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Consumption of a moderately Zn-deficient and Zn-supplemented diet affects soluble protein expression in rat soleus muscle

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Abstract

Zinc deficiency negatively affects muscle function, but there are limited biochemical data identifying the cause of this reduction in function. The objective of the present study was to identify soluble proteins in rat soleus muscle that were responsive to different levels of dietary zinc. Rats ($n=21$) were fed diets containing three concentrations of zinc: 5, 30 and 200 ppm for 42 days. There was no difference in body weights of the rats consuming the 5-ppm zinc diet compared to the rats consuming the 30- or 200-ppm zinc diets; however, bone zinc levels were significantly decreased in the 5-ppm dietary zinc group. Individual soluble protein fractions were isolated from these muscles and the samples were prepared for two-dimensional polyacrylamide gel electrophoresis. The expression levels of four proteins were significantly depressed by dietary Zn depletion and supplementation, S-glutathiolated carbonic anhydrase, myosin light polypeptide 3, heat shock protein 20 and heart fatty acid binding protein. This is the first report that indicates that both Zn depletion and supplementation result in protein expression profiles that may negatively affect skeletal muscle function. These results indicate that there are specific signaling pathways that require proper Zn nutriture for maintaining optimal muscle function and suggest that the consumption of pharmacologic doses of Zn may be detrimental to muscle function.
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1. Introduction

Skeletal muscle contains over half of the whole-body Zn, with estimates of approximately 57%. The next largest pool of Zn within the whole body is the bone, which contains approximately 29% [1]. Although the muscles contain most of the whole-body Zn, muscle Zn levels remain relatively constant during Zn deficiency. Consumption of a diet containing 0.7 mg Zn/kg body weight for 35 days caused a reduction in slow-twitch oxidative fibers and an increase in fast-twitch glycolytic fibers in rat soleus muscle [2]. A separate study in which rats consumed a diet containing 1.1 mg Zn/kg body weight for 28 days also caused a decrease in the size of fibers containing fast ATPase [3]. Consequently, the reduction in body weight observed during Zn depletion could be partly due to this decrease in muscle fiber number and size.

Early studies found a relationship between Zn and muscle function. The Zn concentration of the muscle correlated with the functional activity and color of the muscle. With the use of porcine muscle, the serratus ventralis (dark in color and highly active) contained 72% more Zn on a dry, fat-free basis compared to the lighter colored and less active longissimus dorsi. The higher Zn content was associated with the myofibril and nuclear fraction [4]. Zn simply added to the buffer containing a sartorius muscle preparation resulted in the potentiation of its twitch tension and the prolonging of twitch parameters, with no effect on tetanus tension. Thus, the addition of Zn effectively prolonged the active state of the muscle [5]. In a study which used female subjects, it was found that Zn supplementation (135 mg Zn/24 h for 14 days) resulted in a

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significant increase in both isokinetic strength and isometric endurance as measured using knee extension exercises [6]. A more recent study involving male subjects found that consumption of a Zn-depleted diet (0.3 mg Zn/day) for 33 to 41 days resulted in a significant reduction in the total work capacity of the knee extensor and shoulder extensor and flexor muscles [7].

The available data indicate that Zn deficiency affects muscle function. However, there are only limited biochemical data identifying potential causes for the reduction in work capacity, etc., discussed above. In this report, data are presented indicating that the expression of several soluble proteins from the rat soleus muscle is affected by dietary Zn depletion and supplementation. These proteins, separated by two-dimensional gel electrophoresis and identified by MALDI TOF mass spectrometry, are involved in the composition of muscle fibers, in energy metabolism, and in protection against oxidative stress.

2. Materials and methods

2.1. Animals and diets

Male, Sprague-Dawley weanling rats ($n=21$; Charles River Laboratories, Wilmington, MA, USA) were housed individually in stainless steel wire-bottom cages in a temperature-controlled room (22±2°C) with controlled lighting (0700 to 1900 h). All experiments were approved by the Institutional Animal Care and Use Committee at the United States Army Research Institute for Environmental Medicine. In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals" as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The initial body weight of the rats was 125 to 149 g. The rats were fed a stock diet (LM-485, Harlan Teklad, Indianapolis, IN, USA) for 14 days, then divided into three groups consisting of seven rats each based on equal average body weight for each group. Post hoc power analysis of previous data measuring the effect of changes in dietary Zn on femur Zn levels indicated that five to seven rats per dietary group would be sufficient for detecting significant treatment differences (effect size=0.95; power=0.79) [8]. The rats were then fed test diets containing fixed protein (12% from sprayed dried egg whites) and incremental amounts of Zn carbonate (5, 30, 200 ppm).

