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L-threo 3,4-dihydroxyphenylserine treatment during mouse perinatal and rat postnatal development does not alter the impact of dietary copper deficiency

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Abstract

Dietary copper (Cu) deficiency was induced perinatally in Swiss Albino mice and postnatally in male Holtzman rats to investigate the effect of L-threo 3,4-dihydroxyphenylserine (DOPS) on pup survival and catecholamine levels in a 2×2 factorial design. Mouse dams were placed on one of four treatments 14 days after mating and rats at postnatal day 19 (P19). Treatments were Cu-adequate (Cu +) and Cu-deficient (Cu -) diets with or without DOPS (1 mg/ml) in the drinking water. Mouse pups were killed at P14 and rats at P49. Mortality in Cu - pups was 46% and not significantly improved by DOPS, 39%. A repeat study with mice adding ascorbic acid in the water with DOPS showed no improvement. Compared to Cu + animals, Cu - animals were smaller, anemic and had a 92% reduction in liver Cu. DOPS treatment made no improvement to and in some cases exacerbated the Cu deficiency. Catecholamine levels measured in heart and brain by LCEC showed decreased NE levels and increased DA levels in Cu - animals compared to controls. DOPS treatment did not alter this pattern. Although DOPS was present in treated animal's tissues, survival in mice and catecholamine levels in mice and rats were not altered by the 1 mg/ml dose of DOPS.

Keywords

L-threo 3,4-dihydroxyphenylserine; catecholamines; copper deficiency; development; mice; rats

Introduction

Proper development of all organisms is dependent on timely and adequate levels of several key nutrients during the neonatal periods of life (Wauben and Wainwright 1999). Copper is an essential nutrient for the central nervous system and other biological systems (Davis and Mertz 1987, Prohaska 1987).

Consequences of copper malnutrition on brain development are quite severe. Cases of poor brain development, degeneration of brain tissue, and even death have been reported over several mammalian species. In humans, poor intestinal transport of copper creates a peripheral deficiency known as Menkes' disease, which results in brain degeneration and those afflicted with Menkes' disease rarely live more than three years (Danks et al. 1972). This is due to the loss of the copper efflux transporter ATP7A. Malnutrition in pregnant ewes grazing on pastures low in copper resulted in delivered lambs suffering from neonatal ataxia (Bennetts and

Chapman 1937). Guinea pigs fed a diet deficient in copper showed a reduction in cerebellum size of 50% or more, and rat pups born to copper deficient dams have shown focal necrosis of the cortex, and other neural lesions (Everson et al. 1967, Carlton and Kelly 1969). Prohaska demonstrated in two different early gestation copper deficiency experiments in rats a diminished auditory startle response and abnormal rotorod performance even after being repleted with adequate copper for months (Prohaska and Hoffman 1996, Penland and Prohaska 2004).

Copper's biochemical role is to act as a cofactor in protein function for enzymes such as Cu, Zn-superoxide dismutase, cytochrome *c* oxidase, ceruloplasmin (ferroxidase) and dopamine beta monooxygenase (DBM). Which, if any, of these cuproenzymes might be linked to the neuropathology of copper deficiency is not known. DBM functions to convert dopamine (DA) to norepinephrine (NE) by means of hydroxylation in tissues such as the brain and sympathetic nerves of several other organs. Thomas et al. have shown that most DBM knockout mice, derived from breeding DBM +/- mice, do not survive to birth (Thomas et al. 1995). Fetal tissue from DBM -/- mice was low in NE and elevated in DA. Further work in DBM -/- mice demonstrated an imbalance of NE and DA with NE concentrations being lower and DA concentrations being increased in the heart and most other tissues measured (Thomas et al. 1998). Severe copper deficiency in mice during the neonatal period is associated with pups dying during suckling (Prohaska and Brokate 2002). Brains of copper deficient mice exhibit the same catecholamine pattern, lower NE and higher DA, as DBM -/- mice (Prohaska and Bailey 1993). Is there a connection between decreased NE concentrations and developmental problems and death in developing offspring?

Thomas et al. tested the low NE concentration and death hypothesis in DBM -/- mice using L-threo 3,4-dihydroxyphenylserine (DOPS), a drug that can be directly converted into NE by aromatic L-amino acid decarboxylase (AAD, EC 4.1.1.28) (Thomas et al. 1995). DOPS was supplied in the drinking water to DBM +/- dams and demonstrated that DBM -/- pups could be rescued to birth while subsequent experiments showed subcutaneous injections of DOPS to the pups restored NE concentrations in heart tissue to normal levels (Thomas et al. 1998).

