Impaired Thyroid Hormone Status and Thermoregulation During Cold Exposure of Zinc-Deficient Rats

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Summary

Forty-five male, weaning Sprague-Dawley rats were matched by weight into three groups (n = 15). One group was fed ad libitum a semipurified diet containing all essential nutrients and 30 ppm of zinc (control). A second group was fed ad libitum a similar diet but with a deficient zinc intake of < 1 ppm (ZnD). A third group was pair-fed (PF) the control diet in amounts equal to that consumed by the matched ZnD animals. After 42 days, the animals were fasted for 12 hr then five animals from each group were sacrificed and the remainder was exposed to 3 °C for 6 hr. Rectal temperatures were lower (p < 0.05) in ZnD at 23 °C and during cold exposure. Plasma thyroid hormone (T4) and triiodothyronine (T3) concentrations were reduced (p < 0.05) at room temperature in ZnD rats. During cold exposure, the ZnD animals had depressed (p < 0.05) plasma thyrotropin, T4 and T3 concentrations. Thus, ZnD adversely affects thermoregulatory performance of rats acutely exposed to cold by influencing thyroid hormone metabolism.

Key words

Zinc — Thyroid Hormones — Temperature Regulation — Cold

Introduction

As an essential nutrient, zinc (Zn) is required for many biological processes. Some Zn-dependent functions include cell division, tissue development and growth, behavior, immunocompetence, neurophysiological performance and hormone metabolism (Mills 1989). One aspect of Zn-dependent endocrine function is the alteration of thyroid hormone metabolism and energy expenditure in animals and humans consuming diets low in Zn.

Morley, Gordon and Hershman (1980) reported that severe Zn deficiency in rats was associated with reduced circulating thyroxine (T4) and triiodothyronine (T3) concentrations. Preliminary evidence from human investiga-

Acute exposure in a cold environment stimulates the sympathetic nervous system and thyroid hormone metabolism to initiate non-shivering thermogenesis and to increase heat production (Gale 1973; Fregly 1990). In addition, body fatness or insulation influences the capacity to thermoregulate in the cold (Webster 1974).

The purpose of the present study was to examine the effects of controlled Zn deficiency on circulating thyroid hormones and the thermoregulation of rats at room temperature and during acute cold exposure. The findings suggest that reduced Zn status adversely affects thermoregulatory performance of rats acutely exposed to a cold stressor by impairing thyroid hormone metabolism.

Materials and Methods

Animals

Forty-five weaning, male Sprague-Dawley rats (Harlan-Sprague, Madison, WI) weighing 40–50 g were matched for weight into three experimental groups (n = 15). One group was fed ad libitum a semipurified diet containing 20% (by weight) protein as sprayed egg white, 60% carbohydrate as sucrose and corn starch (45 and 15%, respectively), 10% fat as corn oil, 5% cellulose and all essential nutrients including 30 ppm Zn (control). A second group was fed ad libitum a diet similar in composition but deficient in Zn (ZnD); the Zn concentration was < 1 ppm. Animals in a third group were pair-fed (PF) the control diet in amounts equal to that consumed by the paired ZnD rats. The PF regimen was used to control for effects caused by reduced food intake associated with ZnD while maintaining adequate Zn intake. Food intake was determined daily. The animals were housed individually in stainless steel cages with wire-mesh bottoms in a controlled temperature environment (23–24 °C) with a 12 hr light-dark cycle (lights on at 0600). Distilled-deionized water was available ad libitum to all animals.

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Table 1 Effects of zinc deficiency (ZD) and pair-feeding (PF) on plasma concentrations of thyrotropin (TSH), total thyroxine (T₄) and total triiodothyronine (T₃) of rats maintained at room temperature (23 °C) and exposed to cold (3 °C) for four hours. Values are mean ± SE.

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Group</th>
<th>TSH mU/l</th>
<th>Total T₄ nmol/l</th>
<th>Total T₃ nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 °C</td>
<td>5</td>
<td>Control</td>
<td>66 ± 4</td>
<td>33 ± 4</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZD</td>
<td>53 ± 4</td>
<td>22 ± 3*</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF</td>
<td>59 ± 4</td>
<td>30 ± 3</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>3 °C</td>
<td>10</td>
<td>Control</td>
<td>76 ± 2</td>
<td>49 ± 3*</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZD</td>
<td>58 ± 2*</td>
<td>28 ± 2*</td>
<td>1.1 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF</td>
<td>68 ± 2</td>
<td>42 ± 3</td>
<td>1.6 ± 0.1</td>
</tr>
</tbody>
</table>

*Significantly (p < 0.05) different from other treatment groups at a given temperature.

Temperature measurement and cold exposure

After 42 days, the animals were fasted for 12 hr. Rectal or core temperatures were measured with a calibrated thermistor (YSI model 701, Yellow Springs, OH) that was inserted 10 cm into the rectum. Five animals from each group were anesthetized with pentobarbital (50 mg/kg body weight). Blood was obtained by cardiac puncture and collected into heparinized plastic syringes. The animals then were killed by exsanguination.

