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Effects of Chlorinated Drinking Water on Human Lipid Metabolism

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Atherosclerosis with its complications is the most important health problem affecting American adults. The levels of serum cholesterol, of high and low density lipoproteins, and of apolipoproteins A1, A2, and B are major risk factors for the development of atherosclerotic lesions. Animal studies suggest that chlorinated drinking water may elevate the serum cholesterol. Studies are too limited to confirm or refute this effect in humans. Since millions of humans have and have had daily exposure to chlorinated drinking water, it is essential to study the effects of such exposure on human lipid metabolism. We have begun a protocol to discover whether consuming chlorinated drinking water elevates serum cholesterol and the other lipid components of blood known to be associated with atherosclerosis. This protocol has been designed to improve the chance of observing an effect while preserving the ability to generalize the data.

Introduction

Atherosclerosis with its complications is the most important health problem affecting American adults (*1*). Heart disease, most of which is caused by blockage of the coronary arteries by atherosclerotic plaques, is the most common cause of death in persons over 35 years of age. Cerebrovascular disease, most of which is also caused by atherosclerotic lesions, is among the top 10 causes of death in young and middle-aged adults and equals cancer as the second leading cause of mortality in the aged. These diseases and others caused by atherosclerosis such as aortic aneurysm and peripheral vascular insufficiency also account for much of the morbidity and disability in our population.

The levels of cholesterol and other serum lipid components have been shown to be major risk factors for the development of atherosclerosis. Multiple studies have documented the predictive value of the total cholesterol level (*2*). However, measuring the major human lipoproteins and apolipoproteins provides even more information (*3*). Low-density lipoprotein cholesterol (LDL-C) appears to be the major atherogenic component, and an individual with high levels of this factor is at high risk for cardiovascular disease. In contrast, the level of high-density lipoprotein cholesterol (HDL-C) correlates inversely with atherosclerotic risk. In other words, the higher an individual's HDL-C, the less likely he or she is to develop the atherosclerotic complications detailed above. Recent work suggests that the specific protein components of these lipoproteins, the apolipoproteins, may correlate even better with subsequent cardiovascular disease. Specifically, apolipoproteins A1 and A2, which are the major components of HDL-C, are protective, whereas apolipoprotein B, the major component of LDL-C, is deleterious.

Compelling evidence of the close association between serum cholesterol and atherosclerosis is provided by the Lipid Research Clinic's Coronary Primary Prevention Trial (*4*). In this study, lowering the serum cholesterol by diet and cholestyramine resulted in fewer cardiovascular deaths than in controls among men with high cholesterol and no known disease at the beginning of the trial.

Many factors are known to affect human lipid metabolism. An individual's genetic makeup is of primary importance, and many specific diseases have been described in which single genetic defects result in elevated cholesterol levels and increased atherosclerosis risk. Although these diseases are important, they occur relatively infrequently, and the vast majority of cardiovascular illness occurs in persons without an identifiable genetic defect. Other factors, diet in particular, play a primary role in these persons (*5*). High intake of cholesterol and saturated fats results in increased serum cholesterol and LDL-C. Unsaturated fat consumption tends to lower these levels. Unfortunately, the average American diet is not ideal in this regard, although improvements have occurred in the past two decades (*5,6*).

Many other factors affect the levels of cholesterol and the other important lipid components (*7*). Obesity and smoking are associated with lower HDL-C levels in some persons. Regular physical exercise may increase HDL-C levels. Premenopausal women usually have much higher HDL-C levels than men because of their different hormonal makeup. Many drugs, such as diuretics, beta blockers, niacin, and cholestyramine, are known to affect favorably or detrimentally cholesterol

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Table 1. Animal studies of chlorinated drinking water and plasma cholesterol.

