TRACE COPPER LEVELS IN THE DRINKING WATER, BUT NOT ZINC OR ALUMINUM INFLUENCE CNS ALZHEIMER-LIKE PATHOLOGY

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Abstract

Mounting evidence suggests copper may influence the progression of Alzheimer’s disease by reducing clearance of the amyloid beta protein (A\(\beta\)) from the brain. Previous experiments show that addition of only 0.12 PPM copper (one-tenth the Environmental Protection Agency Human consumption limits) to distilled water was sufficient to precipitate the accumulation of A\(\beta\) in the brains of cholesterol-fed rabbits (1). Here we report that addition of copper to the drinking water of spontaneously hypercholesterolemic Watanabe rabbits, cholesterol-fed beagles and rabbits, PS1/APP transgenic mice produced significantly enhanced brain levels of A\(\beta\). In contrast to the effects of copper, we found that aluminum- or zinc-ion-supplemented distilled water did not have a significant effect on brain A\(\beta\) accumulation in cholesterol-fed rabbits. We also report that administration of distilled water produced a reduction in the expected accumulation of A\(\beta\) in three separate animal models. Collectively, these data suggest that water quality may have a significant influence on disease progression and A\(\beta\) neuropathology in AD.

Keywords

AD-like neuropathology; copper; aluminum; zinc

Introduction

The clinical diagnosis of Alzheimer’s disease (AD) is confirmed only after neuropathologic exam based on the occurrence of characteristic lesions and the exclusion of other dementing conditions. The hallmark neuropathologic features of AD are senile plaques (SP) and neurofibrillary tangles (NFT). A sufficient number - set by convention - of both SP and NFT are required by most to affix the diagnosis of AD. In distinct contrast, there are two...
seemingly mutually exclusive camps of investigators, each of which consider the mechanism and genetic influences leading to the formation of either SP or NFT as integral to the etiology of AD.

The main component of the senile plaque is the amyloid β peptide (Aβ), but a wealth of other compounds also occur in and around SP, including cholesterol and its chaperone in the CNS, apolipoprotein E. Most consider the Aβ peptide to be a metabolic by-product of a larger precursor protein (APP).

Genetic mutations of the APP gene have been associated with excess production and accumulation of Aβ as SP in the brains of individuals with familial AD. Accumulation of Ab is presumed to be a result of genetically induced overproduction, but reduced clearance of the peptide is a likely contributing factor. Investigators have capitalized on these observations in familial AD, by isolating and inserting human genetic material containing human APP mutations into the mouse genome. Investigating the mechanism of SP formation has been facilitated by the development of these transgenic mouse models associated with the over-production of Aβ. Memory deficits and accumulation of SP-like deposits of Aβ occur with increasing age in these transgenic mouse models of AD. A role for reduced clearance as a cause of the accumulation of Aβ in these transgenic mouse models comes from recent immunotherapy studies (1, 2). Introduction of Aβ antibodies into the blood, which need not directly enter the CNS, assist in the removal of Aβ from the brains of such transgenic mice.

The effect of cholesterol on production and accumulation of Alzheimer-like Aβ in the brain has gained considerable prominence in recent years. Transgenic mouse models of AD have been shown to accumulate Aβ earlier or in greater abundance, or both, if administered excess cholesterol in the diet (3–8). Cell culture studies have demonstrated that cholesterol is capable of shifting normal metabolism of APP to production of amyloidogenic peptides, and predominantly of newly synthesized APP (9–14). Analogous cell culture studies have also shown that inhibition of the rate-limiting step of cholesterol synthesis with a statin overcomes the effect of exogenous administration of cholesterol and reduces the level of Aβ produced (10, 11, 13, 15, 16). Similar to the results of cell culture studies, administration of cholesterol-lowering drugs to AD transgenic mice reduced levels of Aβ in the brain (17–19).