The purpose of the current studies in DBM +/- mice was to examine survivability of mice from copper deficient dams administered DOPS through the drinking water. A secondary purpose was to measure catecholamine concentration in the heart and brain to determine if DOPS had a positive impact on these concentrations following dietary copper deficiency.

Materials and methods

Animal care and diets

Swiss Webster albino mice and 19 day old male weanling Holtzman rats were purchased commercially (Harland Sprague Dawley, Indianapolis, IN, USA). Animals were fed either a purified copperadequate (Cu +) or copper-deficient (Cu -) diet (Teklad Laboratories, Madison, WI, USA). The purified diet was similar to the AIN-76A (Anonymous 1977, Anonymous 1980) diet and contained the following major components (g/kg diet): Sucrose, 500; casein, 200; cornstarch, 150; corn oil, 50; cellulose, 50; modified AIN-76 mineral mix, 35; AIN-76A vitamin mix, 10. The Cu + diet contained 5.6mg Cu/kg and 52 mg Fe/kg. Cupric carbonate was omitted from the AIN-76A mineral mix in the Cu - diet and it contained 0.36mg Cu/kg and 53mg Fe/kg by chemical analysis. Animals were given deionized water with or without DOPS. DOPS was a generous gift of Sumitomo Pharmaceuticals Co., Ltd. (Osaka, Japan).

Pregnant mice were placed on treatment at 14 days after mating. Experiment one consisted of a 2 x 2 factorial design with Cu + and Cu - diets with or without DOPS. Dams in treatments

that delivered pups included $n = 2$ Cu + , $n = 4$ Cu - , $n = 3$ Cu + /DOPS and $n = 4$ Cu - /DOPS. DOPS was added, 1 mg/ml (Thomas et al. 1998), to the drinking water in an amber bottle with silicone stoppers and changed every two days for experiment one. Experiment two used the same design but added ascorbate (Sigma Chemical, St. Louis, MO, USA) to all solutions at a concentration of 2mg/ml to follow a previous design (Thomas et al. 1998). Fifteen dams on treatments were divided into four groups: $n = 3$ Cu+ /DOPS, $n = 4$ for the other three groups. Water with DOPS and ascorbate was changed daily for experiment two. Twenty male Holtzman 19 day old rats were placed on the same four treatment protocols as the mice in experiment one (no added ascorbate). They were housed individually in stainless steel cages at $n = 5$ per group. All animals had free access to diet and drinking water and were maintained at 24°C with 55% relative humidity on a 12-h light cycle (0700–1900 h light). All protocols were approved by the University of Minnesota Animal Care Committee.

Male mice were sacrificed at age 14 days postnatal (P14) in experiment one and P15 in experiment two. Rats were sacrificed at P49. All animals were anesthetized with diethyl ether and decapitated. In the mouse experiments, brains, livers, hearts and blood were harvested. In mouse experiment two, pooling of brain and hearts for animals on the Cu - diet was required. The rat experiment utilized the brain, liver, heart, spleen and blood. All blood was collected in a heparinized tube, and all other tissues were weighed and either processed for biochemical analysis or frozen in liquid nitrogen and stored at -80°C until use. Brains were dissected on a chilled glass plate and separated into medulla oblongata/pons, cerebrum and hypothalamus in the rat experiment. The heart was dissected on a chilled glass plate and separated into atria and ventricles in the rat experiment.

Biochemical analysis

Hemoglobin, where measured, was determined spectrophotometrically as cyanmethemoglobin (Prohaska 1983). Plasma was obtained from micro centrifuge tubes and the activity of the cuproprotein ceruloplasmin (EC 1.16.3.1) was measured by following oxidation of o-dianisidine at 37°C (Prohaska 1991).

Cu and iron analysis

Brains, livers and diet were wet-digested with concentrated HNO₃ (Trace Metal Grade, Fischer Scientific, Pittsburgh, PA, USA) and residue was brought to 4.0 ml with 0.1M HNO₃. Samples were analyzed for Cu and Fe by flame atomic absorption spectroscopy (model 1100 B, Perkin-Elmer, Wellesley, MA, USA).