The diet was formulated to approximate that of soldier rations (MREs). The protein content of MREs has decreased from 14% to 12% of total calories [9]. For marines, the reported protein intake (43±7 g/day) is about 75% of the RDA of ~60 g/day for males fed to energy balance [10]. Energy balance has a direct influence on apparent protein needs, with the amount of dietary protein needed to maintain nitrogen balance increasing as energy intake decreases. The % fat in MREs averages between 34% (MRE version VIII, IX, X, X1 and XII) and 38% (MRE version XV) of daily calories [9]. Thus, MREs already exceed the RDA recommendation of 30% total fat. Attempting to further increase caloric content of MREs by increasing the % fat is not desirable, since not only is it atherogenic, but also it would increase MRE weight and package size, both of which are undesirable in the field. Increasing the protein content of the rations would decrease the shelf-life and increase the weight/cube of the package — once deployed, soldiers would choose to take smaller/lighter packages. We use a 12% protein diet to mimic the soldier rations in our animal studies. This permits us to ask soldier-relevant questions, e.g., would supplemental dietary zinc increase/improve translation initiation in muscle tissues?

The diets were prepared according to the LM-485 formulation (80 g cellulose per kilogram of diet) by Research Diets, Inc. (New Brunswick, NJ, USA) [11]. The rats were allowed free access to the test diets and deionized water for 42 days. Body weights were measured daily. Twenty-four-hour food intakes were determined weekly. Spilled food was collected, weighed, and food intakes were corrected accordingly. At Day 43, the rats were sacrificed under CO_2. Blood was extracted from the right ventricle. The soleus skeletal muscle was dissected from both legs, cleaned of adherent tissue, wrapped with gauze soaked in saline and stored at −20°C. The tissue was subsequently stored at −80°C prior to protein extraction. Unused muscles were stored at −80°C for future use in the development of protein extraction methods of different membrane compartments.

2.2. Zinc analysis

Zn concentration was assessed in bone, serum and feed using flame atomic absorption spectroscopy (AAS, Perkin Elmer 2380, Norwalk, CT, USA). For the preparation of serum, the blood was collected via intrathoracic cardiac puncture from anesthetized rats into either Kendall or B-D vacutainer blue-top, trace element-free tubes (>20 ppb Zn by analysis). The whole blood was stored on ice for 30 min to develop the clot, and then it was centrifuged at 1500 rpm for 15 min. The samples were free from hemolysis. The serum sample was stored at −20°C until it was analyzed for Zn by AAS. The serum samples were diluted eightfold with 5% nitric acid prior to AAS analysis.

To isolate the tibia/fibula and femur bones, the intact animals were stored at 4°C overnight, and then the muscle around the bone was removed. The stripped bones were then wrapped in saline-soaked gauze and frozen at −20°C until they were prepared for nitric acid digestion. The bones were thawed to room temperature, and their end-pieces removed by scalpel to obtain the shaft weighing approximately 1.0 g. It was decided to use the shaft, but not the proximal or distal ends that would contain unwanted trabecular bone pieces; with femurs, typically both end-pieces were removed to obtain 1.0 g of the shaft. However, to obtain 1.0 g of tibia/fibula bones some part of the proximal joint was included.

The weights were recorded, and the pieces of bone were then immersed in 5% nitric acid. Digestion was allowed to proceed overnight prior to running the Kjeldhal procedure on a Labconco Micro Digestor (Labconco Corporation, Kansas City, MO, USA) [12]. Both tissues and feed (~1.0 g of each diet) samples were diluted eightfold with 5% nitric acid (trace metal grade, Fisher Scientific, Pittsburgh, PA, USA). Zn AAS was performed by the New Jersey Feed Laboratory (Trenton, NJ, USA). Zn standards, prepared from a reference solution (Fisher Scientific) in 5% nitric acid, were used as an internal control. All analyses were conducted in acid-washed glassware. Recovery tests were performed to confirm the accuracy of above-mentioned method, and the recovery of Zn was 108±1.1% (n=5, CV=2.3%).

2.3. Sample preparation

Individual soleus muscles from individual rats within each diet group were processed separately, rather than being pooled from each leg of a single rat. Each muscle was weighed and then pulverized after freezing in liquid nitrogen. The Soluble/Insoluble Protein Extraction Kit (Bio-Rad Laboratories, Hercules, CA, USA) was used to prepare the soluble protein fraction from a subset of rats (four to five). The number of individual soleus muscles analyzed from each dietary group was five for the 5- and 30-ppm Zn groups, and four for the 200-ppm Zn group. The tissue powder (≤200 mg) was placed in 1-ml lysis buffer containing 0.5 ml protease cocktail (Sigma-Aldrich Corporation, St. Louis, MO, USA) per gram tissue. Following sonication, samples were centrifuged at 15,000×g for 30 min at 4°C. The supernatant was removed and stored at −80°C until isoelectric focusing. The pellet was re-