Studies performed in cholesterol-fed New Zealand white rabbits as an animal model of human coronary artery disease (CAD) – based on the finding of SP in the brains of non-demented individuals with CAD – indicate that dietary cholesterol induces a pronounced accumulation of neuronal Aβ compared to animals fed a normal chow diet (20). Further studies in the cholesterol-fed rabbit revealed at least a dozen features similar to the pathology observed in AD brain including Aβ immunoreactivity, extracellular Aβ plaques, meningeal Aβ immunoreactivity, apolipoprotein E immunoreactivity, cathepsin D immunoreactivity, superoxide dismutase immunoreactivity, microgliosis, apoptosis, vascular activation of superoxide dismutase, mouse endothelial cell antigen immunoreactivity, breaches of the blood brain barrier, elevated brain cholesterol and elevated Aβ concentration (21–26). Recent work suggests that the accumulation of Aβ in the brains of cholesterol-fed rabbits was dependent on the quality of the water the animals were administered (27, 28). Animals given tap water accumulated considerably more Aβ in the brain than animals administered distilled water. Both the intensity of the immunoreactivity observed and the number of neurons affected was greatly diminished in animals given distilled water (27). Morphologically, the neurons with Aβ immunoreactivity often appeared shrunken in size among cholesterol-fed rabbits on tap water compared to animals on distilled water. Investigation of Aβ levels in the rabbit blood suggested reduced clearance from the brain to the blood (27). This was because doubled circulating Aβ levels accompanied minimal brain
accumulation in cholesterol-fed rabbits administered distilled water, while minimal increases of circulating Aβ was accompanied by significant brain accumulation in cholesterol-fed animals allowed tap water. This is analogous to findings in mouse models of AD where the equilibrium of Aβ in the brain and blood may vary with the level of its deposition in the brain (2, 29).

Investigation of the trace metal content in the tap water used in these experiments excluded certain agents thought to be possible contributors to the etiology of AD including zinc (30–35), aluminum and mercury (32, 36), but not copper. The copper ion has recently gained attention because it may play a role in promoting Alzheimer’s disease (30, 33, 37–40).

Based on the premise that it was the copper ion in tap water that promoted accumulation of Ab in the cholesterol-fed rabbit brain, we determined the effect of adding trace levels of copper ion to distilled water given to cholesterol-fed animals compared to cholesterol-fed animals given unaltered distilled water. As observed in cholesterol-fed animals given tap water (containing copper ion) compared to animals administered distilled water, addition of 0.12 PPM copper ion to distilled water promoted the neuronal accumulation of Aβ (28).

Initial assessment of the cholesterol-fed rabbit’s ability to acquire complex memory using the eye-blink behavioral paradigm yielded conflicting results. The cholesterol-fed rabbits, allowed local tap water (Morgantown WV), actually performed somewhat better than animals on normal chow (diet without cholesterol) (41). These animals exhibited limited neuronal accumulation and no extracellular deposition of Aβ (41), similar to cholesterol-fed animals administered distilled drinking water. Analysis of Morgantown, WV tap water revealed negligible levels of copper ion. Subsequent studies indicated that introduction of copper ion into the drinking water of cholesterol-fed rabbits produced an 80% deficit in the ability of animals to acquire complex memory compared to animals fed the same diet and allowed unaltered distilled drinking water (28). As with tap water, it was suggested that copper ion in the drinking water led to the inhibition of Aβ clearance from the brain, over-produced as a result of elevated cholesterol, thus leading to its accumulation and the subsequent memory deficits.

The purpose of the present experiment was to: (1) investigate the effects of adding copper to the drinking water in three additional animal models of Alzheimer’s disease – spontaneously hypercholesterolemic Watanabe rabbits, cholesterol-fed Beagles and PS1/APP transgenic mice as well as replicate and extend the effects of adding copper to the drinking water in cholesterol-fed New Zealand White (NZW) rabbits; (2) determine the effects of aluminum- and zinc-supplemented distilled water on Aβ accumulation in the brains of cholesterol-fed NZW rabbits; and (3) determine the effects of distilled water on the expected accumulation of Aβ in Watanabe rabbits, cholesterol-fed Beagles and PS1/APP mice. Watanabe rabbits retain a genetic mutation deleting the LDL receptor causing elevated circulating cholesterol levels. Beagles exhibit age-related increases in Aβ deposition as SP-like structures starting at about 14 years of age. SP-like deposition of Aβ starts between 2 and 3-months of age in PS1/APP (line 8.9) mice fed a normal control diet.

