptors of nuclear fractions of livers were determined by using standard methods.<sup>24</sup> A 50  $\mu\text{L}$  aliquot containing approximately 100  $\mu\text{g}$  of protein was incubated with 400  $\mu\text{L}$  of buffer consisting of 20 mmol TRIS-HCl/L, 2 mmol EDTA/L, 50 mmol NaCl/L 10% (vol/vol) glycerol, and 0.16 mmol DTT/L at pH = 8.2, and 50  $\mu\text{L}$   $^{125}\text{I}$ -T<sub>3</sub> (DuPont New England Nuclear, Boston, MA, USA) at 37°C for 50 min, followed by another incubation at 4°C for 24 hr. Incubant (500  $\mu\text{L}$ ) then was applied to a hydroxyalkoxypropyl dextran (Type IV, Sigma Catalogue #H6258) column, pretreated with methanol, incubation buffer, and 3% non-fat dried milk. The columns were washed with 1 mL of incubation buffer; the eluent, containing the bound fraction, was collected and counted in a gamma counter. Data are expressed as fmol  $^{125}\text{I}$  bound/mg of protein.

### Body composition

Each animal was prepared for direct chemical analysis by removing the hair, viscera, paws, and tail. The carcass was weighed and frozen for later analyses. Three aliquots of finely ground and homogenized samples from each animal were used for proximate chemical analyses. Dry matter was determined by oven-drying at 105°C. Protein was assayed by the micro-Kjeldahl method, and total fat was estimated by the Foss-let procedure.<sup>25</sup>

All chemical reagents, unless otherwise indicated, were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Vitamin-

free casein was purchased from ICN Biochemicals (Costa Mesa, CA, USA).

### Statistical methods

Data are presented as the mean  $\pm$  standard error of the mean (SE). A one-way analysis of variance<sup>26</sup> was used to determine the effects of graded dietary Cu on various indices of Cu status and enzyme activities. A repeated measures analysis of variance<sup>26</sup> was used to examine the effects of time and dietary Cu on body temperature. Significant differences among groups were identified with Tukey contrasts.<sup>27</sup>

## Results

### Somatic and biochemical evidence of copper deficiency

Dietary Cu did not affect final body weight (Table 1). The ratio of heart weight to body weight also was not affected by dietary copper (3.13  $\pm$  0.14, 3.04  $\pm$  0.04 and 3.10  $\pm$  0.08 mg/g for CuA, CuM, and CuD, respectively). The constancy of body weight was paralleled by the estimated protein and water content of the carcass. In contrast, the ash content was decreased ( $P < 0.05$ ) and the fat content was increased ( $P < 0.05$ ) in the rats fed the Cu-restricted diets.

Plasma cholesterol concentration increased ( $P < 0.05$ ) with Cu deprivation (Table 2). Hematocrit and hemoglobin were less ( $P < 0.05$ ), although within the range of normal values, in the rats fed the copper-restricted diets as compared with the rats fed the CuA diet. Ceruloplasmin and plasma copper concentrations declined ( $P < 0.05$ ) in response to graded dietary Cu.

Tissue Cu status also was impacted by dietary Cu (Table 3). The liver Cu concentration decreased ( $P < 0.05$ ) and the iron concentration increased ( $P < 0.05$ ) when dietary Cu was decreased. Cytochrome c oxidase and glutathione peroxidase activities decreased ( $P < 0.05$ ) in both the liver and BAT when dietary Cu was restricted.

### Body temperature measurements

There was no effect of time on the repeated measurements of body temperature (Table 4). The overall mean body temperature determined on three successive days was decreased more ( $P < 0.05$ ) in the rats fed the CuD diet than in the rats fed either the CuM or CuA diets. The temperature of the CuA animals was greater ( $P < 0.05$ ) than that of the CuM rats.