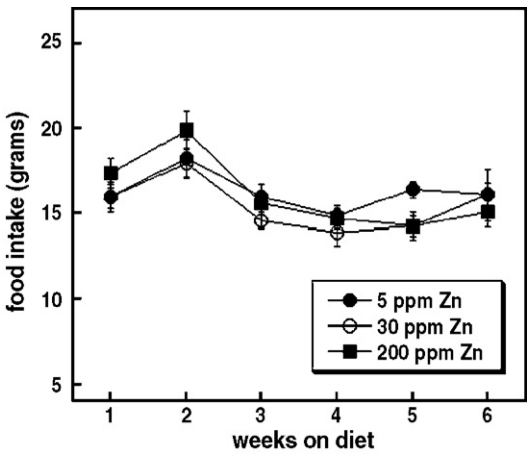

Fig. 1. Twenty-four-hour food intake measurements determined once a week during the 6 weeks of food consumption. Each data point represents the mean±S.E.M. (n=7) of the weight of the diet consumed for that day. The lowercase letter above the data points at Week 5 indicates that the rats consuming the 5-ppm Zn diet exhibited significantly increased intake compared to those consuming the 30-ppm Zn diet (P=.042) and the 200-ppm Zn diet (P=.017).

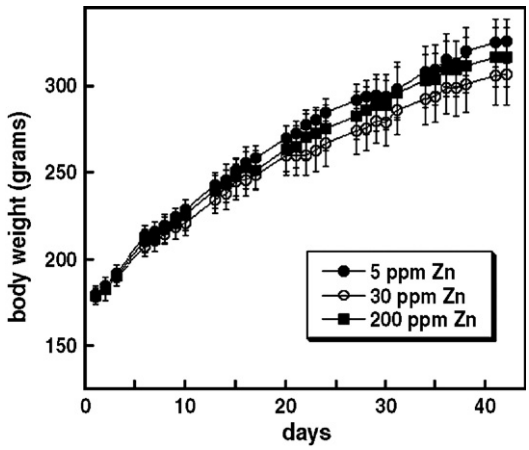

Fig. 2. The body weights of the rats consuming the purified diets containing different Zn concentrations were measured for 42 days. The data points and error bars represent the mean±S.E.M. for each day (n=7). There were no significant differences between the growth of the control rats and those consuming any of the purified diets containing different Zn concentrations.

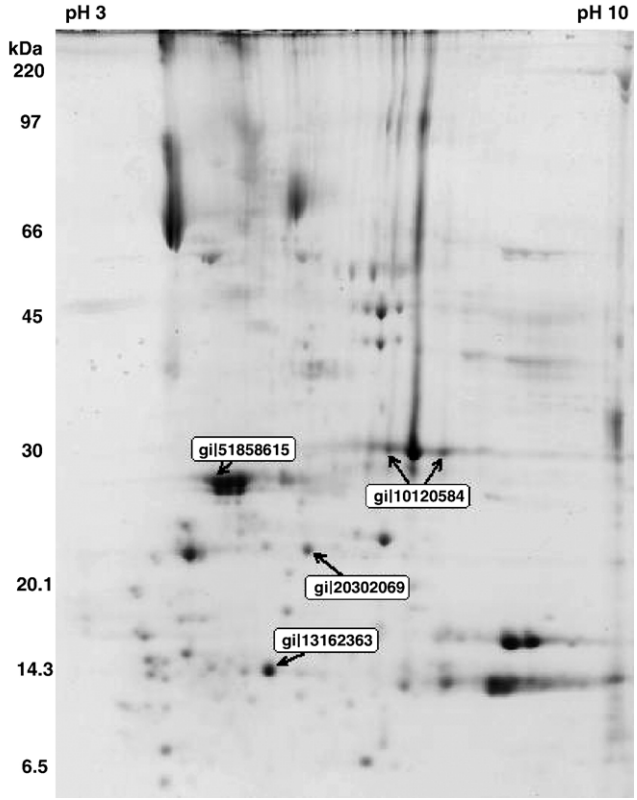

Fig. 3. Two-dimensional 10% to 20% gradient polyacrylamide gel representative image of soleus soluble protein separation from a rat in the 30-ppm Zn dietary group. The molecular weight markers (in kilodalton) migrated as indicated by the numbers to the left of the gel image. The pH is indicated at the top of the gel image, with the acidic end of the IPG strip (pH 3) oriented to the left and the basic end of the IPG strip (pH 10) oriented to the right. The arrows labeled with the accession numbers indicate the proteins that were subsequently identified by MALDI TOF mass spectrometry and which were significantly reduced in the rats consuming the 5- or 200-ppm Zn diets.

extracted, the supernatant discarded and the pellet also stored at −80°C. The protein content of the soluble fractions was measured using the RC/DC protein assay (Bio-Rad Laboratories) [13].

