Lack of Effect of Drinking Water Chlorine on Lipid and Thyroid Metabolism in Healthy Humans

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Animal studies and a single human epidemiological study have suggested that chlorine in drinking water may raise the level of blood cholesterol. The purpose of this study was to determine whether a 4-week exposure to drinking water chlorine (1.5 L per day) at a concentration of 20 ppm (ppm = mg/L) under controlled conditions would alter circulating parameters of lipid metabolism in healthy humans. Thirty men and thirty women each completed an 8-week protocol during which diet (600 mg cholesterol per day, 40% calories as fat) and other factors known to affect lipid metabolism were controlled. For the first 4 weeks of the protocol, all subjects consumed distilled water. For the second 4 weeks, half of the subjects were assigned randomly to drink 1.5 L per day of chlorinated water (20 ppm), while the others continued drinking distilled water. Four blood samples were collected from each subject at the end of each 4-week study period. Compared to the control group, those subjects given chlorine showed no significant changes in total plasma cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, or apolipoproteins A1, A2, or B. There was a trend toward low serum thyroxine and triiodothyronine levels in men given chlorine, though thyroid-stimulating hormone levels were unchanged. This trend, if real, was not clinically significant. Thus, short-term exposure to chlorinated drinking water at 20 ppm appears to have no significant impact on parameters of lipid or thyroid metabolism in healthy humans.

Introduction

Exposure of humans to chlorinated drinking water disinfectants is almost universal in the United States (1). Chlorine is used by the vast majority of public water systems that disinfect their water. Even persons who consume water from untreated sources may be exposed to chlorine in foods (2,3) or in swimming pools. Revis and colleagues have studied the effect of drinking water chlorine on lipid metabolism and thyroid function in white Carneau pigeons (4). Pigeons consuming a high-fat, high-cholesterol, low-calcium diet experienced a significant increase in serum cholesterol and a decrease in serum thyroxine when given chlorinated water to drink. These effects were observed at low concentrations of drinking water chlorine in the range consumed by humans.

Investigations of the effects of chlorine on human lipid and thyroid metabolism are limited. Lubbers and colleagues exposed healthy human volunteers to low and high concentrations of chlorine and other chlorinated disinfectants for brief intervals and observed no effect on total cholesterol levels (5). More prolonged exposure to 5 ppm chlorine also had no apparent effect. However, diet and the intake of other liquids possibly containing chlorine were uncontrolled, so these studies do not exclude the possibility that chlorine affects human lipid metabolism.

Wones and colleagues conducted a 15-week dose-response trial of drinking water chlorine (0, 2, 5, 10 ppm) in 19 healthy men and observed a clinically small increase (3%) in total plasma cholesterol levels at chlorine concentrations of 5 ppm and 10 ppm (6). However, no control group was studied, and it is possible that the observed increase was due to the protocol diet or other factors and not to the chlorine.

Zeighami and colleagues reported an epidemiological study of 40 small Wisconsin communities, 20 of whom chlorinated their water supply and 20 of whom did not chlorinate their water supply (7). Women living in communities with chlorinated water supplies appeared to have slightly higher total cholesterol levels (about 5%) than women living in communities with unchlorinated supplies. There was no significant difference found in men. While these findings are intriguing, they suggest only an association between chlorine in drinking water and serum cholesterol levels in women. Prospective experimental data are needed to determine whether this association is causal or coincidental.

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Thus, published reports in humans are insufficient to either confirm or exclude an effect of drinking water chlorine on human lipid or thyroid metabolism. The purpose of this study was to determine if drinking water containing 20 ppm chlorine affected lipid or thyroid metabolism in healthy women unselected for baseline total cholesterol levels or in healthy men selected to have baseline total cholesterol levels above the 50th percentile for their age.

**Methods**

This study was a randomized, controlled, parallel trial of 8 weeks' duration. The smell and taste of chlorine at 20 ppm obliterated the blinding of the subjects or investigators. The study protocol is illustrated in Figure 1. The protocol consisted of a 4-week dietary stabilization period during which all subjects drank distilled water buffered with sodium monophosphate to a pH of 8.0. The purposes of the baseline period were to achieve stabilization on a high-fat, high-cholesterol diet and to allow the effects of prior exposure to chlorinated water to dissipate. The baseline period was followed by a 4-week treatment period during which half of the subjects was assigned randomly to continue consuming buffered distilled water (distilled group), while the other half was assigned to consume 1.5 L per day of drinking water containing 20 ppm (ppm = mg/L) chlorine buffered with sodium monophosphate to a pH of 8.0 (chlorine group). The trial was conducted in the General Clinical Research Center (GCRC) at the University of Cincinnati Hospital and was approved by its Institutional Review Board; all subjects gave written informed consent to participate.