Species	Diet	Dose range, ppm	Effect[a]
White Carneau pigeons	Normal	0,0.1,10,15,30	Increased cholesterol at 30 ppm
	Calcium-deficient	0,0.1,10,15,30	Increased cholesterol at 10 ppm and above
	Calcium-deficient, high fat, high cholesterol	0,0.1,10	Increased cholesterol at 0.1 and 10 ppm
Rabbits	Calcium-deficient	0,0.1,15	Increased cholesterol at 15 ppm
	Calcium-deficient, high fat	0,0.1,15	Increased cholesterol at 15 ppm
Rats	Unspecified	0,0.1,10	Increased cholesterol at 10 ppm

[a] Statistically significant changes ($p \leq 0.05$).

and lipoprotein levels. Also, many unrelated diseases will alter human lipid levels. For example, elevated cholesterol is a well-known effect of hypothyroidism and the nephrotic syndrome, and people with diabetes mellitus will often have elevated lipid levels. Thus, many factors can influence an individual's lipid profile at any one time.

Several studies in animals, detailed later in this paper, suggest that consumption of chlorinated drinking water increases plasma cholesterol. In view of the known risks of elevated lipid levels, these studies are of major concern. Exposure to chlorinated water is almost universal in this country as well as in many others (*8*). Most public systems use chlorine as a disinfectant and as an aid to other treatment processes. Even persons who consume water from untreated wells can be exposed to chlorinated water in swimming pools, in canned and bottled beverages, and even from certain foods. The exposure to chlorinated water is so prevalent and so continuous from infancy to old age that any effect it might have on human cholesterol metabolism would have important implications for cardiovascular health.

The literature on the effects of chlorinated drinking water on lipid metabolism is very limited. The only studies available for review are several studies of experimental animals and one study in humans.

Studies of the effects of chlorinated drinking water on cholesterol have been conducted in white Carneau pigeons and New Zealand rabbits by Revis and colleagues and in rabbits and rats by Douglas and colleagues (*9,10*). The results of these studies are summarized in Table 1. Chlorinated drinking water consistently elevated these animals' plasma cholesterol levels, although relatively high concentrations of chlorine were necessary to induce these results in those animals on normal diets. However, several manipulations of the diet dramatically lowered concentrations of chlorine required to produce levels more typical of human exposure levels. Specifically, a diet low in calcium and/or one high in cholesterol or saturated fats made the animals sensitive to the effects of chlorine. Other factors may also be important. Water at pH 8.5 enhanced the observed effects, whereas water at pH 6.5 lessened them. Dietary iodine may also play a role, although this work is very preliminary (N. W. Revis, personal communication).

Unfortunately, lipid metabolism in these animals is very different in many respects from lipid metabolism in humans. Therefore, whether these interesting results can be generalized to humans remains an unanswered question. Limited human data showing no effect of chlorinated drinking water on serum cholesterol are available. However, this research was a screening study and was not designed to investigate lipid metabolism in detail. Therefore, its negative results may not be informative about potential effects of chlorine on serum cholesterol.

Bianchine and colleagues conducted a study of chlorine dioxide and four other drinking water disinfectants to assess their health and metabolic effects on humans (*11,12*). Chlorine was one of the four other disinfectants studied and actually served as a control in this research. Two experiments were conducted. Phase I studied the effects of single exposures to various concentrations of chlorinated water, ranging from 0.1 to 24 ppm. One liter of water was consumed on day 1 of the protocol, and blood was collected on day 2 for many tests including total cholesterol. On day 4 of the protocol, another 1-L sample containing the next higher concentration of chlorine was consumed, blood was collected on day 5, and so on. In phase II of the project, the volunteers consumed ½ L of 5 ppm chlorinated water daily for 12 weeks, and blood was collected weekly for cholesterol measurements and other tests. The individuals' diets and intake of other liquids that could contain chlorine before and during the study were not specified.

No statistically significant effect on serum cholesterol was found in either the phase I or the phase II studies. Although this result is encouraging, the study was not designed to assess critically the effects of chlorinated drinking water on human lipid metabolism, and the limitations of the project make any conclusions premature.