2.4. Two-dimensional gel electrophoresis

Isoelectric focusing, and the second dimension separation of the isoelectic focused proteins, was performed as previously described [14,15]. The gels were fixed in 40% methanol, 10% acetic acid. The separated proteins were stained with Bio-Safe Coomassie Blue G250 protein stain (Bio-Rad Laboratories). The images of the stained gels were digitized and the densities determined using Phoretix 2D software (Nonlinear Dynamics, Newcastle upon Tyne, UK).

2.5. Data analysis

Data were analyzed using one-way ANOVA (StatCrunch 4.0, Integrated Analytics LLC). Post hoc analysis was performed using the multiple comparisons *t*-test ($P \leq .05$; Statmost 1.01, Dataxiom Software, Los Angeles, CA, USA).

2.6. Mass spectrometry

MALDI TOF mass spectrometry was performed at the University of Georgia Proteomic Resource Facility. Differentially expressed protein spots were manually excised from the gels, digested with trypsin, and the peptide masses obtained by MALDI TOF mass spectrometry, as reported previously [14,15].

3. Results

The pattern of food intake of the rats during the course of the 42-day feeding period was similar among the three dietary Zn groups (Fig. 1). The 24-h food intake of the rats consuming the 5-ppm Zn diet exhibited a slight, but significant, increase at Week 5. However, that difference was not evident during the preceding week, nor was it sustained for the following week.

The dietary treatments did not affect the growth of the rats (Fig. 2). At the time of sacrifice, no rats in the 5-ppm Zn group exhibited growth retardation, anorexia or dermal lesions on the extremities, tail or around the eyes. Despite the lower protein content of the experimental diets (12%), the growth rates of these rats were not different from a group of rats receiving LM-485 diets containing approximately 20% protein ($n = 7$, data not shown).

Rats fed the 5-ppm Zn diet exhibited significantly lower ($P < .05$) bone Zn concentrations (expressed as μg Zn/g wet wt tissue) than rats fed the 30-ppm Zn diet [tibia/fibula: 101.1±3.2 (S.E.M.) vs. 67.4±2.8 (S.E.M.); femur:

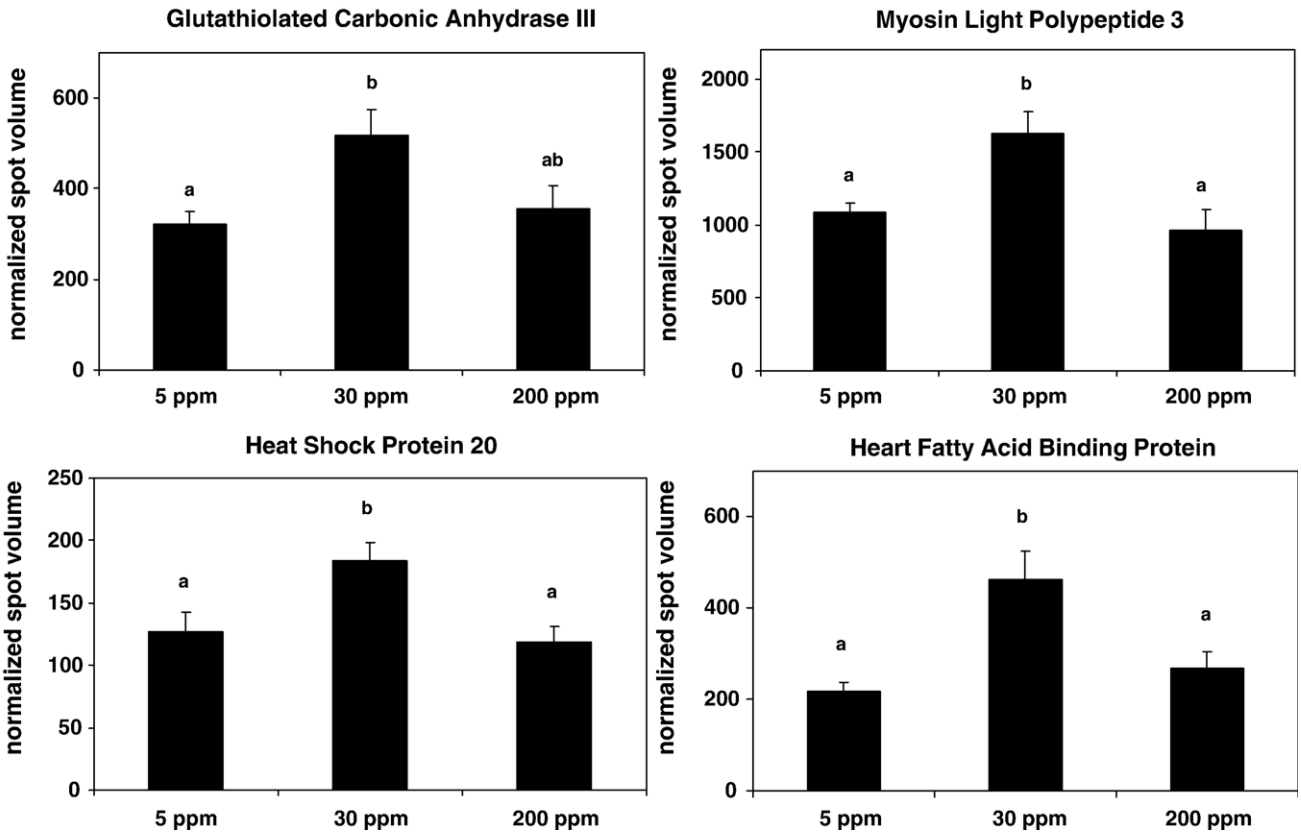

Fig. 4. Bar charts of the normalized volumes of proteins that exhibited Zn-dependent differential protein expression (mean±S.E.M.). The sample size for each Zn diet group is follows: 5 ppm Zn: $n = 5$; 30 ppm Zn: $n = 5$; 200 ppm Zn: $n = 4$. The bars that are significantly different ($P < .05$) from each other have different lowercase letters above the error bars.

131.6±6.6 (S.E.M.) vs. 83.9±2.5 (S.E.M.), respectively]. Consumption of the 200-ppm diet did not result in any further significant increase in bone Zn content [tibia/fibula: 115.8±4.3 (S.E.M.); femur: 149.9±5.4].

Serum Zn concentrations (expressed as μg Zn/ml serum) of rats fed the 5- and 30-ppm Zn diets were not significantly different (0.83±0.06 vs. 1.03±0.04, respectively; $P>.05$). Consumption of the 200-ppm Zn diet, however, caused a significant increase in serum Zn levels [1.28±0.06 (S.E.M.); $P<.05$].

Two-dimensional polyacrylamide gel electrophoresis was used to separate soluble proteins from the soleus muscle. Over 100 soluble proteins were separated by this proteomic method. Fig. 3 shows the representative protein separation pattern from a single 30-ppm soleus muscle preparation. The arrows in Fig. 3 point to protein spots whose identities were determined by MALDI TOF mass spectrometry, including the spots that were differentially expressed as a function of dietary Zn intake. Fig. 4 shows the mean normalized volume±S.E.M. of the spots whose expression was sensitive to dietary Zn and whose identities were determined by MALDI TOF mass spectrometry (5 ppm Zn: $n=5$; 30 ppm Zn: $n=5$; 200 ppm Zn: $n=4$). A significant treatment effect was observed for four proteins. Post hoc analysis determined that the expression of S-glutathiolated carbonic anhydrase III, myosin light polypeptide 3, heat shock protein 20 and heart fatty acid binding protein was significantly reduced in rats consuming the 5-ppm Zn diet compared to the 30-ppm Zn diet. Rats consuming the 200-ppm Zn diet exhibited significantly reduced expression of three of these proteins compared to the 30-ppm Zn diet group: myosin light polypeptide 3, heat shock protein 20 and heart fatty acid binding protein. Expression of S-glutathiolated carbonic anhydrase III was reduced in the 200-ppm Zn diet group compared to the 30-ppm Zn diet group, but the difference did not reach significance ($P=.073$).

4. Discussion

This study investigated the effects of dietary Zn on protein expression in rat skeletal muscle. The two-dimensional expression profile of the soleus muscle soluble subproteome indicates the appearance of four proteins (myosin light polypeptide 3, heat shock protein 20, S-glutathiolated carbonic anhydrase III and heart fatty acid binding protein) that were significantly reduced in rats consuming either the moderate-Zn depletion or supplementation diets. The decrease in these proteins may adversely affect skeletal muscle function in vivo, resulting in biochemical changes such as myofibril protein degradation that leads to muscle atrophy, or by altering energy metabolism via decreased fatty acid binding protein expression [16] or antioxidant tone via decreased expression of glutathiolated carbonic anhydrase III.

Results from recent in vitro and in situ studies indicate that high levels of Zn induce the formation of reactive oxygen species and increase lipid peroxidation [17,18]. The mechanism may involve the Zn translocating to the mitochondria and the activation of p53 [18]. The decrease in the expression of the proteins discussed in the current study may be associated with the formation of Zn-induced reactive oxygen species in the rat solei, or in the nerves innervating the solei, of the Zn supplemented animals. However, the hypothesis that dietary supplementation with 200 ppm Zn causes increased formation of reactive oxygen species in muscle tissue or at the neuromuscular junction was not tested in this study.

Much of the research examining the role of Zn status in animal health has focused on frank Zn deficiency. The literature is replete with short-term feeding studies in rats following Zn-deficient (typically 0–2 ppm) or Zn-adequate (20–50 ppm) diets [19–22]. However, the effects of dietary Zn on protein expression in growing rats have not previously been investigated in a dose–response study design, using both a moderately Zn-deficient diet (5 ppm) and a supplemental-Zn diet (200 ppm), for an extended duration (6 weeks). Very few studies have documented the effects of moderate Zn deficiency, which may not be diagnosed clinically because of the lack of reliable Zn status indicators and overt symptoms of deficiency. Moderate Zn deficiency may occur even in developed nations, as classical studies have demonstrated increased growth of Zn-supplemented children from middle to upper socioeconomic classes in the United States and Canada [23,24].