This study used a chlorine concentration 10 times higher than that routinely found in public water supplies because previous work by our group found no effect at 2 ppm and only a possible, small effect at 5 ppm or 10 ppm (6). Because of the results of the epidemiological study cited above (7), and because the effects of chlorine on lipid metabolism in females had not been experimentally determined previously, we first studied healthy women unselected for baseline cholesterol levels. Because the impact of drinking water chlorine on lipid metabolism was clinically unimpressive in our previous study of unselected men (6), we next chose to study men with baseline cholesterol levels above the 50th percentile to determine whether elevated baseline cholesterol might be associated with an increased responsiveness to chlorinated drinking water.

Inclusion criteria for the women's group included: healthy females between the ages of 18–65 years; no medications (including birth control pills); and no concurrent illnesses. Inclusion criteria for the men's group were identical to the women's except that screening cholesterol levels for males were required to be greater than the 50th percentile but less than the 90th percentile for their age (8).

The study diet was designed individually to satisfy the food preferences of each subject and to be isocaloric. Each subject's diet plan was adjusted as necessary by a GCRC diettian to maintain the subject at his or her initial weight. The diet consisted of 20% protein, 40% carbohydrate, and 40% fat. It contained 600 mg of cholesterol/day and a polyunsaturated to saturated fat ratio of 0.4. Dietary calcium was restricted to 80% of the minimum daily requirement for American adults.

The characteristics of the study diets were designed to mimic Revis' study in pigeons (4). Therefore, the study diets were relatively high in total fat, saturated fat, and cholesterol. Despite the high fat and cholesterol content, such diets are not unusual for many Americans. Only over the last decade have Americans consumed less fat and cholesterol on average (9). The calcium restriction also reflects nutritional patterns in this country since many people, especially women and older Americans, consume a diet containing 80% or less of the recommended daily allowance of calcium (10). Study diets were formulated to avoid foods that contain chlorine (i.e., bleached flour). In summary, the study diets were designed to maximize the potential for observing a small effect of chlorine on lipid and thyroid metabolism, minimize the influence of extraneous dietary confounders, and preserve generalizability.

All food was prepared in the GCRC kitchen. Two daily meal plans were used for each subject so that each person ate the same

![Figure 1](image-url)
meals every other day for 56 days. Subjects were required to eat at least two meals and drink no less than 1.5 L of drinking water each day. These meals and the mandatory drinking water were consumed in the GCRC under observation. A third meal and a snack were packed for those subjects who wanted to consume them at home or at work. Subjects were instructed not to eat or drink anything which had not been provided for them.

Other lifestyle factors which might affect lipid or thyroid levels were controlled as well as possible. Subjects were instructed not to drink alcohol. If they smoked cigarettes, they were asked to keep their smoking constant throughout the study. They kept diaries of exercise activities and were asked to keep their exercise habits constant. Subjects were required to refrain from swimming during the study to avoid exposure to chlorine from pool water.

Drinking water for each subject was prepared by standard procedures. Water was distilled and passed through an activated charcoal filter to remove any trace organic impurities. Before launching the study, samples of the resulting purified water were submitted to the Environmental Protection Agency (EPA) Health Effects Research Laboratory in Cincinnati and were judged to be free of trace organic impurities. A highly concentrated solution of chlorine (250 ppm) was prepared by bubbling chlorine gas through water alkalized to pH 8.0 with sodium hydroxide. A concentrated sodium monophosphate buffer solution at pH 8.0 was prepared in the GCRC Core Laboratory. Each day, GCRC dietary staff mixed aliquots of these concentrated solutions with distilled water to prepare that day’s drinking water for each subject. The final pH and chlorine concentration of one subject’s drinking water was checked each day to assure that subjects actually received the appropriate concentration of chlorine. Each subject’s drinking water was prepared and stored in a well-marked thermos. Studies of these thermos containers documented that the chlorine concentration was stable for at least 24 hr and that loss of chlorine due to evaporation did not occur.

Subjects were also provided with a thermos of distilled water free of chlorine and buffer which they could drink at their option in addition to the 1.5 L of study water which they had to drink. Consumption of mandatory and optional water was monitored by daily checks of subjects’ thermoses. Twenty-four-hour fluid intakes were recorded daily.