**Table 1** Effects of graded dietary copper on body compositional variables of rats\*

	Body wt (g)	Carcass wt (g)	Protein (g)	Water (g)	Ash (g)	Fat (g)	Fat (%)
CuA	317 $\pm$ 4	258.6 $\pm$ 3.6	54.5 $\pm$ 1.0	174.4 $\pm$ 2.8	10.2 $\pm$ 0.4 <sup>a</sup>	19.4 $\pm$ 0.6 <sup>a</sup>	7.5 $\pm$ 0.7 <sup>a</sup>
CuM	326 $\pm$ 5	261.1 $\pm$ 3.6	56.9 $\pm$ 1.1	170.4 $\pm$ 2.5	8.9 $\pm$ 0.4 <sup>b</sup>	24.4 $\pm$ 1.5 <sup>b</sup>	9.3 $\pm$ 0.9 <sup>b</sup>
CuD	318 $\pm$ 7	262.4 $\pm$ 5.9	55.7 $\pm$ 1.8	170.1 $\pm$ 3.1	9.3 $\pm$ 0.4 <sup>b</sup>	26.1 $\pm$ 1.3 <sup>b</sup>	10.2 $\pm$ 0.8 <sup>b</sup>

\*Values are mean  $\pm$  SE. Values in a column with different superscripts are significantly different,  $P < 0.05$  (Tukey's contrasts).

**Table 2** Effects of dietary copper restriction on blood biochemical indices of copper status\*

	Hematocrit (L)	Hemoglobin (g/L)	Plasma		
			Cholesterol (mmol/L)	Ceruloplasmin (mg/L)	Copper (μmol/L)
CuA	0.46 ± 0.01 <sup>a</sup>	153 ± 1 <sup>a</sup>	78 ± 4 <sup>a</sup>	47.6 ± 3.6 <sup>a</sup>	1.14 ± 0.08 <sup>a</sup>
CuM	0.43 ± 0.01 <sup>b</sup>	148 ± 2 <sup>b</sup>	92 ± 6 <sup>b</sup>	33.4 ± 2.6 <sup>b</sup>	0.87 ± 0.05 <sup>b</sup>
CuD	0.43 ± 0.01 <sup>b</sup>	142 ± 1 <sup>b</sup>	100 ± 4 <sup>b</sup>	6.4 ± 2.6 <sup>c</sup>	0.40 ± 0.01 <sup>c</sup>

\*Values are mean ± SE. Values in a column with different superscripts are significantly different, *P* < 0.05 (Tukey's contrasts).

**Thyroid hormone status**

Serum T<sub>4</sub> concentrations were decreased (*P* < 0.05) and TSH concentrations increased (*P* < 0.05) in the CuM and CuD rats as compared with the CuA rats (Table 5). Circulating T<sub>3</sub> concentrations were less (*P* < 0.05) in the Cu-restricted animals.

Dietary Cu restriction adversely impacted peripheral thyroid hormone metabolism (Table 5). Hepatic type I 5'-monodeiodinase activity decreased (*P* < 0.05) in the animals fed CuM and CuD diets. In addition, BAT type II 5'-monodeiodinase activity decreased (*P* < 0.05) in the animals fed the Cu-restricted diets.

Liver T<sub>3</sub> receptors were influenced by dietary Cu. Nuclear T<sub>3</sub> binding in the liver increased (*P* < 0.05) in the Cu-restricted as compared with the CuA animals.

**Discussion**

The present study was designed to examine the role of thyroid hormone status and metabolism on the body temperature of male rats fed diets varying in Cu content. It demonstrates graded mild hypothermia at thermoneutral ambient temperature in response to dietary Cu restriction. Although the decrease in body temperature between the CuA and the CuD rats was 1°C, the magnitude of the body temperature of the CuD was at the limit of the homeothermy for the Sprague-Dawley rat.<sup>28</sup> To our knowledge, this is the first report of impaired thermoregulatory function in Cu-deficient rodents.

One mechanism for this functional impairment is altered thyroid hormone metabolism. Circulating T<sub>4</sub> concentrations are decreased in response to dietary Cu restriction; concomitantly, TSH concentrations are increased. These observations suggest an intact thyrotropic axis that responds with

negative feedback signals (i.e., increased TSH) when circulating T<sub>4</sub> decreases in CuD. These findings indicate a failure of the thyroid gland to either synthesize or secrete adequate T<sub>4</sub> in Cu-deprived animals and reflect primary hypothyroidism.

These observations are consistent with the previous finding of altered thyroid function in CuD rats.<sup>13</sup> In response to a thyrotropin-releasing hormone challenge, CuD weanling rats responded with a similar increase in TSH as did CuA rats. However, serum T<sub>4</sub> response was blunted in CuD rats indicating an impairment in T<sub>4</sub> production or release from the thyroid gland.