The remaining 10 animals in each group were placed in an environmental chamber maintained at 3 ± 0.1 °C (mean ± SE), 50% relative humidity with an air flow of less than 15 m/min for 4 hr without food or water. Rectal temperature was measured at room temperature (23 °C) and every hour during the cold exposure. The animals then were killed and blood was obtained as described above.

Measurement of tissue zinc

Liver samples were removed after sacrifice and wet ashed by using standard procedures (AAAC 1960). Liver Zn content was determined by atomic absorption spectrophotometry and was verified by using a certified standard. Bovine liver standards (National Institute of Standards and Technology, Washington, D.C., Standard Reference Material #1577a) were analyzed for Zn concentration to evaluate our methodologic accuracy. The analyzed Zn concentration of the liver standard was 126 ± 1 μg/g as compared to the certified value of 130 ± 13 μg/g.

Hormone assays

The measurement of thyrotropin (TSH), T₄ and T₃ in plasma was performed by using radioimmunoassays. The TSH analytical procedure has been described elsewhere (Roohk, Azukizawa, Dik Stethen, Ogihara and Hershman 1979). The T₄ and T₃ concentrations were determined by using commercial kits (Diagnostic Products Corporation, Los Angeles, CA).

Body composition

Each animal was prepared for direct chemical analysis by removing all hair and gut contents. The rats were weighed and frozen for later analysis. Three aliquots of finely ground and homogenized sample were used for proximate chemical analysis. Dry matter was determined by oven-drying at 105 °C. Protein was determined by the micro-Kjeldahl method, and total fat by the Foss-let procedure (AAAC 1980).

Fig. 1 Rectal temperatures of rats with different zinc status at room temperature (23 °C) and during acute cold exposure at 3 °C. Asterisks indicate a significant (p < 0.05) difference between the zinc-deficient group and other treatment groups.

Statistical analysis

A one-way analysis of variance (SAS 1989) was used to determine a significant difference among treatments. Differences between treatments were determined by the Scheffé post hoc test (Scheffé 1959).

Results

Zinc deficiency depressed growth. The ZnD and PF consumed less (p < 0.05) food (6.9 ± 1.0 and 7.0 ± 1.1 g/d, respectively) than the control rats (16.1 ± 0.7 g/d). The ZnD rats (94 ± 3 g) weighed less (p < 0.05) than the PF (116 ± 2 g) and control animals (290 ± 5 g). The PF animals also weighed less (p < 0.05) than the control rats.

These differences in body weight did not parallel the observed differences in body composition. The percent of body weight as fat was similar (p > 0.05) for the ZnD and PF (10 ± 2 and 12 ± 1%, respectively) but less (p < 0.05) than that for the controls (22 ± 1%).

Liver Zn concentration, an index of zinc status, was lower (p < 0.05) in the ZnD (1.25 ± 0.05 μmol/g dry weight) than in the PF or control animals (1.53 ± 0.04 and 1.59 ± 0.03 μmol/g dry weight, respectively).

Zinc deficiency influenced circulating thyroid hormone concentrations (Table 1). At room temperature, the ZnD rats had a reduced (p < 0.05) plasma T₄ concentration, with a tendency for diminished plasma TSH and T₃ concentrations, relative to control animals. In the cold, however, TSH, T₄ and T₃ concentrations were reduced (p < 0.05) in the ZnD rats as compared to PF and control animals.

These alterations in plasma hormone concentrations were associated with differences in body temperature (Figure 1). At 23 °C, the ZnD rats had rectal or core temperatures that were lower (p < 0.05) than the control and PF animals. During cold exposure, the ZnD animals demonstrated a marked decline (p < ) in core temperature resulting in the
lowest (p < 0.05) body temperatures throughout the cold exposure. The average rate of cooling of central body temperature was greater (p < 0.05) in ZnD (0.1 ± 0.001 °C/hr) than in control and PF rats (0.04 ± 0.001 and 0.04 ± 0.001 °C/hr, respectively).

Discussion and Conclusions

The findings of the present study indicate that severe zinc deficiency impairs thyroid hormone status and adversely affects thermoregulatory function during acute cold exposure. These effects apparently are independent of the influence of the starvation associated with Zn deficiency.

When young, growing animals are fed a diet low in Zn, growth is depressed as the result of cyclical feeding behavior and a reduction in food intake (Chesters and Quarterman 1970). This coincides with a reduced activity of Zn-dependent enzymes needed for cell division and growth (Williams and Chesters 1970).