All subjects reported in the fasting state each morning to the GCRC for measurement of weight, blood pressure, pulse rate, and temperature by a GCRC staff nurse. Blood was drawn weekly for total cholesterol, triglycerides, HDL cholesterol (HDL-C), apolipoproteins A1, A2 and B, thyroxine (T4), triiodothyronine (T3), T3 resin uptake, and thyroid-stimulating hormone (TSH). During weeks 4 and 8, blood samples were obtained on 4 consecutive days. On day 0, 28, and 56, safety tests (serum electrolytes, blood urea nitrogen and creatinine, glucose, liver transaminases and alkaline phosphatase, calcium and phosphorous, urinalysis, white and red blood cell counts, reticulocyte count, and methemoglobin) were performed. A single measurement of glucose 6-phosphate dehydrogenase (G6PD) was made at entry to detect individuals who might be susceptible to hemolytic anemia due to the oxidizing effects of chlorine.

All blood samples were drawn from the antecubital veins of supine subjects. Lipid, lipoprotein, and apolipoprotein analyses were collected in sodium/potassium ethylenediaminotetraacetate (EDTA, 1 mg/mL) anticoagulated tubes, and the plasma was separated quickly from the cells by centrifugation. The plasma samples were frozen at −20°C and analyzed as a batch for each subject. Total cholesterol, triglycerides, and HDL cholesterol were measured by microenzymatic procedures, standardized and monitored through the Center for Disease Control’s Lipid Standardization Program (7). HDL was isolated using the modified heparin-manganese chloride procedure (12). Interference in the enzymatic procedure was eliminated by the addition of 8 mEq/L of EDTA to the cholesterol reagent (13). Apolipoproteins (Apo) A1 and B were quantitated by electroimmunoassay (14) and Apo A2 by enzyme-linked immunosorbent assay (15). LDL cholesterol (LDL-C) was calculated as total cholesterol minus HDL-C minus triglycerides divided by 5. The other tests listed above were performed by the clinical laboratories of the University of Cincinnati Hospital using standard procedures which are available upon request.

Results of the lipid and thyroid function tests were analyzed as follows. For each subject, the mean of the four consecutive daily measurements during week 4 was considered as the “baseline” value and the mean of the four daily measurements during week 8, the “treatment” value. The treatment value minus the baseline value was the “change” value for each person. The primary analysis compared the mean of the change values for the distilled groups to the mean of the change values for the chlorine group using a two-sample t-test. A secondary analysis using the lipid, lipoprotein, and thyroid data from all 8 weeks was performed also using repeated measures analysis of variance. All p-values were two-tailed. Sample size determinations made before the study using variance data from a previous study (6), an alpha of 0.05, and a beta of 0.20 indicated that 11 subjects would be needed in each group to observe a change in total cholesterol of 5%.

Results

Thirty-one women entered and thirty completed the study protocol. One woman was dropped from the study on day 23 due to noncompliance with diet. Entry data for the women in the control and the chlorine groups are presented in Table 1. No significant differences in age, weight, or total cholesterol existed between the groups. Fourteen women in the distilled group and 12 in the chlorine group were premenopausal.

Baseline, treatment, and change lipid and thyroid function test results for the women in the chlorine and distilled water groups are shown in Table 2. There were no differences between the groups for these parameters at baseline (two-sample t-tests). There were no differences between the chlorine and distilled groups for changes between weeks 4 and 8 in any of the lipid or thyroid parameters. Similarly, the analysis using the data from all weeks showed no effect of chlorine on any of these parameters. No abnormalities in any of the safety tests occurred in either group. Using the variance data from this trial and assuming an alpha of 0.05, this study had a 73% chance of detecting a 5% change (9.5 mg/dL) in total cholesterol.

Thirty-two men began and 31 completed the protocol, and usable data were available for 30. One subject was eliminated from the study on day 6 due to a seizure. This subject had a distant history of a seizure disorder, but he was on no anticonvulsant medications at the time of entry and his seizure disorder was thought to be inactive. One subject in the chlorine group com-
completed the protocol but his data were not included in the analyses because he developed a viral gastrointestinal illness during the fourth week of the study that led to a major decrease in his cholesterol values so that his baseline period results were not representative of his usual state. Entry data for the men are presented in Table 1. The mean age of the chlorine group was slightly lower than that of the distilled group, but there were no differences in weight or total cholesterol at entry.

Baseline, treatment, and change lipid and thyroid function test results for the men in the chlorine and distilled water groups are
shown in Table 3. No significant differences between the groups at baseline were evident (two-sample t-tests). There were no clearly significant differences between the groups either by comparing treatment (week 8) to baseline (week 4) or by repeated measures ANOVA using the weekly data. The small decreases in thyroid and triiodothyronine in the chlorinated group relative to the distilled water group were judged not to be meaningful because of borderline statistical significance and because thyroid-stimulating hormone levels did not change. Meaningful changes in thyroid and triiodothyronine should be accompanied by changes in thyroid-stimulating hormone levels. The absence of such changes suggests that no physiologic impact on thyroid metabolic status occurred as a result of chlorine ingestion. No adverse changes in any of the safety tests were seen in either group. Using the variance data from this trial and assuming an α of 0.05, this study had a 79% chance of detecting a 5% change in total cholesterol.

