The effect of dietary fat, antioxidants, and alcohol on serum lipoprotein concentrations and aortic fatty streak formation in the C57BL/6 mouse model of atherosclerosis.

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"You scientists *think* too much," blurted Miss Pefko. She laughed idiotically. Dr Breed's friendliness had blown every fuse in her mental system.

A winded, defeated-looking woman in filthy coveralls trudged beside us, hearing what Miss Pefko said. She turned to examine Dr. Breed, looking at him with helpless reproach. She hated people who thought too much. The fat woman's expression implied that she would go crazy on the spot if anybody did any more thinking.

"I think you will find," said Dr. Breed, "that everybody does about the same amount of thinking. Scientists simply think about things one way, and other people think about things in others."

"Ech," gurgled Miss Pefko emptily. "I take dictation from Dr. Horvath and it's just like a foreign language. When I used to come home from school Mother used to ask me what happened that day, and I'd tell her. Now I come home from work and she asks me the same question, and all I can say is-" Miss Pefko shook her head and let her crimson lips flap slackly-

"I dunno, I dunno, I dunno."

"If there's something you don't understand," urged Dr. Breed, "ask Dr. Horvath to explain it."

He turned to me. "Dr. Hoenikker used to say that any scientist who couldn't explain to an eight-year-old what he was doing was a charlatan."

"Then I'm dumber than an eight-year-old," Miss Pefko mourned. "I don't even know what a charlatan is."

from 'Cat's Cradle' by Kurt Vonnegut Jr. Victor Gollancz Ltd. London. 1963

Abstract

In this research programme the effect of dietary fat, alcohol, and antioxidants on the serum lipoprotein profile and the development of atherosclerosis was studied in a series of experiments, primarily using the C57BL/6 mouse model of atherosclerosis. These mice, when fed a special diet, develop fatty streaks (thought to be the earliest lesion of atherosclerosis) in the intima of the aortic sinus within 15 weeks. Another mouse model of early atherogenesis, the human apoB-transgenic mouse model, was also used but was not found to possess any clear advantages over the non-transgenic C57BL/6 model. Pilot *in vitro* studies using isolated human low density lipoprotein (LDL) and macrophage cell cultures were also performed.

The effects of different dietary fats on serum lipoprotein concentrations are generally well known, however, their role in atherogenesis remains controversial. To provide further information on this, atherogenic diets containing different proportions of saturated and unsaturated fats supplied from a variety of commercial fats/oils were fed to C57BL/6 mice for 15 weeks. In contrast to the results of human studies, a high proportion of saturated fatty acids in the diet of these mice increased the ratio of serum high density lipoprotein (HDL) to total cholesterol. Although diets high in saturated fat also reduced atherogenesis in the mouse model, the literature suggests that dietary fats produce dissimilar changes in the lipoprotein profile of humans. Therefore, whether or not saturated fatty acids will have similar effects in humans remains unknown. Another group of dietary fats which have been suggested to reduce atherogenesis are the conjugated linoleic acids. However, despite producing a less atherogenic lipoprotein profile, the inclusion of conjugated linoleic acids in the experimental diet promoted fatty streak formation in C57BL/6 mice.

Epidemiological evidence suggests that dietary antioxidants may reduce atherogenesis. To investigate this experimentally, vitamin E and butylated hydroxytoluene (BHT) were examined using the C57BL/6 mouse model. Compared to controls, vitamin E lowered serum total cholesterol concentration but did not reduce fatty streak formation, while BHT lowered the ratio of serum HDL to total cholesterol and increased fatty streak formation. Because both these antioxidants were found to affect key enzymes involving lipid metabolism, it is impossible to use data from these studies to determine whether or not their

antioxidant properties influenced atherosclerosis.

The ability of dietary antioxidants to protect LDL particles from oxidation was investigated. Human subjects were given 6g of raw garlic, 2.4g of aged garlic extract, or 0.8g vitamin E each day for 7 days. Supplementation with vitamin E greatly increased the resistance of the isolated LDL to oxidation. Less, but still significant, protection was provided by aged garlic extract, but raw garlic was without effect.

There are reports in the literature that moderate consumption of alcohol by human subjects increases serum HDL cholesterol concentration and decreases atherosclerosis risk. To investigate the effect of alcohol in the mouse model, C57BL/6 mice were given water containing 3.1% alcohol in the form of either red or white wine. In contrast to humans, dietary alcohol lowered the proportion of serum cholesterol contained in the HDL fraction and promoted fatty streak formation in the C57BL/6 mouse model. While it is likely that some of the increased atherogenesis was attributable to lowered serum HDL cholesterol concentrations, data from this study suggested that dietary alcohol also influenced atherogenesis independently of the serum lipoprotein profile. No differences were observed between mice receiving alcohol from either red or white wine, suggesting that the greater quantity of antioxidants contained in red wine did not influence serum lipoprotein concentration or fatty streak formation in this model.

In conclusion, it is probable that differences in lipid metabolism between humans and C57BL/6 mice resulted in some of the dietary factors altering the serum lipoprotein concentration of mice in a way which would be considered unlikely to occur in humans. Changes in the serum lipoproteins probably then contributed to the seemingly anomalous increases in fatty streak formation that was observed in some experiments. These results illustrate some of the problems involved in investigating a disease which only occurs naturally in humans. Data derived from studies using animal and *in vitro* models may be able to provide useful information regarding human atherosclerosis. However, because it is unknown how accurately animal and *in vitro* models of atherosclerosis represent the human disease, the results of such studies must be interpreted with caution.

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