Specifically, lipoproteins and apolipoproteins were not measured. Thus, important changes in HDL-C and LDL-C could have occurred without a change in the total cholesterol value. The many factors known to affect lipid metabolism, such as diet and exercise, were uncontrolled in this study. Any effect or lack of effect could have been caused by alterations in these parameters coincidental with the project. Also, no chlorine-free washout period was observed before initiating the study. Since exposure to chlorine in the environment is so prevalent, this research could not compare the results of consuming chlorine-treated water to those of consuming unchlorinated water, but rather compared the effects of consuming a known dose of chlorine to those of consuming another unknown dose. Also, the study

had only 10 volunteers per group and intergroup differences could easily have been dwarfed by intragroup variation.

Additionally, the exposure in the phase I portion of the project was too brief to assess the long-term effects of chlorine. The concentration of chlorine in phase II was adequate, but since only ½ L was consumed, the total daily dose was relatively small and may have seen insufficient to induce any effects. Thus, this project provided necessary and important baseline data on water disinfectants, but it cannot rule out a significant effect of chlorine on human metabolism.

In the studies by Revis and colleagues, intestinal absorption of cholesterol appeared to be increased in animals given chlorinated water to drink (*9*). There was no evidence that the synthesis of cholesterol in the animals was increased. Preliminary work by Bercz indicates that levels of thyroid hormone may be decreased in animals exposed to chlorine-containing water disinfectants (P. Bercz, personal communication). Since hypothyroidism is a well-known cause of elevated serum cholesterol in humans, these data are provocative.

Methodology for Controlled Trial

Because of the effects that chlorine in drinking water may have on human lipid metabolism and atherosclerosis risk, the Environmental Protection Agency (EPA) and the General Clinical Research Center (GCRC) at the University of Cincinnati College of Medicine have begun to study this issue. Our project is designed to maximize the chances of observing an effect of chlorine on serum lipids while preserving the ability to generalize from the data.

The General Clinical Research Center at the University of Cincinnati College of Medicine is a specialized inpatient and outpatient unit funded by the Division of Research Resources of the National Institutes of Health. The unit is designed for and has extensive experience in careful metabolic balance studies. The staff has much experience in this demanding kind of research and has participated in a number of careful studies in environmental and occupational pollutants. The GCRC has its own kitchen, its own core laboratory, its own inpatient hospital ward, its own computer system, and has at its disposal the full resources of the University of Cincinnati hospital including a nationally standardized lipid laboratory.

A time-line for this project is shown in Figure 1. We will study 20 healthy men between the ages of 18 and 60 on a 15-week protocol as illustrated. Each volunteer will serve as his own control. The specific components of the study are listed in Table 2, and the rationale for these components discussed below.

We chose to study healthy subjects so that the results can be generalized to the population at large and so that the confounding effects of other diseases can be minimized. We plan to use male volunteers, since the small monthly fluctuations of lipids that occur during the menstrual cycle could make interpretation of the results more difficult. Persons on medications known to affect lipids will be excluded. Subjects may smoke but they will be instructed to maintain their habits before and during the protocol. The amount of regular exercise will be recorded, and every effort will be made to keep this constant. No alcohol intake will be allowed during the protocol.

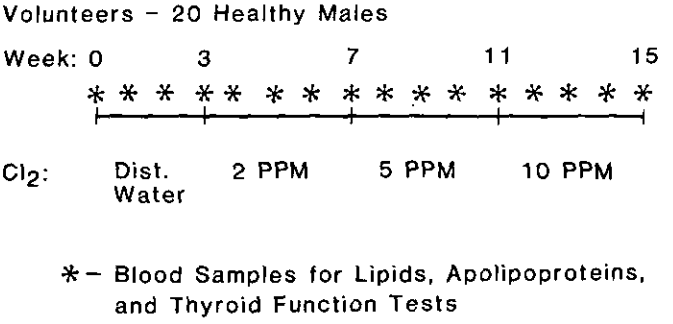

FIGURE 1. Chlorinated drinking water and human lipid metabolism, University of Cincinnati GCRC study.