The data for both the men and the women were examined qualitatively to determine whether any identifiable subgroup might have been affected by the drinking water chlorine even though the overall analyses showed no effect. Formal statistical analyses of subgroups were not possible due to the small numbers of subjects in each subgroup. No subgroups based on age, race, baseline total cholesterol, or source of prestudy drinking water appeared to be uniquely affected by the chlorine.

The drinking water was well tolerated by the subjects. Three women experienced loose stools at the beginning of the 4-week chlorine treatment period that resolved by the seventh day of treatment. One woman experienced intermittent loose stools during both the 4-week baseline period and the 4-week chlorine treatment period. This subject weighed 45 kg and the loose stools may have been related to the ingestion of a larger-than-usual volume of water. Two men in the distilled group and two men in the chlorine group reported gastrointestinal complaints while on the protocol. In the distilled group, one subject had a single episode of diarrhea during the fifth week, while the other subject had intermittent diarrhea and mild abdominal cramps during the last 4 weeks of the study. In the chlorine group, one subject had one episode of constipation during the 8 weeks and one subject had 3 days of loose stools at the beginning of the chlorine treatment period that resolved by the fifth week. Two women subjects who received chlorine were found to be G6PD deficient at entry into the study, but they experienced no detectable hemolysis while drinking chlorinated water.

| Parameter                        | Number of subjects | Group   | Baseline Mean ± SEM | Treatment Mean ± SEM | Change ± SEM | p-value |
|----------------------------------|--------------------|---------|--------------------|----------------------|--------------|---------|
| Total cholesterol, mg/dL         | 15                 | Distilled | 215.5 ± 8.11 | 222.4 ± 7.09 | +6.90 | 0.12    |
|                                  | 15                 | Chlorine | 201.7 ± 7.88 | 202.8 ± 9.10 | +1.10 |         |
| Total triglycerides, mg/dL       | 15                 | Distilled | 76.2 ± 7.21 | 81.8 ± 8.24 | +5.60 |         |
|                                  | 15                 | Chlorine | 78.1 ± 5.67 | 79.8 ± 7.21 | +1.70 |         |
| HDL cholesterol, mg/dL           | 15                 | Distilled | 48.9 ± 3.75 | 49.1 ± 3.88 | +0.20 |         |
|                                  | 15                 | Chlorine | 49.6 ± 2.97 | 49.4 ± 2.93 | -0.20 |         |
| LDL cholesterol, mg/dL           | 15                 | Distilled | 151.4 ± 7.10 | 156.9 ± 6.30 | +5.50 |         |
|                                  | 15                 | Chlorine | 136.5 ± 6.85 | 137.5 ± 8.29 | +1.00 |         |
| LDL/HDL ratio                    | 15                 | Distilled | 3.34 ± 0.26 | 3.48 ± 0.29 | +0.14 |         |
|                                  | 15                 | Chlorine | 2.86 ± 0.18 | 2.89 ± 0.21 | +0.03 |         |
| Apolipoprotein A1, mg/dL         | 15                 | Distilled | 128.0 ± 4.56 | 132.8 ± 4.23 | +4.80 |         |
|                                  | 15                 | Chlorine | 130.0 ± 4.48 | 131.2 ± 4.06 | +1.20 |         |
| Apolipoprotein A2, mg/dL         | 15                 | Distilled | 44.6 ± 2.35 | 45.0 ± 2.07 | +0.40 |         |
|                                  | 15                 | Chlorine | 44.9 ± 1.16 | 44.9 ± 1.60 | 0.00 |         |
| Apolipoprotein B, mg/dL          | 15                 | Distilled | 129.6 ± 6.19 | 136.5 ± 5.69 | +6.90 |         |
|                                  | 15                 | Chlorine | 118.4 ± 5.18 | 117.3 ± 5.86 | -1.10 |         |
| T4, μg/dL                        | 15                 | Distilled | 7.94 ± 0.26 | 8.15 ± 0.27 | +0.21 |         |
|                                  | 15                 | Chlorine | 7.97 ± 0.47 | 7.86 ± 0.41 | -0.11 |         |
| T3, μg/dL                        | 15                 | Distilled | 136.6 ± 5.25 | 138.8 ± 4.79 | +2.20 |         |
|                                  | 15                 | Chlorine | 138.1 ± 8.29 | 133.1 ± 8.53 | -5.00 |         |
| T3 Resin uptake, %               | 15                 | Distilled | 93.3 ± 2.41 | 92.6 ± 2.50 | -0.70 | 0.61    |
|                                  | 15                 | Chlorine | 99.1 ± 2.67 | 99.0 ± 2.56 | -0.10 |         |
| Thyroid-stimulating hormone, μU/mL| 15                 | Distilled | 2.38 ± 0.20 | 2.36 ± 0.16 | -0.02 |         |
|                                  | 15                 | Chlorine | 2.34 ± 0.19 | 2.44 ± 0.25 | +0.10 | 0.38    |