Catecholamine analysis

Catecholamines were extracted from tissues by adding 9 volumes of cold 0.05M HClO₄ (Fisher) with added dihydroxybenzylamine (DHBA), (Sigma), at 300pmol/ml. The tissue was then homogenized (Tissuemizer, Tekmar Co., Cincinnati, OH, USA) for 30 sec at half maximum speed. The homogenate was centrifuged at 14,500 × g for 15min at 4°C (Model J25I, JA25.50 rotor, Beckman Instruments). After centrifugation, 1ml of the supernatant was transferred to a micro centrifuge tube containing 15 mg of alumina and 0.5 ml of 3M Tris (pH 8.6). The tube was rotated for 15min (Multipurpose Rotator, Scientific Industries Inc, Springfield, MA, USA). Following rotation, the tube was centrifuged for 30 sec and the supernatant was aspirated off. About 1ml of deionized water was added and the tube was then rotated for 5min. The wash sequence was repeated once more. Following removal of the last wash, 200 µl of 60% 0.2M acetic acid and 40% 0.04M phosphoric acid was added to elute the catecholamines from the alumina. The tube was shaken for 10 sec and rotated for 5 min. Following a 30 sec centrifugation, the final supernatant was transferred to a micro insert in an auto sampler vial (National Scientific Company, Duluth, GA, USA) and then into the autosampler (SP8880, Spectra-Physics, San Jose, CA, USA) for analysis or frozen at -20°C.

Catecholamines were fractionated using reverse-phase ion-pairing HPLC with electrochemical detection. Originally, a 4.6×250 -mm analytical column (Ultrasphere ODS $5\mu\text{M}$, Beckman Instruments) was used in conjunction with a mobile phase composed of 0.06M methyl sulphonic acid (Aldrich, Milwaukee, WI, USA), 0.03M phosphoric acid (Fischer), 0.3mM 1-octanesulfonic acid (Sigma) and 0.1mM EDTA (Sigma) pH 2.5 (Bauch 1989). A second column, 4.6×250 -mm (ODS II $5\mu\text{M}$, Regis, Morton Grove, IL, USA), used a mobile phase composed of 0.55mM 1-octanesulfonic acid, 0.1mM EDTA, and 0.1M NaH_2PO_4 (Mallinckrodt, St. Louis, MO) with 4% methanol (Fisher) and adjusted to pH 3 with phosphoric acid (Temam and Prohaska 1999). Both columns were preceded by a 3.2×15 -mm cartridge guard column (Aquapore ODS $7\mu\text{M}$, Brownlee, Perkin Elmer). A column heater (BioAnalytical Systems (BAS) West Lafayette, IN, USA LC-22A) set at 32°C warmed the sample prior to detection. Amperometric detection (BAS LC-4B) utilized a glassy-carbon electrode set at 0.7 V versus Ag/AgCl in a flow cell housing (BAS 17A). Output was recorded and integrated (Peak Simple Chromatography Data Systems, Las Vegas, NV, USA).

Statistical analysis

Data were analyzed by two-way ANOVA (diet and treatment) and significant interactions were compared by Fisher PLSD test, $\alpha = 0.05$: In addition, Student's unpaired *t*-test, $\alpha = 0.05$; was also used when comparing two groups. Data was analyzed with the aid of Statview 4.5 (Abacus Concepts, Berkeley, CA, USA).

Results

Mouse experiment one

Survival of mice in experiment one was lower in dams on the Cu – diet. DOPS had no apparent impact. The Cu – /water group had a mortality rate of 46% (11/24) (95% CI $42 \pm 32\%$). The Cu – /DOPS group had a mortality rate of 39% (14/36) (95% CI $39 \pm 41\%$). Cu + /water (0/18) group and Cu + /DOPS (0/19) group had no pup mortality.

Seven different markers of copper status were analyzed by factorial ANOVA in the P14 mice of experiment one to determine if DOPS had any effect (Table I). Body weight results show a significant interaction between diet and treatment; the Cu – /DOPS mice weighed less than the Cu – /water group. Heart/body weight was altered by both diet and treatment. The Cu + diet mice had lower ratios than the Cu – mice, and the DOPS treated mice had lower ratios than those drinking only deionized water. Brain/body weight ratios showed a significant interaction with the highest values in the smallest mice. Liver copper concentration was higher in Cu + mice, whereas liver iron content was higher in Cu – mice. Liver Fe was higher in Cu – /water mice than Cu – /DOPS mice. Hemoglobin concentration and ceruloplasmin activity showed no significant interactions but did confirm that Cu – groups had lower plasma ceruloplasmin activity and anemia.