Dietary Cu restriction also impacted peripheral thyroid hormone metabolism. Similar to a previous report,<sup>29</sup> a significant decrease in hepatic type I 5'-monodeiodinase activity was found in the hypothyroid, Cu-restricted rats. Interestingly, in both the present and previous<sup>29</sup> studies, the ratio of T<sub>4</sub>/T<sub>3</sub> was not affected by dietary Cu restriction. This observation is apparently the outcome of an effect of hypothyroidism on type II 5'-monodeiodinase activity. Hypothyroidism is associated with increased circulating 3,3',5'-triiodothyronine (rT<sub>3</sub>) which exerts a negative feedback control on type II 5'-monodeiodinase activity in some peripheral tissues.<sup>30</sup> Plasma rT<sub>3</sub> concentrations are increased in Cu-deficient rats.<sup>29</sup> Thus, decreased T<sub>4</sub> production and reduced conversion of T<sub>4</sub> to T<sub>3</sub> maintained a relatively constant T<sub>4</sub>/T<sub>3</sub> ratio among the rats fed graded dietary Cu.

Differences in dietary Cu also may influence peripheral thyroid hormone metabolism by altering catecholamine synthesis. The Cu-containing enzyme, dopamine-β-monooxygenase, regulates the synthesis of norepinephrine from dopamine in the sympathetic nervous system.<sup>31</sup> Copper-deficient rodents have decreased concentrations of norepinephrine and increased concentrations of dopamine in

**Table 3** Liver mineral concentrations and enzyme activities of liver and brown adipose tissues of rats fed diets varying in copper content\*

	Liver		Cytochrome c oxidase (nmol/min/mg of protein)		Glutathione peroxidase (nmol NADPH/min/mg of protein)	
	Cu (μg/g)	Fe (μg/g)	Liver	BAT	Liver	BAT
	CuA	5.0 ± 0.1 <sup>a</sup>	390 ± 48 <sup>a</sup>	211 ± 10 <sup>a</sup>	1606 ± 113 <sup>a</sup>	1868 ± 62 <sup>a</sup>
CuM	4.5 ± 0.2 <sup>b</sup>	359 ± 22 <sup>a</sup>	162 ± 8 <sup>b</sup>	957 ± 109 <sup>b</sup>	1520 ± 71 <sup>b</sup>	116 ± 4 <sup>b</sup>
CuD	2.5 ± 0.01 <sup>c</sup>	757 ± 42 <sup>b</sup>	151 ± 7 <sup>b</sup>	717 ± 105 <sup>c</sup>	1311 ± 81 <sup>c</sup>	108 ± 4 <sup>c</sup>

\*Values are mean ± SE. Values in a column with different superscripts are significantly different, *P* < 0.05 (Tukey contrasts).

**Table 4** Body temperatures (°C) of rats fed graded dietary copper\*

	Day			Average
	1	2	3	
CuA	38.2 ± 0.1	38.3 ± 0.1	38.2 ± 0.1	38.2 ± 0.1 <sup>a</sup>
CuM	38.0 ± 0.1	37.7 ± 0.1	37.6 ± 0.1	37.7 ± 0.1 <sup>b</sup>
CuD	37.3 ± 0.1	37.1 ± 0.1	37.1 ± 0.1	37.2 ± 0.1 <sup>c</sup>

\*Values are mean ± SE. Values in a column with different superscripts are significantly different,  $P < 0.05$  (Tukey's contrasts).

many tissues, including the central nervous system and peripheral tissues.<sup>32</sup> Studies have shown reduced rates of norepinephrine synthesis in the hearts of Cu-deficient rats; supplementation of Cu-deficient rats rapidly restores cardiac norepinephrine content.<sup>33</sup> Importantly,  $\alpha_1$ -adrenergic stimulation is needed to increase BAT type II 5'-monodeiodinase activity in response to a cold stressor.<sup>34</sup> The findings of reduced body temperature and decreased type II 5'-monodeiodinase activity are consistent with a regulatory role of Cu in norepinephrine synthesis.

To investigate further the effects of Cu deficiency on thyroid hormone metabolism, nuclear binding of  $T_3$  was examined. An inverse relationship between nuclear receptor binding of  $T_3$  and Cu status was observed. This finding is consistent with an up-regulation of  $T_3$  receptors in response to the decreased circulating concentrations of both  $T_4$  and  $T_3$  and indicates a cellular adaptation to hypothyroidism.