**Methods**

**Watanabe Rabbits**

Adolescent male Watanabe rabbits (3000–4000g) were housed in the rabbit facility at Sun Health Research Institute, Sun City, AZ (SHRI) operating under the guidelines of the USDA with a 12:12 light cycle, at 67 ± 7°F, and 45–50% humidity. Animals were randomly assigned to one of three groups as a sub-set of a larger IACUC approved experimental protocol. One group of animals was administered normal chow and allowed tap water ad libitum (N=4). One group of animals was administered normal chow and allowed distilled
water ad libitum (N=4). One experimental group of animals was administered normal chow and allowed distilled water supplemented with 0.12 PPM copper ion (as copper sulfate) ad libitum (N= 4; Arrowhead distilled drinking water). Control diet was commercially obtained from Purina Mills, Inc. (Laboratory Rabbit Diet). Dietary food intake was limited to one cup per day (8 oz) and ad libitum water consumption varied between 32 and 40 oz/day.

Animals were sacrificed ten weeks after initiating the experimental dietary (food and water) protocol. Animals were administered a cocktail of Ketamine and Xylazine (IM; 45–75 mg/kg and 5–10 mg/kg respectively) on the day of sacrifice and the brain was perfused via the heart under pressure with 120 mls of 4% paraformaldehyde at a constant rate of 5 ml/min using a constant pressure pump. The brain was removed and further fixed by immersion in 4% paraformaldehyde for 2 weeks before sectioning. Fifty-micron vibratome sections of hippocampus and hippocampal cortex of the brain were immunostained with \( \beta \)-amyloid antibody (10D5; provided by Dr. Dale Schenk of Elan Pharmaceuticals) using published peroxidase-antiperoxidase immunohistochemical methods (42).

**Dogs**

Beagle dogs were housed in the animal facility Case of Western Reserve University, Cleveland OH (CWRU) under an IACUC approved experimental protocol. Thirteen year-old post menopausal female dogs were allotted randomly to one of the following four groups: (1) high fat – 4% cholesterol 0.4% cholate Purina diet (HFC), plus distilled water (n=3); (2) HFC plus copper supplemented (0.2 gram/liter CuSO\(_4\)) distilled water (n=3); 3) control Purina diet plus distilled water (n=3); 4) control Purina diet plus copper supplemented distilled water (n=2). Two 5 year-old beagle dogs were fed with HFC plus distilled water. At the end of the four-month feeding period, all beagles were euthanized with phenobarbital (30 mg/kg). The chest was opened to expose the heart and aorta. Butterfly needles were placed at the apex of heart and the ascending internal carotid to perfuse the brain. The vena cava was clipped. About 800 ml of 4% paraformaldehyde were used to perfuse each animal under a biochemical hood at the animal resource center at CWRU. A full necropsy was performed on each animal.

The brain and other organs were collected in 4% paraformaldehyde. All experimental procedures were performed in accordance with National Institute of Health (NIH), and CWRU guidelines for the use and the care of laboratory animals. Fifty-micron vibratome sections of hippocampus and hippocampal cortex of the brain were immunostained with \( \beta \)-amyloid antibody as noted above (42).

**PS1/APP mice**

Breeding pairs of the PS1 (PS1M146L, line 8.9) and APP (APPK670N, M671L, Tg2576) transgenic mice were provided by Dr Karen Duff at the Center for Dementia Research, Nathan Kline Institute. The animals were housed in the ALAC-approved facility at the Nathan Klein Institute, Orangeburg, NY with a 12h/12h light/dark cycle and free access to food and water. Equal number of male and female double transgenic PSAPP mice (PS1M146L, line 8.9 X Tg2576) were randomized to tap water (n=9; 4 females –5 males), double distilled water (n=9, 5 females – 4 males) or double distilled water containing 0.12ppm copper sulfate (n=8, 4 females – 4 males) starting at 11 weeks of age. Controlled water was administered for a 6-week period prior to sacrifice. The average daily water consumption was 19ml (+/− 1ml). After 6 weeks of treatment the animals were anesthetized with a ketamine/xylazine cocktail and sacrificed by transcardial perfusion with 0.1M phosphate buffer. Brains were quickly removed; one hemi-brain was postfixed in 4% paraformaldehyde/0.1M phosphate buffer overnight and the other was snap frozen and stored at −80C for future use.

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Fixed hemi-brains were transmitted to SHRI for immunohistological processing. Three adjacent fifty-micron vibratome sections of hemi-brain were immunostained with β-amyloid antibody as noted above (42). Mean cortical plaque number and plaque size (πR² in mm) were calculated for each hemi-brain.