NE and DA concentrations of the heart and brain were quantitated by HPLC (Figure 1). While no significant interactions occurred in the heart or brain between the four groups, there were diet effects between the Cu + and the Cu – groups. In the heart, Cu + mice had higher NE concentrations than Cu – mice. The DA values were opposite with Cu – mice having higher concentrations than Cu + mice. The same pattern held true for brain suggesting lower DBM activity in Cu – mice. Treatment with DOPS did not affect catecholamine levels. DOPS concentrations in the hearts averaged 0.19 nmol/g in the Cu + animals and 0.09 nmol/g in the Cu – animals and were not statistically different.

Mouse experiment two

In mouse experiment two, survival was worse than mouse experiment one with mortality occurring in all litters. The Cu – /DOPS mice were impacted the greatest with mortality rates of 73% (30/41) (95% CI $77 \pm 27\%$) while mortality in the Cu – /AA mice was 58% (21/36) (95% CI $54 \pm 26\%$). The two Cu + groups were slightly impacted with the Cu + /DOPS mortality at 8% (2/24) (95% CI $7 \pm 9\%$) and the Cu + /AA mortality at 3% (1/35) (95% CI $3 \pm 4\%$).

Four copper status markers were evaluated in experiment two in pups at P15, due to Cu – litter mortalities (Table II). Body weights were significantly affected by diet but not DOPS treatment. Cu + mice were heavier than the Cu – mice. The heart weight to body weight ratio showed a significant interaction effect. The Cu + mice ratios were lower than the Cu – mice ratios, and the Cu – /DOPS was significantly lower than the Cu – /AA group. A significant diet interaction was seen in brain weight to body weight ratios and in ceruloplasmin activity measurements. In the brain weight to body weight ratios, values for the Cu + mice were lower than Cu – mice. Ceruloplasmin activities were higher in Cu + mice than Cu – mice.

Quantitation of catecholamines in the brain was complicated by sample losses resulting in the Cu + /DOPS mice having an $n = 1$: Ignoring this group, one way ANOVA and post-hoc analysis of brain catecholamines indicated that the Cu + /AA group had higher NE concentrations than the Cu – /AA group (Figure 2). Brain DA was significantly higher in Cu – /AA mice than Cu – /DOPS mice. Heart NE and DA values confirm the results observed in experiment one. Cu + mice have higher NE and lower DA than Cu – mice.

Cu deficiency was associated with lower NE levels in hearts of mouse dams compared to Cu adequate dams, mean (nmol/g) $\pm$ sem (n), Cu + 4.32 ± 0.39 (4), Cu – 2.54 ± 0.47 (7), $P < 0.05$. Heart catecholamines of dams were not influenced by DOPS in the drinking water. DOPS concentration in the Cu – /DOPS dam hearts was found to be 1.81 ± 1.3 (3) nmol/g. Analyses were not performed on Cu + /DOPS dams.

Postnatal rat experiment

In the rat experiment, eight markers of copper status were examined (Table III). Analysis of body weights showed diet and treatment effects. Cu + rats weighed more than Cu – rats and DOPS-treated animals weighed less than rats drinking deionized water. The ventricle weight to body weight ratio analysis showed a significant interaction with the Cu + animals having the lowest ratios. Cu – /water rats had a lower ventricle/body weight ratio than and Cu – /DOPS rats. The brain weight to body weight ratios show significant diet and treatment effects with Cu + rats having lower ratios than Cu – rats and DOPS treated rats groups having higher ratios than water treated rats. The spleen weight to body weight ratios did not show any significant interactions or main effects.

Liver copper concentrations showed a diet effect with values in Cu + rats higher than Cu – rats. Liver iron concentrations also showed a diet effect with higher concentrations in Cu – rats than in Cu + rats. The hemoglobin and ceruloplasmin analysis showed a diet effect with Cu + rats having higher hemoglobin and ceruloplasmin activity than Cu – rats.

Catecholamine analysis showed no significant interactions or main effects for medulla/pons NE and DA (Figure 3). In the ventricle, a diet effect was detected for NE concentrations. Cu + rats had greater NE concentrations than Cu – rats. A significant interaction occurred with DA concentrations. The Cu + rats had the lowest DA concentrations while the Cu – /DOPS rats had lower DA concentrations than Cu – /water rats. Spleen results showed a significant interaction in NE concentrations. Cu + rats had greater concentrations than Cu – rats and Cu – /DOPS rats had higher concentrations than Cu – /water rats. Spleen DA concentrations showed a diet effect with Cu – rats having greater DA concentrations than Cu + rats.