The reduction in hepatic type I 5'-monodeiodinase activity was associated with decreased activity of glutathione peroxidase, a selenium-containing enzyme,<sup>35</sup> suggesting an indirect influence of dietary Cu on tissue selenium status. The mechanism of the interaction of Cu deficiency and tissue depletion of glutathione peroxidase is unknown at present. It has been suggested that Cu deficiency may indirectly affect selenogluthathione peroxidase because of reductions in Cu-zinc superoxide dismutase activity<sup>36</sup> and/or alterations in glutathione metabolism.<sup>37</sup>

Another hypothesis is that Cu deficiency may be involved in the regulation of selenoprotein synthesis independently of the antioxidant defense system. As seen with Se-glutathione peroxidase, type I 5'-monodeiodinase is expressed from mRNA containing a unique codon for selenocysteine that is required for enzymatic activity.<sup>38</sup> In

addition, there is a stem-loop structure involving the UGA codon and an adjacent sequence necessary for selenocysteine incorporation into some bacterial enzymes, including formate dehydrogenase and glycine reductase.<sup>39</sup> In rats and humans, type I 5'-monodeiodinase requires the 3' untranslated region, the primary sequence of which is not totally similar to that of Se-glutathione peroxidase, but can substitute for it.<sup>40</sup> Mutations in the stem-loop structure prevent or cause a reduction in the translation of type I 5'-deiodinase.<sup>38</sup> This selenocysteine insertion sequence is a critical structural characteristic that is indispensable for proper translation for type I 5'-deiodinase and Se-glutathione peroxidase.<sup>38</sup> Furthermore, these findings suggest the possibility of a common, regulatory pathway for these enzymes at the molecular level. The reduced hepatic Se-glutathione peroxidase mRNA concentration and enzyme activity found in Cu-deficient animals strongly suggest that Cu may play an important role in selenoenzyme metabolism.<sup>36</sup>

In addition to the role of thyroid hormone metabolism on temperature regulation, cytochrome c oxidase, a Cu-containing enzyme, may be important. This enzyme, which regulates electron transport in the mitochondria and hence energy status of the cell, has been shown to be reduced in the livers and skeletal muscles of children with Menkes' Kinky Hair Syndrome.<sup>13,14</sup> These children are characterized by the clinical signs of hypothermia, reduced spontaneous activity, hypotonia, and lethargy. Similarly, decreased cytochrome c oxidase activities in liver and BAT paralleled the reduced body temperatures of the rats in response to graded dietary Cu.

The relationship between reduced organ cytochrome c oxidase activity and altered energy metabolism in Cu deficiency is unclear. Despite significant decreases in cytochrome c oxidase activity in a variety of organs, disturbances in steady-state levels of adenine nucleotides have not been observed universally. Studies in suckling mice<sup>41</sup> and weanling rats<sup>42</sup> found no alteration in cardiac ATP levels. However, myocardial oxygen consumption rates were significantly decreased, although ADP:O ratios were unaffected, in Cu deficiency.<sup>43</sup> Reduced hepatic ATP concentrations have been found in older Cu-deficient rats, particularly when anemia was not present.<sup>44,45</sup> This suggests that alterations in energy metabolism may occur in rats that are not severely Cu-deficient. In contrast, one study<sup>46</sup> did not find decreases in hepatic ATP concentration in Cu-deficient rats.

**Table 5** Effects of dietary copper on circulating thyroid hormone concentrations, organ monodeiodinase activities and hepatic soluble thyroid hormone receptors\*

	$T_4$ (nmol/L)	$T_3$ (nmol/L)	TSH ( $\mu$ U/L)	Monodeiodinase activity mmol <sup>125</sup> I/min/mg of protein		Liver $T_3$ receptors (fmol <sup>125</sup> I/mg of protein)
				Liver	BAT	
CuA	55 ± 1 <sup>a</sup>	0.97 ± 0.03 <sup>a</sup>	0.55 ± 0.03 <sup>a</sup>	1426 ± 56 <sup>a</sup>	289 ± 10 <sup>a</sup>	4.14 ± 0.41 <sup>a</sup>
CuM	46 ± 1 <sup>b</sup>	0.83 ± 0.02 <sup>b</sup>	0.62 ± 0.04 <sup>b</sup>	799 ± 28 <sup>b</sup>	231 ± 17 <sup>b</sup>	5.53 ± 0.29 <sup>b</sup>
CuD	40 ± 1 <sup>c</sup>	0.72 ± 0.03 <sup>c</sup>	0.74 ± 0.03 <sup>c</sup>	770 ± 43 <sup>b</sup>	186 ± 20 <sup>b</sup>	7.27 ± 0.38 <sup>c</sup>

\*Values are mean ± SE. Values in a column with different superscripts are significantly different,  $P < 0.05$  (Tukey's contrasts).