The data show that nutritional status of the rats was affected by the different Zn diets without changing their growth or food consumption. Consequently, the changes observed in the muscle protein expression are occurring due to the differences in Zn in the diet and not growth impairment or differences in caloric intake. Proteomic analyses of skeletal muscle have been used to identify protein expression occurring as a function of muscle type and disease state [25], but this is the first study that has used differential proteomic analysis to identify muscle proteins affected by dietary Zn deficiency and supplementation.

There have been a limited number of studies investigating the effects of Zn deficiency on muscle function. Functional analysis indicates that dietary Zn depletion in humans (0.3 mg Zn/day) caused a significant reduction in the total work capacity, though not affecting the peak force generated by the muscle [7]. Following depletion for 12 to 14 days, the work capacity of the knee extensor was reduced by almost 30%, and that of the shoulder extensor and flexor was reduced by almost 25%. Zn depletion was continued for an additional 21 to 27 days, then the subjects were repleted for an additional 22 to 24 days. Importantly, muscle function was not restored at this time, indicating that the effects of Zn deficiency persisted. One hypothesis is that Zn depletion causes reduced muscle lactate dehydrogenase activity, thereby reducing the clearance of lactic acid during exercise and negatively affecting muscle endurance [7].

Other effects of Zn deficiency that would affect muscle function include the effects of Zn deficiency on the composition of the cellular membranes, including nerve and muscle. In chicks, dietary Zn deficiency caused peripheral neuropathy and a reduction in the sciatic nerve conduction velocity. Nerve conduction velocity was not restored to normal after a week of repletion following 3 weeks of depletion [26]. Similar results were observed in guinea pigs. In addition, the activity of Na,K-ATPase was reduced in Zn-deficient animals [27]. The effects of Zn deficiency on nerve conduction may further affect the response of the muscle to stimulation due to exercise and affect the fiber composition of the muscle and its ability to adapt [3].

The glutathiolation of proteins protects them from modification by oxidation and occurs almost immediately following the initiation of cellular oxidation. Glutathione, besides becoming oxidized and protecting the cell against damage, binds to sulfhydryls on proteins and protects them from oxidation as well [28]. The presence of S-glutathiolated carbonic anyhdrase III suggests that under normal conditions muscle may be under oxidative stress. Since the amount of S-glutathiolated carbonic anyhdrase III is reduced in the Zn-depleted rats, then the muscle may be more susceptible to damage due to oxidative stress compared to rats consuming normal dietary Zn levels. A recent study in humans found that, in red blood cells, total carbonic anhydrase activity, carbonic anhydrase I and II activities were significantly lower in men consuming a diet low in Zn [29]. The reduced activity of red blood cell carbonic anhydrase correlated reduced physiologic responses to peak and submaximal exercise, including oxygen uptake, carbon dioxide output and the respiratory exchange ratio. The biochemical changes in muscle protein expression that were observed in the rats consuming the 5-ppm Zn diet used in the current study are in line with the changes observed in the study of red blood cells from human males consuming the low-Zn diet by Lukaski [29], as both would result in impaired responses to exercise.

The composition of the muscle fiber type is dependent on the metabolic nature of the muscle, slow twitch vs. fast twitch, oxidative vs. glycolytic. Myosin light polypeptide 3 expression is decreased in cardiomyocytes following hypoxia preconditioning [30]. The decrease in the expression of this protein in soleus skeletal muscle following Zn deficiency and supplementation may be an adaptive response as the muscle fibers change from an oxidative to a glycolytic configuration.

Heat shock protein 20 is also named α-crystalline-related, heat shock protein B6. These small heat shock proteins form larger, mixed oligomeric complexes [31–33] and are actin-binding proteins [34,35]; their association with actin being phosphorylation dependent via cGMP protein kinase [36]. These proteins are highly expressed in soleus, and other skeletal muscles, containing primarily fibers consisting of slow type 1 myosin heavy chain [37]. The expression of these small heat shock proteins occurs in response to changes in the stimulus. For example, the amount of heat shock protein 20 decreases following limb immobilization or inactivation by denervation [37–41]. Neural activity-independent factors such as neurotrophic factors are not involved [28], indicating that it is the loading of the muscle that affects expression of heat shock protein 20. This protein has been proposed as a marker for muscle atrophy [40]. However, since the rats consuming the 5- or 200-ppm Zn diet did not exhibit a reduction in their growth rate and body weight, muscle atrophy is not suspected in these rats. Atrophy of the soleus muscles in these rats, however, was not measured.