DOPS was measured in four rat tissues, serum, ventricle, spleen and medulla/pons (Table IV). No difference in DOPS levels existed in any of the tissues between Cu + and Cu - rats. Concentration of DOPS was highest in the ventricle but still well below NE levels. Levels of DOPS in brain tissue were less than a tenth of those in the heart. Ventricle DOPS levels in the rats were similar to heart values in mouse dams.

Discussion

Previous work has demonstrated copper is necessary for survival through gestation. Timing of dietary Cu deficiency during pregnancy affects pup survival. Dams placed on the Cu deficient diet at embryonic day 13 (E13) had pup mortality before weaning while dams placed on the diet at E19 had no pup deaths before weaning (Prohaska and Brokate 2002). Cu status indicators (tissue Cu levels, anemia, cardiac hypertrophy and survival) in the current experiments that began at E14, all confirm that the diet treatment for mice was effective at producing severe Cu deficiency. Indicators in rats also are consistent with severe Cu deficiency.

Genetic deletion of the mouse copper transporter, CTR1, (Kuo et al. 2001, Lee et al. 2001) and mouse DBM result in mouse embryonic death. DBM +/- dams treated with DOPS produced viable -/- pups but -/- pup NE levels were lower than +/- and +/- offspring (Thomas et al. 1995).

In the current experiments with DBM +/- mice, DOPS treatment did not reduce the mortality associated with perinatal dietary Cu deficiency. It was determined that DOPS is stable for 48 h, the time between fresh doses, without added ascorbate. Ascorbate was included in the initial rescue experiments with DOPS using DBM -/- mice (Thomas et al. 1995). Therefore, when no effect of DOPS was noted in the first experiment, ascorbate was added to all solutions and the study repeated. However, mortality is still not impacted by DOPS even with added ascorbate.

Thus, the same concentration of DOPS in the water (1 mg/ml) is sufficient to rescue Cu adequate DBM -/- pups but insufficient to have an impact on DBM +/- pups suffering from dietary Cu deficiency. Perhaps, the dose used in the current lactational model was insufficient to alter NE in the pups. HPLC data would support this possibility. Data for the current studies showed that DOPS concentrations in heart tissue of the dams is 10 times higher than pups and well below NE levels in both cases. It is also possible that mouse strain differences or timing of delivery of NE to critical tissues was different in the two studies. Also, mortality of perinatal Cu deficiency may be unrelated to changes in catecholamines.

It is well known that addition of ascorbate to the diet of Cu deficient rodents exacerbates signs of Cu deficiency (Hunt and Carlton 1965). Recent work has shown ascorbate supplemented to cells deficient in Cu induces cell death through apoptosis (Raymond and Johnson 2004). Results from the current study show that ascorbate addition enhanced cardiac hypertrophy in the Cu deficient mice. DOPS seemed to attenuate this process so a well established model of cardiac hypertrophy, postnatal dietary Cu deficiency in rats, was tested.

Ascorbate was not included in the rat studies. DOPS treatment did not attenuate cardiac hypertrophy in the rat model. If anything, DOPS treatment exacerbated signs of Cu deficiency in the rat model. The concentration of DOPS in heart tissue of the rats is similar to the levels measured in mouse dams, suggesting that metabolism of DOPS is similar in these two mammals.

Results of the current studies in mice support previous research that indicates limiting DBM activity following dietary Cu deficiency. In the perinatal mouse models for brain and heart, there is a decrease in NE, an elevation in DA, and a marked increase in the DA/NE ratio. These

observations were made previously in similar mouse models (Prohaska et al. 1990, Prohaska and Bailey 1993). Administration of DOPS to mouse dams in the current experiments during the latter part of gestation and lactation failed to significantly alter catecholamine metabolism in the pups and dams. Not surprising, dietary Cu deficiency did alter brain catecholamines in the postnatal rat model (Prohaska et al. 1990).