Starvation has been shown to influence thyroid hormone metabolism. Rats starved for up to six days had reductions in circulating TSH, T4 and T3 concentrations relative to nonfasted animals (Harris, Fang, Azizi, Lipworth, Vagenakis and Braverman 1978; Connors, DeVito and Hedge 1985). Similarly, rodents undergoing partial food restriction (a 33% reduction in food intake) for up to 12 days demonstrated significant reductions in plasma T3 concentration (van Doorn, van der Heide and Roelfsema 1984). It has been suggested that the starvation or semistarvation-induced alterations in thyroid hormone metabolism are related to a decrease in the stimulatory input of thyrotropin-releasing hormone (TRH) at the pituitary (Connors, DeVito and Hedge 1985).

Morley, Gordon and Hershman (1980) reported significantly reduced serum T4 concentrations in young rats fed a diet containing less than 1 ppm Zn for 19 days in comparison to PF and control animals fed Zn-adequate diets. The ZnD and PF animals had significantly reduced serum T3 concentrations with the ZnD values significantly less than those of the PF. Serum TSH concentrations were slightly less for the ZnD and PF than for controls. Hypothalamic TRH concentration was significantly decreased in ZnD. These results were consistent with a reduction of TRH biosynthesis and release by the hypothalamus and a possible inhibition of T4 to T3 conversion.

Similarly, plasma T4 concentration at 23 °C was significantly less in the ZnD than the control and PF rats in the present study. Also, TSH and T3 concentrations were depressed in ZnD and PF relative to controls. These observations may be attributed to a combined effect of chronic Zn deficiency and semistarvation.

In contrast to the animals maintained at 23 °C, the effect of ZnD per se on thyroid hormones was demonstrated in the cold-exposed animals. In addition to an inability to maintain core temperature during the cold exposure, the ZnD rats had a small but significant decrease in plasma TSH concentration. Plasma T4 and T3 concentrations were also significantly depressed in the ZnD rats. Although the ZnD animals exhibited a small relative increase in the concentration of these hormones, compared to the concentration in animals maintained at 23 °C, it was negligible in comparison to that seen in the control and PF animals.

Other factors suggest an important role of Zn in regulating thermoregulatory performance. The finding of similar relative body fatness in the ZnD and PF rats, with significantly depressed food Zn concentration and reduced core temperatures in the ZnD group, indicates a role of Zn status, independent of caloric restriction, in regulating thyroid hormone metabolism. Thus, apparently rats with reduced food intake and body insulation similar to the ZnD rats, but adequate Zn status, exhibit a pattern of thyroid hormone response to acute cold exposure similar to control animals.

The physiologic response of rodents acutely exposed to cold is to increase non-shivering thermogenesis by activation of the brown adipose tissue (BAT) by mechanisms involving the sympathetic nervous system (Himms-Hagen 1985). The adrenergic activity serves to uncouple oxidative phosphorylation in BAT and increase heat production (Cannon and Nedergaard 1985). It also acts to stimulate the production of T3 in BAT by increasing the activity of type II 5′-monodeiodinase without changes in circulating T4 or T3 concentrations (Silva and Larsen 1985). Furthermore, BAT-produced T3 is required for the full response of uncoupled oxidative phosphorylation and BAT 5′-deiodinase is essential in providing the appropriate BAT T3 content at euthyroid plasma concentrations of T4 and T3 for cold-induced thermogenesis in rodents (Bianco and Silva 1987).

The exact role of Zn in regulating thyroid hormone metabolism is not known. Some sites of regulation of thyroid hormone metabolism have been studied in animals with different Zn status. The observations of Morley, Gordon and Hershman (1980) suggested that peripheral deiodination of T4 was a possible site of Zn influence. Subsequent investigations (Fujimoto, Ino, Higashi, Matsuda, Kashihara and Nakashima 1986; Oliver, Sachan, Su and Applehans 1987) yielded conflicting results on the in vitro hepatic monodeiodination of T4 to T3 in ZnD rats.

Recently, an inhibition of processing of pro-TRH peptides has been reported in the pituitaries and extrahypothalamic brains of ZnD rats (Pekary, Lukaski, Menas and Hershman in press). These findings suggest that ZnD impairs the biosynthesis of TRH precursors with a C-terminal glycine probably by reducing the activity of Zn-containing post-translational processing enzymes such as membrane-associated endopeptidase and carboxypeptidase H.

Another putative mechanism of Zn action in thyroid hormone metabolism is at the cell membrane. It has also been shown that Zn may be needed for thyroid hormone attachment to receptors (Coward and Wilson 1984; Ramirez, Halwer, Shapiro and Sachs 1991).

Although the mechanism of action of Zn in thyroid hormone metabolism is not well understood, Zn may be required for some aspects of it. Additional investigation is needed to clarify this important biological function of Zn.
The results of the present study indicate that rats fed a diet very low in Zn and maintained at 23 °C demonstrate a mild hypothermia and have circulating TSH and thyroid hormone concentrations indicative of chronic semistarvation in combination with Zn deficiency. When exposed to cold, however, Zn-deficient rats have a blunted response in thyroid hormone metabolism and impaired thermoregulatory performance. The physiological mechanism associated with this observation remains to be determined.

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