Heart fatty acid binding protein is involved in shuttling long chain fatty acids within the cytosol of heart and skeletal muscle. Studies using heart fatty acid binding protein null mice found that the solei from these rodents hydrolyzed and esterified less triacylglycerol and oxidized less palmitate [16]. Although the muscles from these mice exhibited an increase in fatty acid oxidation during contraction, their metabolism remained significantly lower than solei from wild-type mice [16].

The mechanism for the Zn-dependent reduction in the amounts of the proteins discussed above may be related to their ubiquitination and subsequent proteosomal degradation via the MURF family of ubiquitin ligases [42]. Future experiments will need to be designed to determine the specific role these changes in the soluble protein fraction of skeletal muscle play in the physiological effects of Zn depletion on contractility and metabolism.

References

[1] Jackson MJ. Physiology of zinc: general aspects. In: Mills CF, editor. Zinc in human biology. London: Springer-Verlag; 1989. p. 1–14.
[2] O'Leary MJ, McClain CJ, Hegarty PVJ. Effect of zinc deficiency on the weight, cellularity and zinc concentration of different skeletal muscles in the post-weanling rat. Br J Nutr 1979;42:487–95.
[3] Maltin CA, Duncan C, Wilson AB, Hesketh JE. Effect of zinc deficiency on muscle fibre type frequencies in the post-weanling rat. Br J Nutr 1983;50:597–604.
[4] Hambidge KM, Casey CE, Krebs NF. Zinc. In: Mertz W, editor. Trace Elements in Human and Animal Nutrition. New York: Academic Press; 1986. p. 1–137.
[5] Isaacson A, Sandow A. Effects of zinc on responses of skeletal muscle. J Gen Physiol 1978;46:655–77.
[6] Knotkrewski M, Gudmundsson M, Backstrom P, Mandroukas K. Zinc and muscle strength and endurance. Acta Physiol Scand 1982;116:309–11.
[7] Van Loan MD, Sutherland B, Lowe NM, Turnlund JR, King JC. The effects of zinc depletion on peak force and total work of knee and shoulder extensor and flexor muscles. Int J Sport Nutr 1999;9:125–35.
[8] Chu Y, Mouat MF, Harris RBS, Coffield JA, Grider A. Water maze performance and changes in serum corticosterone levels in zinc-deprived and pair-fed rats. Physiol Behav 2003;78:569–78.
[9] Sherman DE. Nutrition content of the meal, ready-to-eat 1988–1999. TN-02/3. Natick, MA: USARIEM; 2002.
[10] Hoyt RW, Buller M, DeLaney J, Stultz D, Warren K, Hamlet M, et al. Warfighter physiological status monitoring (WPSM): Energy balance