The dose and route of DOPS administration in the current mouse and rat experiments were not sufficient to alter catecholamine pool sizes. Steady state concentration of DOPS in the plasma of rats and mouse dams (data not shown) was less than 1nmol/ml despite the supply of DOPS in the drinking water of 1mg/ml (greater than 5000 nmol/ml). This suggests that DOPS was poorly absorbed or actively metabolized by these two mammals. Metabolism of DOPS by AAD in the peripheral nervous system has an impact on DOPS levels in the brain. In fact, Thomas et al. determined AAD must be inhibited with carbidopa to observe an NE increase in the brain, likely due to elevated DOPS (Thomas et al. 1995). This may be why the rat brain DOPS levels in the current studies were much lower than heart or spleen levels.

Injections of DOPS into DBM knockout mice, 4–8 months old, at a dose of 1mg/g body weight restored NE in heart tissues to levels comparable to control animals. Inhibition of AAD via addition of carbidopa caused an increase in brain NE levels in the null mice (Thomas et al. 1995). Perhaps carbidopa treatment will be necessary to elevate NE in brain tissue of our experimental rodents in future experiments. In unpublished work, Cu deficient rats injected with the same dose of DOPS (1 mg/g) without carbidopa also demonstrated a restoration of NE in heart tissue. Future work with DOPS in developing Cu deficient mice and rats needs to be oriented towards delivery of a larger dose to see an increased efficacy to specific brain regions to compensate for peripheral nervous system metabolism. Further work will also be necessary to elucidate the molecular mechanisms for enhanced neonatal mortality following perinatal Cu deficiency.

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References

- Anonymous. Anonymous Report of the AIN Ad Hoc Committee on standards for nutritional studies. *J Nutr* 1977;107:1340–1348. [PubMed: 874577]
- Anonymous. Anonymous Second report of the AIN Ad Hoc Committee on standards for nutritional studies. *J Nutr* 1980;110:1726.
- Bauch HJ, Struwer E, Kelsch U. A general method for the quantitative determination of catecholamines in body fluids using off-line sample pretreatment and HPLC-ED analysis. *Chromatographia* 1989;28:78–84.
- Bennetts HW, Chapman FE. Copper deficiency in sheep in Western Australia: A preliminary account of the etiology of enzootic ataxia of lambs and an anemia of ewes. *Aust Vet J* 1937;13:138–149.
- Carlton WW, Kelly WA. Neural lesions in the offspring of female rats fed a copper-deficient diet. *J Nutr* 1969;97:42–52. [PubMed: 5763444]
- Danks DM, Campbell PE, Stevens BJ, Mayne V, Cartwright E. Menkes's kinky hair syndrome. An inherited defect in copper absorption with widespread effects. *Pediatrics* 1972;50:188–201. [PubMed: 5045349]
- Davis, GK.; Mertz, W. Copper. In: Mertz, W., editor. Trace elements in human and animal nutrition. San Diego CA: Academic Press; 1987. p. 301–364.
- Everson GJ, Tsai HC, Wang TI. Copper deficiency in the guinea pig. *J Nutr* 1967;93:533–540. [PubMed: 6082665]