The diet of the volunteer will be strictly controlled, and all meals will be eaten in the GCRC. Specifically, the diet will be isocaloric, and weight will be measured daily to ensure that no weight change occurs over the 15 weeks. The diet will contain 600 mg of cholesterol daily, which is approximately twice the recommended intake and significantly more than that consumed on average by Americans (*5*). Of the calories consumed, 20% will come from protein, 40% from fat, and 40% from carbohydrates. Current recommendations suggest that no more than 30% of calories should come from fat (*5*). The polyunsaturated/saturated fat ratio will be 0.4, which is lower than that which is recommended, but which is not atypical for American diets. The calcium in the diet will be controlled at 80% of the Recommended Daily Allowance (*13*). Thus, the diet mimics the low calcium, high fat, and high cholesterol diet used in some of the animal studies, but is not very different from the diets of many Americans.

Subjects will receive all their beverages and drinking water from the GCRC staff. They must drink at least 1.5 L/day of the study water. Any intake desired above

Table 2. Diet and water specifications for the GCRC study of chlorinated drinking water and human lipid metabolism.

	Specification
Diet	Isocaloric 20% Protein, 40% carbohydrate, 40% fat 600 mg cholesterol daily 0.4 Polyunsaturated: saturated fat ratio 80% Minimum Daily Requirement of calcium
Water	Prechlorination water distilled and carbon filtered. Buffered at pH 8.0 by NaH_2PO_4 Appropriate chlorine content (chlorine gas bubbled through distilled water alkalinized to pH 8.0 by NaOH) Mandatory 1.5 L of study water per day. Any additional water will be distilled.

that level will be distilled water. This water intake approximates the total daily fluid consumption for most persons, and it ensures that the dose of chlorine we deliver is adequate. The water itself will be distilled in facilities in the hospital and will be circulated through a carbon and bacteria filter in the GCRC before use. All the study waters during both control and study periods will contain a constant, small amount of monobasic sodium phosphate and sodium hydroxide to keep the pH of the water at 8.0. This value was chosen since the effect of chlorine appears to be more intense at basic pH and because U.S. drinking waters are typically alkaline. A concentrated chlorine solution at pH 8.0 will be made by bubbling chlorine gas through distilled water alkalinized by sodium hydroxide. Appropriate dilutions of this solution with addition of the above buffer solution will be prepared daily in the GCRC kitchen and given to the subjects in thermoses from which less than 10% of the chlorine has been observed to disappear over several days. The concentrations will be 0, 2, 5, and 10 ppm. These exposures are somewhat higher than what could be expected from most water systems but not by more than one order of magnitude. They were chosen because effects in animals have been seen at similar concentrations, because Bianchine's results confirm their safety, and because the data generated at these levels would be most useful for regulatory purposes. The 4-week study interval for each concentration should provide ample time to observe any effect, since all interventions of which we are aware have observable effects in such a period of time (*14*).

Each subject will have a completed history and physical examination at the beginning of the study and periodically thereafter. Blood for total cholesterol and triglycerides, lipoproteins, and apolipoproteins A1, A2, and B will be collected weekly in the fasting state. Multiple samples will be collected at the end of the control and study periods to allow computation of variance. Thyroid function tests will be performed periodically, also for reasons discussed above. Safety tests, including methemoglobin, blood counts, and measures of kidney, liver, and bone function, will be performed twice during each interval.

We have designed an intensive quality assurance program for this study that will supplement the extensive quality control procedures already in force in the GCRC. The drinking water will be tested daily for chlorine content and pH by standard methods to ensure the dose prescribed is the dose delivered. Dietary control procedures are and have been standard policy. The lipid laboratory at the University of Cincinnati is nationally standardized.

Statistical analysis of the cholesterol data will be performed using two-way analysis of variance by standard methods (*15*).

The project has been approved by the College of Medicine's Committee on Human Research. All subjects will give fully informed consent before admission to the study.

The first volunteers will begin the protocol September 4, 1985. Completion of this first phase of the cooperative agreement is anticipated in late 1986.

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