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In addition to the liver, decreased cytochrome c oxidase activity was found in BAT of Cu-deficient rats. To our knowledge, this is the first report of an adverse effect of Cu restriction on this tissue. Because of the requirement for uncoupled ATP production in this tissue for the maintenance of thermal homeostasis,<sup>3</sup> it is important to delineate whether adenosine nucleotide concentrations are impacted by restricted dietary Cu.

The ratio of BAT to body weight was affected by dietary Cu. This ratio was decreased ( $P < 0.05$ ) in CuD ( $0.70 \pm 0.04$  mg/g) as compared with CuM ( $0.78 \pm 0.03$  mg/g) and CuA ( $0.79 \pm 0.04$  mg/g). Although these data are influenced by the ability to dissect out all BAT from the interscapular space and to remove all visible white adipose tissue and muscle from the BAT, they suggest a reduced thermogenic potential in CuD rats.

In the present study, anemia was not induced with the Cu-restricted diets. Anemia has been shown to impair thermoregulatory function of iron-deficient rodents because of the reduction of oxygen-carrying capacity and the possible regulation by iron status of type I 5'-monodeiodinase activity.<sup>47</sup> Thus, the finding of hypothermia in the present study is related to tissue Cu depletion rather than an interaction between Cu and iron as is seen with anemic Cu-deficient animals.

In contrast to many other studies of metabolic effects of Cu deficiency, the present study used a model of young, adult rats, as compared with weanling animals. This approach was taken to induce marginal Cu deficiency as may be found among humans. In addition, this experimental model provided an opportunity to avoid the anemia of severe Cu deficiency and the attendant complication of reduced oxygen-carrying capacity while eliciting altered tissue iron metabolism as seen with the increased hepatic iron concentration.

The effect of dietary Cu on body composition may provide some insight into the role of Cu on whole body energy status. Although final body weight was not affected by dietary Cu, the chemical composition of the carcass was impacted. Fat content, either in absolute or relative values, was significantly increased in response to dietary Cu restriction. This observation suggests decreased energy expenditure in the Cu-deprived as compared with the CuA rats and is consistent with the markedly reduced cytochrome c oxidase activities in the tissues. Despite the increased insulation, body temperatures were less in the Cu-restricted relative to the CuA rats.

The effects of Cu restriction on indirect measures of energy utilization and body composition assessment were examined recently in male weanling rats.<sup>48</sup> After 7 weeks, rats fed the CuD diet ( $9 \mu\text{mol Cu/kg}$ ) had reduced rates of growth despite similar daily energy intakes as rats fed CuA diet ( $102.2 \mu\text{mol/kg}$ ). Body fat content (absolute and expressed as a percentage of body mass) was decreased in CuD rats ( $65.7$  vs.  $51.5$  g and  $22.7$  vs.  $19.6\%$ ). The authors conclude that Cu deficiency in weanling rats results in a preferential utilization of stored fat as an energy source.

The discrepancies between the findings of the present study and the observations of Hoogeveen et al.<sup>48</sup> may be explained by differences in experimental designs and methods. Responses to restricted dietary Cu may vary on the

basis of age and developmental status of the rats. In addition, methodological differences (chemical analysis versus indirect estimation) may contribute to differences in body composition assessments. Each of these factors need clarification to delineate the effects of Cu restriction on energy metabolism and energy stores of animals.

In summary, dietary Cu restriction was associated with hypothyroidism and decreased peripheral thyroid hormone metabolism; it resulted in hypothermia at  $24^\circ\text{C}$ . The impairment in type I 5'-monodeiodinase activity apparently is associated with an indirect effect of Cu deficiency on selenium metabolism in the liver. Reduced type II 5'-monodeiodinase activity in BAT apparently is related to the induced hypothyroidism and reduced dopamine  $\beta$ -monooxygenase, a Cu-containing enzyme, activity in the sympathetic nervous system. In future studies, it will be useful to determine the functional response of Cu-deficient animals to an acute cold stressor and to discern the role of dopamine- $\beta$ -monooxygenase and the adrenergic component of the sympathetic nervous system on thermoregulatory performance.

## Acknowledgments

The authors wish to thank Kim Michelsen, Mary Ellen Sleeper, and Eugene Korynta for excellent technical assistance throughout this project.

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