- Hunt CE, Carlton WW. Cardiovascular lesions associated with experimental copper deficiency in the rabbit. *J Nutr* 1965;87:385–393. [PubMed: 5841854]
- Kuo YM, Zhou B, Cosco D, Gitschier J. The copper transporter CTR1 provides an essential function in mammalian embryonic development. *Proc Natl Acad Sci USA* 2001;98:6836–6841. [PubMed: 11391004]
- Lee J, Prohaska JR, Thiele DJ. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. *Proc Natl Acad Sci USA* 2001;98:6842–6847. [PubMed: 11391005]
- Penland JG, Prohaska JR. Abnormal motor function persists following recovery from perinatal copper deficiency in rats. *J Nutr* 2004;134:1984–1988. [PubMed: 15284387]
- Prohaska JR. Changes in tissue growth, concentrations of copper, iron, cytochrome oxidase and superoxide dismutase subsequent to dietary or genetic copper deficiency in mice. *J Nutr* 1983;113:2048–2058. [PubMed: 6312000]
- Prohaska JR. Functions of trace elements in brain metabolism. *Physiol Rev* 1987;67:858–901. [PubMed: 3299411]
- Prohaska JR. Changes in Cu,Zn-superoxide dismutase, cytochrome c oxidase, glutathione peroxidase and glutathione transferase activities in copper- deficient mice and rats. *J Nutr* 1991;121:355–363. [PubMed: 1848285]
- Prohaska JR, Bailey WR. Persistent regional changes in brain copper, cuproenzymes and catecholamines following perinatal copper deficiency in mice. *J Nutr* 1993;123:1226–1234. [PubMed: 8391562]
- Prohaska JR, Bailey WR, Gross AM, Korte JJ. Effect of dietary copper deficiency on distribution of dopamine and norepinephrine in mice and rats. *J Nutr Biochem* 1990;1:149–154. [PubMed: 15539197]
- Prohaska JR, Brokate B. The timing of perinatal copper deficiency in mice influences offspring survival. *J Nutr* 2002;132:3142–3145. [PubMed: 12368408]
- Prohaska JR, Hoffman RG. Auditory startle response is diminished in rats after recovery from perinatal copper deficiency. *J Nutr* 1996;126:618–627. [PubMed: 8598546]
- Raymond LJ, Johnson WT. Supplemental ascorbate or alpha-tocopherol induces cell death in Cu-deficient HL-60 cells. *Exp Biol Med* 2004;229:885–894.
- Teman PT, Prohaska JR. Copper-deficient rats have altered plasma dihydroxyphenylacetic acid and spleen catecholamine levels. *Nutr. Neurosci* 1999;2:103–111.
- Thomas SA, Matsumoto AM, Palmiter RD. Noradrenaline is essential for mouse fetal development. *Nature* 1995;374:643–646. [PubMed: 7715704]
- Thomas SA, Marck BT, Palmiter RD, Matsumoto AM. Restoration of norepinephrine and reversal of phenotypes in mice lacking dopamine beta-hydroxylase. *J Neurochem* 1998;70:2468–2476. [PubMed: 9603211]
- Wauben IP, Wainwright PE. The influence of neonatal nutrition on behavioral development: A critical appraisal. *Nutr Rev* 1999;57:35–44. [PubMed: 10079701]

Figure 1.

Effects of perinatal Cu deficiency and DOPS treatment on mouse brain and heart NE and DA levels. Each bar represents the mean $\pm$ SEM ($n = 8$) of P14 mice. Heart and brain catecholamine levels were significantly affected by diet but not DOPS treatment when analyzed by factorial ANOVA, $P < 0.05$.

Figure 2.

Effects of perinatal Cu deficiency and DOPS treatment with ascorbate on mouse brain and heart NE and DA levels. Each bar represents the mean $\pm$ SEM ($n = 4$) of P15 mice. Brain Cu + /DOPS ($n = 1$) and Cu + /AA ($n = 3$); had fewer samples. Brain catecholamine data was analyzed by one-way ANOVA excluding the Cu + /DOPS ($n = 1$) sample. NE was significantly higher for the Cu + /AA mice than Cu - /AA mice. For DA the level in Cu - /AA mice was significantly higher than the Cu - /DOPS mice. Heart DA levels of Cu + groups were significantly lower than Cu - groups by factorial ANOVA, $P < 0.05$.

Figure 3.

Effects of postnatal Cu deficiency and DOPS treatment on rat brain, cardiac ventricle and spleen NE and DA levels. Each bar represents the mean $\pm$ SEM ($n = 5$) of P49 rats. Cardiac ventricle NE levels and spleen DA levels of Cu + groups were significantly different than Cu - groups by factorial ANOVA, $P < 0.05$. A significant interaction occurred in cardiac ventricle DA levels ($P < 0.001$) and spleen NE levels ($P = 0.009$): Bars with unlike superscripts are significantly different, $P < 0.05$, by Fisher PLSD test.

Table 1
Effects of perinatal Cu deficiency and L-threo-3,4-dihydroxyphenylserine (DOPS) on P14 mice.

Group	Body weight, g	Heart/BW, mg/g	Brain/BW, mg/g	Liver Cu, µg/g	Liver Fe, µg/g	Hemoglobin, g/l	Ceruloplasmin, units/l
Cu + /water	6.89 ± 0.47 ^b	8.53 ± 0.37	52.1 ± 2.49 ^a	19.4 ± 2.39	25.4 ± 1.07 ^b	113 ± 2.32	40.5 ± 1.66
Cu - /water	8.61 ± 0.70 ^{ab}	9.85 ± 0.69	42.7 ± 2.08 ^b	1.62 ± 0.15	33.1 ± 2.15 ^a	72.6 ± 4.34	0.97 ± 0.31
Cu + /DOPS	9.04 ± 0.52 ^a	6.85 ± 0.25	41.4 ± 1.80 ^b	24.4 ± 0.85	27.1 ± 0.67 ^b	129 ± 4.87	40.2 ± 1.48
Cu - /DOPS	7.39 ± 0.32 ^{ab}	9.51 ± 0.31	50.6 ± 1.56 ^a	2.10 ± 0.21	26.5 ± 1.42 ^b	78.8 ± 3.46	0.91 ± 0.25
Diet	NS	<0.001	NS	<0.001	0.012	<0.001	<0.001
Drug	NS	0.028	NS	0.032	NS	0.007	NS
Diet*Drug	0.002	NS	<0.001	NS	0.022	NS	NS