**New Zealand white rabbits**

Adolescent male New Zealand white rabbits (3000–4000g) were housed in the rabbit facility at SHRI operating under the guidelines of the USDA with a 12:12 light cycle, at 67 ± 7° F, and 45–50% humidity. Animals were randomly assigned to one of six groups as a subset of a larger IACUC approved experimental protocol. One group of animals was administered normal chow and allowed distilled water with 0.12 PPM copper (N=8), 0.36 PPM zinc (as sulfate, N=3) or 0.36 PPM Aluminum (as sulfate, N=3) ad libitum (Arrowhead distilled drinking water). Another group of animals was administered 2% cholesterol diet and allowed distilled water with 0.12 PPM copper ion (as sulfate, N=16), 0.36 PPM zinc (as sulfate, N=5) or 0.36 PPM Aluminum (as sulfate, N=5) ad libitum. Control and cholesterol diets were commercially obtained from Purina Mills, Inc. (Laboratory Rabbit Diet with and without 2% cholesterol). Dietary food intake was limited to one cup per day (8 oz) and ad libitum water consumption varied between 32 and 40 oz/day.

Animals in each group were sacrificed ten weeks after initiating the experimental dietary (food and water) protocol. On the day of sacrifice, animals were administered a cocktail of Ketamine and Xylazine (IM; 45–75 mg/kg and 5–10 mg/kg respectively). Anesthetized animals were secured to a stainless steel surgical apparatus, the heart was exposed and a butterfly needle was inserted in the left apex, and blood was collected in purple top (EDTA) vacutainer tubes for chemical analysis. Thereafter, a needle attached to the perfusion apparatus was inserted and secured in the left apex of the heart, the vena cava was incised and perfusion was initiated. Animals were perfused under pressure with 120 mls of 4% paraformaldehyde at a constant rate of 5 ml/min using a constant pressure pump. A full necropsy was performed on each animal.

The brain was removed and further fixed by immersion in 4% paraformaldehyde for 2 weeks before sectioning. Fifty-micron vibratome sections of hippocampus and hippocampal cortex of the brain were immunostained with β-amyloid antibody (10D5; provided by Dr. Dale Schenk of Elan Pharmaceuticals) using published peroxidase-antiperoxidase immunohistochemical methods (42).

**Water analysis**

Water was analyzed by US Filters (Vivendi Environment), an EPA Certified Water Quality Testing Laboratory for levels of Arsenic (EPA 200.9), Mercury (EPA 245.1), and organics (total organic carbo-TOC; SM5310C) as special studies, and for a ‘Standard A’ assessment (EPA 200.7, EPA 300.0) to include levels of aluminum, calcium, magnesium, sodium, potassium, barium, strontium, iron, copper, manganese, zinc, chloride, sulfate, nitrate, fluoride, and silica.

**Results**

**Watanabe rabbits**

As noted above Watanabe rabbits retain a genetic mutation deleting the LDL receptor causing elevated circulating cholesterol levels. Accumulation of Aβ was observed first in the hippocampus of Watanabe rabbits eating normal diet and drinking tap water (containing > 0.2 PPM copper ion) starting at 6 months of age. Similar to animals provided tap water (not shown), 6-month old Watanabe rabbits administered distilled water supplemented with 0.12
PPM copper ion for 3 months exhibited significant accumulation of Aβ in superior temporal cortex (STC) and hippocampus, while accumulation was nearly absent in animals administered unaltered distilled drinking water for the following 3-month period (Figures 1 and 2, respectively).

The mean number of Aβ immunoreactive neurons in the STC and hippocampus was reduced in Watanabe rabbits administered distilled water compared to both the tap water and copper supplemented water groups (Table 1). The reduction did not achieve significance in the STC, but did for the hippocampus between the distilled water and copper supplemented groups (Table 1). A clear difference in the intensity of Aβ immunoreactivity occurred between the distilled water and the tap water and copper supplemented groups (Figures 1 and 2).