*Mean ± SEM for each parameter.  
Change = treatment (week 8) - baseline (week 4).  
*p (Change (distilled) = change (chlorine)) is less than the value shown.
Discussion

The short-term (4 weeks) consumption of chlorinated drinking water (20 ppm) does not affect circulating parameters of lipid or thyroid metabolism in healthy women unselected for baseline total cholesterol levels or in healthy men with baseline total cholesterol levels above the median for their age. These data in humans are contrary to results from investigations in animals showing an increase in serum cholesterol after administration of various doses of chlorinated drinking water (0.1–30 ppm) (2, 4). Our findings support previous uncontrolled results in humans by Lubbers and colleagues (5) who found no significant effect on serum cholesterol from either a single-dose exposure to chlorinated water (0.1 ppm to 24 ppm) or from a 12-week exposure to 0.5 L/day of 5 ppm chlorinated water.

The results of these studies suggest that the 3% increase in total cholesterol we observed in a previous, uncontrolled study (6) was not due to the ingestion of drinking water chlorine but was more likely due to the protocol diet. The men in the distilled group in the study reported here experienced a 3% increase in total cholesterol between the end of the baseline and the end of the treatment periods despite the fact that they did not receive any chlorine. Indeed, the men who consumed chlorine experienced a smaller increase in total cholesterol between the end of the baseline and the end of the treatment period (not statistically significant).

Our results also fail to confirm the possible association found by Zeighami et al. (7) between drinking water chlorine and blood cholesterol in women. These conflicting results could be explained in one of several ways. It may be that drinking water chlorine has no effect on human lipid levels and the association found in the epidemiological study reflects other unknown factors that are related to both drinking water characteristics and blood cholesterol levels. Alternatively, it may be that drinking water chlorine does cause an increase in blood cholesterol levels but that we missed such an effect in this experimental study because the period of exposure was too brief, our subjects were too healthy or too young, or too few subjects were studied to detect a very small change.

One unanticipated result was a fall in total cholesterol from entry to baseline in the men’s chlorine group. We expected total cholesterol levels to increase from entry to baseline because we were feeding subjects high-fat and high-cholesterol diets. This occurred as expected in both women’s groups and in the men’s distilled group. The unexpected fall between entry and baseline in the men’s chlorine group could have been a chance result or could have been related to the prestudy diets of the subjects. This finding, while interesting, does not invalidate our conclusions because the principal end point was the change between baseline and treatment, not between entry and treatment. Indeed, had we compared entry to treatment, it would appear that chlorine had a cholesterol-lowering effect in men.

The principal limitations of this study were the relatively brief baseline and treatment periods and the good health of the subjects studied. Almost all subjects consumed chlorinated drinking water from local water supplies before entry into the protocol. The 4-week baseline period may not have been long enough to achieve adequate washout of the effects of previously ingested chlorine. Alternatively, the 4-week treatment period may have been too brief to see an effect. The length of the baseline and study periods were chosen based on the clinical observation that most factors that affect blood cholesterol levels, including diet, do so within a time span of 4 weeks (16). Also, practical considerations involved with studying humans limited the maximum possible length of exposure. The other possible limitation of this study was that the subjects, by design, were healthy. Indeed, the average baseline total cholesterol level in women in the study was below 200 mg/dL. The men were selected to have higher baseline levels but they also were uniformly healthy except for mildly increased cholesterol levels. It may be that people who are not as healthy overall as our subjects would be more affected by drinking chlorinated water.

In summary, this randomized, controlled trial failed to show any meaningful effect of drinking water chlorine at a concentration of 20 ppm on parameters of lipid or thyroid metabolism in healthy men and women. While the results of animal studies and an epidemiological survey are interesting and merit further study, the consistent lack of prospective, experimental evidence in humans linking drinking water chlorine to changes in parameters of lipid and thyroid metabolism argues that major changes in water disinfection practices in response to concerns about hypercholesterolemia are not warranted at this time.

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