Values are means ± SEM (n = 8). Data were analyzed by factorial ANOVA. Significant interactions were evaluated by Fisher PLSD test and unlike superscripts are significantly different $P < 0.05$.

Table II

Effects of perinatal Cu deficiency and L-threo-3,4-dihydroxyphenylserine (DOPS) with ascorbate on P15 mice.

Group	Body weight, g	Heart/BW, mg/g	Brain/BW, mg/g	Ceruloplasmin, units/L
Cu +/ascorbate	7.74 ± 0.65	7.76 ± 0.41c	48.9 ± 3.82	43.3 ± 2.37
Cu - /ascorbate	5.97 ± 0.73	13.9 ± 1.52a	61.5 ± 5.30	0.42 ± 0.22
Cu + /DOPS	7.14 ± 0.66	8.54 ± 0.23bc	52.6 ± 3.83	41.5 ± 4.38
Cu - /DOPS	4.81 ± 0.58	10.6 ± 0.22b	69.6 ± 5.78	1.57 ± 0.54
Diet	0.009	< 0.001	0.009	< 0.001
Drug	NS	NS	NS	NS
Diet*Drug	NS	0.027	NS	NS

Values are means ± SEM ($n = 4$). Data were analyzed by factorial ANOVA.

Significant interactions were evaluated by Fisher PLSD test and unlike superscripts are significantly different $P < 0.05$.

Table III
Effect of postnatal Cu deficiency and L-threo-3,4-dihydroxyphenylserine (DOPS) on P49 male rats.

Group	Body weight, g	Ventricle/BW, mg/g	Brain/BW, mg/g	Spleen/BW, mg/g	Liver Cu, µg/g	Liver Fe, µg/g	Hemoglobin, g/l	Ceruloplasmin, units/l
Cu +/water	282 ± 10.3	4.03 ± 0.01b	6.43 ± 0.33	3.71 ± 0.15	3.65 ± 0.10	70.2 ± 3.35	158 ± 2.63	149 ± 8.64
Cu - /water	278 ± 11.1	6.56 ± 0.06c	6.92 ± 0.16	3.75 ± 0.20	0.61 ± 0.05	104 ± 10.5	125 ± 16.0	2.84 ± 0.82
Cu + /DOPS	268 ± 4.7	4.17 ± 0.02b	7.27 ± 0.14	3.87 ± 0.22	3.67 ± 0.09	72.7 ± 4.10	156 ± 1.88	160 ± 8.54
Cu - /DOPS	227 ± 11.0	9.61 ± 0.90a	8.12 ± 0.40	3.16 ± 0.19	0.41 ± 0.06	111 ± 7.17	104 ± 11.1	2.44 ± 2.02
Diet	0.037	< 0.001	0.029	NS	< 0.001	< 0.001	0.001	< 0.001
Diet*Drug	0.004	0.010	0.002	NS	NS	NS	NS	NS
Diet*Neuro	NS	0.016	NS	NS	NS	NS	NS	NS

Values are means ± SEM (*n* = 5). Data were analyzed by factorial ANOVA. Significant interactions were evaluated by Fisher PLSD test and unlike superscripts are significantly different *P* < 0.05.

Table IV

Concentration of L-threo-3,4-dihydroxyphenylserine (DOPS) in tissue of P49 male rats.

	Cu+	Cu –
Serum (nmol/ml)	0.07 ± 0.03	0.28 ± 0.14
Ventricle (nmol/g)	1.85 ± 0.05	1.15 ± 0.39
Spleen (nmol/g)	0.37 ± 0.06	0.54 ± 0.05
Medulla/Pons (nmol/g)	0.05 ± 0.01	0.09 ± 0.01

Values are means ± SEM ($n = 4$). Data were analyzed by Student's t -test, $\alpha = 0.05$. No dietary treatment effects were detected.