Dogs

Beagles exhibit age-related increases in Aβ deposition as SP-like structures starting at about 14 years of age. In both 5- and 11-year old Beagles we found premature accumulation of Aβ in both young and old animals after administering dietary cholesterol and distilled water compared to age-matched animals on a control diet and distilled drinking water. In 11-year old cholesterol-fed animals, drinking distilled water with high levels of copper ion caused deposition of Aβ in excess of that induced by drinking unaltered distilled water (Figure 3). The number of Aβ immunoreactive pyramidal neurons in the hippocampal cortex in animals administered distilled water with copper added and fed normal diet was not increased compared to animals administered distilled water and normal diet. Extracellular Aβ deposits were more common in the older animals and those who received cholesterol and copper supplementation (Figure 3).

Mice

SP-like deposition of Aβ starts between 2 and 3-months of age in PS1/APP (line 8.9) mice fed a normal control diet. At 11 weeks of age PS1/APP mice fed a control diet were started on local (Orangeburg, NY) tap water (containing 0.19 PPM copper ion), distilled water or distilled water supplemented with copper (0.12 PPM). After 6 weeks of controlled water intake we examined the extent of Aβ deposition (Figure 4) and found that the mean number of SP-like Aβ deposits was similar in all three groups, but there was a marginally significant (p < 0.06) reduction in the mean size of SP-like deposits in mice provided distilled water compared to those drinking tap water or distilled water containing copper ion (Table 2).

New Zealand White rabbits

We identified a significant increase in the number of Aβ immunoreactive neurons in cholesterol-fed rabbits administered local (Sun City, AZ) tap water (routinely containing > 0.21 PPM copper ion) compared to animals maintained on distilled drinking water (Table 3). A similar significant increase was observed among animals fed a cholesterol diet and administered distilled drinking water supplemented with 0.12 PPM copper (Table 3 and Figure 5). It is important to note that there was no significant increase in the number of Aβ immunoreactive neurons in cholesterol-fed animals administered distilled drinking water supplemented with either zinc or aluminum (Table 3 and Figure 5). There was also no difference in those animals fed normal chow and administered distilled water with or without added zinc or aluminum compared to animals drinking unaltered distilled water.

Discussion

The influence of copper has recently gained attention in AD, particularly due to its identified interactions with the hallmark lesions of the disorder, neurofibrillary tangles (NFT) and
amyloid-β containing senile plaques (SP). Copper may play a role in promoting Alzheimer’s disease (30, 33, 37–40), as chelation of CNS copper and zinc reduces the levels of Aβ in the brains of transgenic mouse models of AD (43). The protein tau has been shown to bind the copper ion, which may facilitate assembly into paired helical tau filaments (PFT) as an intermediate in later NFT formation (44). Aβ has been shown to bind copper and Aβ reduces copper ++ to copper + independent of peptide aggregation state (45). Furthermore, APP and Aβ may participate in copper metabolism, increased production of APP and Aβ has been associated with reduce CNS copper levels, while increasing copper levels tend to reduce brain Aβ levels (46). It has also been shown that APP and Aβ bind copper (38, 39); this binding of copper produces more soluble aggregates of Ab (33) which may promote hydrogen peroxide formation (30) and the neurotoxicity of Aβ (37).

In mice producing Aβ there are reduced copper levels in brain and oral administration of copper returns brain copper levels to normal and reduces Aβ production, and suggests that reduced levels of copper in AD may be overcome by supplementation of Cu (ongoing trial) (47). In contrast to this concept, epidemiologic data suggest that increased dietary copper increases the progression of AD compared to the progression of AD among individuals on a low copper diet (Martha Morris, University of Chicago, personal communication).

Squitti et al. have reported a significant increase in circulating copper in AD and a trend for increased ceruloplasmin (48). These authors also reported a significant negative correlation in AD between increased copper/ceruloplasmin and decreased scores on the MMSE, AVLT-A7 and the clock draw (48). Similarly, we have shown that there are significant increases in blood copper and ceruloplasmin (its chaperone) in AD compared to age matched control (49) and that increases were related to decreasing performance on the MMSE and AVLT-A7 in controls and the clock draw among individuals with MCI, in that circulating copper/ ceruloplasmin levels are increased in controls with lower performance on the AVLT-A7 and MMSE compared to controls with near perfect performance and remain elevated in MCI and AD as cognitive ability progressively deteriorates.