and thermal status during a 10-day cold weather U.S. Marine Corps infantry officer course field exercise. TN-02/02. Natick, MA: USARIEM; 2001.
[11] Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents — final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[12] Volpe SL, Huang H-W, Larpadisorn K, Lesser II. Effect of chromium supplementation and exercise on body composition, resting metabolic rate and selected biochemical parameters in moderately obese women following an exercise program. J Am Coll Nutr 2001;20:293–306.
[13] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[14] Mouat MF, Kolli K, Orlando R, Hargrove JL, Grider A. The effects of quercetin on SW480 human colon carcinoma cells: a proteomic study. Nutr J 2005;4:11–7.
[15] Chu Y, Mouat MF, Coffield JA, Orlando R, Grider A. Expression of P2X(6), a purinergic receptor subunit, is affected by dietary zinc deficiency in rat hippocampus. Biol Trace Elem Res 2003;91: 77–87.
[16] Binas B, Han X-X, Erol E, Luiken JJFP, Glatz JFC, Dyck DJ, et al. A null mutation in H-FABP only partially inhibits skeletal muscle fatty acid metabolism. Am J Physiol Endocrinol Metab 2003;285:E481–9.
[17] Lin AM, Chen KB, Chao PL. Antioxidative effect of vitamin D3 on zinc-induced oxidative stress in CNS. Ann NY Acad Sci 2005;1053: 319–29.
[18] Rudolf E, Rudolf K, Cervinka M. Zinc induced apoptosis in HEP-2 cancer cells: the role of oxidative stress and mitochondria. Biofactors 2005;23:107–20.
[19] Eberle J, Schmidmayer S, Erben RG, Stangassinger M, Roth HP. Skeletal effects of zinc deficiency in growing rats. J Trace Elem Med Biol 1999;13:21–6.
[20] Hosea HJ, Taylor CG, Wood T, Mollard R, Weiler HA. Zinc-deficient rats have more limited bone recovery during repletion than diet-restricted rats. Exp Biol Med 2004;229:303–11.
[21] Ovesen J, Moller-Madsen B, Thomsen JS, Danscher G, Mosekilde L. The positive effects of zinc on skeletal strength in growing rats. Bone 2001;29:565–70.
[22] Rossi L, Migliaccio S, Corsi A, Marzia M, Bianco P, Teti A, et al. Reduced growth and skeletal changes in zinc-deficient growing rats are due to impaired growth plate activity and inanition. J Nutr 2001;131:1142–6.
[23] Hambidge KM, Hambidge C, Jacobs M, Baum JD. Low-levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediatr Res 1972;6:868–74.
[24] Gibson RS, Vanderkooy PDS, MacDonald AC, Goldman A, Ryan BA, Berry M. A growth-limiting, mild zinc-deficiency syndrome in some southern Ontario boys with low height percentiles. Am J Clin Nutr 1989;49:1266–73.
[25] Okumura N, Hashida-Okumura A, Kita K, Matsubae M, Matsubara T, Takao T, et al. Proteomic analysis of slow- and fast-twitch skeletal muscles. Proteomics 2005;5:2896–906.
[26] O'Dell BL, Conley-Harrison J, Browning JD, Besch-Williford C, Hempe JM, Savage JE. Zinc deficiency and peripheral neuropathy in chicks. Proc Soc Exp Biol Med 1990;194:1–4.
[27] Terril-Robb LA, Clemons DJ, Besch-Williford C, O'Brien DP, O'Dell BL. Morphophysiologic characterization of peripheral neuropathy in zinc-deficient guinea pigs. Proc Soc Exp Biol Med 1996;213:50–8.
[28] Mallis RJ, Hamann MJ, Zhao W, Zhang T, Hendrich S, Thomas JA. Irreversible thiol oxidation in carbonic anhydrase III: protection by S-glutathiolation and detection in aging rats. Biol Chem 2002;383:649–62.
[29] Lukaski HC. Low dietary zinc decreases erythrocyte carbonic anhydrase activities and impairs cardiorespiratory function in men during exercise. Am J Clin Nutr 2005;81:1045–51.
[30] Xu FF, Sun S, Liu XH. Pilot study of proteomic changes of cardiomyocyte induced by hypoxia preconditioning. Acta Chimi Sin 2006;64:543–50.
[31] Sugiyama Y, Suzuki A, Kishikawa M, Akutsu R, Hirose T, Waye MM, et al. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. J Biol Chem 2000;275:1095–104.
[32] Bova MP, McHaourab HS, Han Y, Fung BK. Subunit exchange of small heat shock proteins. Analysis of oligomer formation of alphaA-crystallin and Hsp27 by fluorescence resonance energy transfer and site-directed truncations. J Biol Chem 2000;275:1035–42.
[33] Benndorf R, Sun X, Gilmont RR, Biederman KJ, Molloy MP, Goodmurphy CW, et al. HSP22, a new member of the small heat shock protein superfamily, interacts with mimic of phosphorylated HSP27 (3DHSP27). J Biol Chem 2001;276:26753–61.
[34] Brophy CM, Lamb S, Graham A. The small heat shock-related protein, HSP20, is an actin-associated protein. J Vasc Surg 1999;29:326–33.
[35] Tessier DJ, Komalavilas P, Panitch A, Joshi L, Brophy CM. The small heat shock protein (HSP) 20 is dynamically associated with the actin cross-linking protein actinin. J Surg Res 2003;111:152–7.
[36] Brophy CM, Knoepp L, Xin JD, Pollock JS. Functional expression of NOS 1 in vascular smooth muscle. Am J Physiol Heart Circ Physiol 2000;278:H991–7.
[37] Inaguma YHK, Kato K, Nishida Y. cDNA cloning of a 20-kDa protein (p20) highly homologous to small heat shock proteins: developmental and physiological changes in rat hindlimb muscles. Gene 1996;178:145–50.
[38] Huey KA, Thresher JS, Brophy CM, Roy RR. Inactivity-induced modulation of Hsp20 and Hsp25 content in rat hindlimb muscles. Muscle Nerve 2004;30:95–101.
[39] Isfort RJ, Hinkle RT, Jones MB, Wang F, Greis KD, Sun Y, et al. Proteomic analysis of the atrophying rat soleus muscle following denervation. Electrophoresis 2000;21:2228–34.
[40] Isfort RJ, Wang F, Greis KD, Sun Y, Keough TW, Bodine SC, et al. Proteomic analysis of rat soleus and tibialis anterior muscle following immobilization. J Chromatogr B Analyt Technol Biomed Life Sci 2002;769:323–32.
[41] Isfort RJ, Wang F, Greis KD, Sun Y, Keough TW, Farrar RP, et al. Proteomic analysis of rat soleus muscle undergoing hindlimb suspension-induced atrophy and reweighting hypertrophy. Proteomics 2002;2:543–50.
[42] Witt SH, Granzier H, Witt CC, Labeit S. MURF-1 and MURF-2 target a specific subset of myofibrillar proteins redundantly: towards understanding MURF-dependent muscle ubiquitination. J Mol Biol 2005;350:713–22.