Regardless of the mechanism by which copper interacts with Aβ in the brain, it seems clear that circulating copper may influence the clearance of Aβ from the brain. This effect is apparent in four animal models of AD, and not reproduced by either zinc or aluminum.

Acknowledgments

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References


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Figure 1.
Amyloid β staining in superior temporal cortex of Watanabe rabbits administered distilled drinking water (upper panel) or distilled water with 0.12 PPM copper ion added (lower panel).
Figure 2.
Amyloid β staining in hippocampus from Watanabe rabbits administered distilled drinking water (upper panel) or distilled water with 0.12 PPM copper ion added (lower panel).
Figure 3.
Amyloid β staining in Beagle brain. 5 year old animals on normal chow and distilled water (A), 11 year old animal on normal chow and distilled water (B), 11 year old animal on cholesterol and distilled water (C), 5 year old animal on cholesterol and distilled water (D), 11 year old animals on cholesterol and distilled drinking water with 120 PPM copper ion added.
Figure 4.
Amyloid β staining in PS1/APP mice maintained on distilled water to drink (upper panel), New York tap water (middle panel), or distilled water with 0.12 PPM copper ion added (lower panel).
Figure 5.
Amyloid β staining in temporal cortex of New Zealand white rabbits. Animals were fed a normal diet and administered distilled drinking water supplemented with 0.12 PPM copper ion (A), 0.35 PPM aluminum ion (C) or zinc ion (E), or fed a 2% cholesterol diet and administered distilled drinking water supplemented with 0.12 PPM copper ion (B), 0.35 PPM aluminum ion (D) or zinc ion (F).
**Table 1**

Mean number of neurons (± SEM) exhibiting Aβ immunoreactivity in the superior temporal cortex (STC) and hippocampus of Watanabe rabbits fed normal chow and administered unaltered distilled drinking water or distilled water supplemented with 0.12 PPM copper ion. (Analysis of Sun City tap water indicated a copper level of 0.21 PPM.)

<table>
<thead>
<tr>
<th>Region water</th>
<th>N</th>
<th>STC</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap</td>
<td>4</td>
<td>57.5 ± 8.5</td>
<td>43.0 ± 4.0</td>
</tr>
<tr>
<td>Distilled</td>
<td>4</td>
<td>39.6 ± 6.1</td>
<td>32.5 ± 1.0</td>
</tr>
<tr>
<td>Distilled with 0.12 PPM copper ion</td>
<td>4</td>
<td>57.3 ± 7.6</td>
<td>59.0 ± 6.8*</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to animals administered distilled water
Table 2

Cortical plaque volume (mm²) in PS1/APP mice (17 weeks old) after administration of New York tap water, distilled water or distilled water with 0.12 PPM copper ion as sulfate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Plaque Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>9</td>
<td>1.63 ± 0.05*</td>
</tr>
<tr>
<td>Tap water</td>
<td>9</td>
<td>1.82 ± 0.08</td>
</tr>
<tr>
<td>Copper ion added</td>
<td>8</td>
<td>1.84 ± 0.05</td>
</tr>
</tbody>
</table>

* p = 0.06
Table 3

Number of neurons exhibiting Aβ immunoreactivity in the superior temporal cortex (/mm²) of New Zealand white rabbits fed 2% cholesterol diet or normal chow, and allowed distilled drinking water with and without 0.12 PPM copper ion, 0.36 PPM zinc ion or 0.36 PPM aluminum.

<table>
<thead>
<tr>
<th>Diet Water ad libitum</th>
<th>Normal chow</th>
<th>Cholesterol diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>23.8 ± 4.8</td>
<td>54.6 ± 4.2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>17.3 ± 4.6</td>
<td>33.6 ± 4.3</td>
</tr>
<tr>
<td>DW/0.12 PPM copper</td>
<td>33.8 ± 8.3</td>
<td>65.4 ± 7.8</td>
</tr>
<tr>
<td>DW/0.36 PPM zinc</td>
<td>17.7 ± 5.1</td>
<td>29.8 ± 4.9</td>
</tr>
<tr>
<td>DW/0.36 PPM aluminum</td>
<td>16.6 ± 4.8</td>
<td>27.9 ± 5.2</td>
</tr>
</tbody>
</table>

a p < 0.0001 compared to respective control group fed normal chow;
b p < 0.05 compared to cholesterol-fed rabbits allowed distilled water