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Reduction of plasma lipid and homocysteine levels by pyridoxine, folate, cobalamin, choline, riboflavin, and troxerutin in atherosclerosis

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Summary

Elevated plasma homocysteine and lipid levels are risk factors for atherosclerosis. The plasma levels of homocysteine, determined in acid hydrolyzates of plasma, were found to be correlated with total cholesterol ($r = 0.47$, $P < 0.001$), triglycerides ($r = 0.40$, $P < 0.01$), and body mass index ($r = 0.42$, $P < 0.01$) in 52 males, aged 30–60. A group of 12 male survivors of acute myocardial infarction was given pyridoxine, folate, cobalamin, choline, riboflavin, and troxerutin for 21 days. The plasma concentrations of homocysteine and α-amino adipic acid declined to 68% ($P < 0.001$) and 57% ($P < 0.001$) of the pretreatment values, and the cholesterol, triglycerides, and LDL apo B declined to 79% ($P < 0.001$), 68% ($P < 0.01$), and 63% ($P < 0.001$) of the pretreatment values, respectively. The results suggest a new strategy for control of the metabolic abnormalities in atherosclerosis through the use of naturally occurring, non-toxic nutrients which minimize homocysteine accumulation.

Key words: Homocysteine; Plasma lipids; Pyridoxine; Folate; Cobalamin; Choline; Riboflavin; Troxerutin

Introduction

Elevated plasma lipids, especially low density lipoprotein and total cholesterol, are associated with increased risk of atherosclerosis and ischemic heart disease. Elevated plasma homocysteine, both free and bound to proteins of plasma, has also been associated with increased risk of atherosclerosis [1–5]. Recently, very high concentrations of homocysteine were found in acid hydrolyzates of plasma in ischemic heart disease [6]. Parenteral homocysteine increases the cholesterol, low density lipoprotein, phospholipids, and triglycerides of plasma, when administered to rabbits in the

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thiolactone form of the molecule [7]. In the present study the possible correlation between elevation of plasma lipids and homocysteine was investigated in human subjects, using our method for determining total plasma homocysteine.

According to the homocysteine theory of arteriosclerosis, elevated plasma homocysteine initiates arteriosclerotic plaques by intimal injury, destruction of elastin, deposition of proteoglycans and lipoproteins, fibrosis and calcification [8]. Elevation of plasma homocysteine may be produced by deficiency of pyridoxine [9], cobalamin or folic acid [10]. Choline deficiency produces atheromatous changes in experimental animals [11]. Choline is a source of methyl groups which remethylate homocysteine to methionine, and riboflavin is a co-factor in the conversion of methylenetetrahydrofolate to methyltetrahydrofolate, a direct precursor of methyl cobalamin, the coenzyme which methylates homocysteine to methionine [8,12]. Troxerutin inhibits the conversion of ascorbate to dehydroascorbate [13,14], the substance which participates in the oxidation of the sulfur of homocysteine in the metabolism of homocysteine thiolactone to homocysteic acid and phosphoadenosine phosphosulfate [15]. This metabolic pathway is responsible for sulfation of proteoglycans which bind lipoproteins in plasma [16] and within developing atherosclerotic plaques [17].

In the present study patients with ischemic heart disease were given pyridoxine, folic acid, cobalamin, choline, riboflavin, and troxerutin, substances which facilitate homocysteine catabolism. Highly significant reductions of plasma homocysteine, cholesterol, triglycerides, and low density lipoprotein were observed. The findings suggest a new strategy for the control of atherosclerosis and ischemic heart disease through the combined use of naturally occurring, non-toxic nutrients which minimize homocysteine accumulation.

Materials and Methods

To study the correlation between plasma lipids and homocysteine, 52 male subjects, aged 30–60, were chosen randomly from our study population. Twenty-six subjects had coronary heart disease, and 26 were free of heart disease. The mean body mass indices, according to Quetelet (weight (kg)/height (m^2)), were 26.2 ± 2.2 and 24.2 ± 2.1, respectively. Plasma from heparinized blood samples was obtained after an overnight fast. Triglycerides and total cholesterol were determined by the Boehringer–Mannheim enzymatic methods. Homocysteine was determined in acid hydrolyzates of whole plasma by automated amino acid analysis (Beckman 119C1 with 126 Data System Integrator), as previously described [6]. Correlation coefficients were calculated among values for plasma homocysteine, triglyceride, total cholesterol, and body mass index. Statistical significance was accepted at the $P = 0.05$ level, calculated from the Student t-test.

To study the effect of substances facilitating homocysteine catabolism, 22 male subjects, aged 40–60, with ischemic heart disease were randomly divided into treated and control groups. All of these subjects were survivors of acute myocardial infarction, 2–3 months after the acute phase. All had characteristic electrocardiographic changes, according to the Minnesota code. Twelve subjects were in the treated group, and 10 served as controls. The mean body mass index values in treated and control groups were 27.3 and 27.2 kg/m^2 before treatment and 26.6 and 26.3 kg/m^2 after treatment, respectively. The age distributions of the treated and control groups were similar. All patients received the same amount of normal hospital diet which was monitored daily. The mean daily caloric intake was 2200 ± 150 kcal/day, providing 55% of calories as carbohydrates, 30% as fat, and 15% as protein. All subjects were ambulatory, and the physical activity of both groups was similar. Informed consent was obtained from each subject in accordance with the guidelines established by the Committee of the Institute of Cardiology and the Institute of Food and Nutrition, which approved the study. These subjects were all patients at the Institute of Cardiology, Warsaw, Poland.

Subjects in the treated group received the following oral medications: pyridoxine, 150 mg/day for 21 days, folic acid, 10 mg/day for 21 days, cyanocobalamin with intrinsic factor (IF 12), 0.3 mg/day for the first 7 days, choline citrate, 2 g/day for 21 days, riboflavin, 18 mg/day for the

first 2 days and 9 mg/day for the remaining 19 days, and troxerutin (Venoruton), 1 g/day for 21 days. Pyridoxine and cyanocobalamin were given thrice daily, and folic acid, choline, riboflavin, and troxerutin were given twice daily. The compounds were given together after meals. No other drugs were employed, except for vasodilators, which were given to both treated and control groups.

Homocysteine, α-amino adipic acid, total cholesterol, low density lipoprotein (LDL) apo B, high density lipoprotein (HDL)-cholesterol, and triglycerides were determined in plasma samples after an overnight fast. Homocysteine and α-amino adipic acid were determined, as previously described [6]. LDL apo B was measured by radial immunodiffusion [18]. HDL-cholesterol was determined after precipitation of lipoprotein containing apo B with $MnCl_2$. The results are shown as mean ± SD. Statistical significance was accepted at the $P = 0.05$ level, as calculated from the Student t-test. All chemical analyses were conducted at the National Institute of Food and Nutrition, Warsaw, Poland.

Results

In a series of 52 male subjects plasma homocysteine levels were found to be correlated with total cholesterol ($r = 0.47$, $P < 0.001$), triglycerides ($r = 0.40$, $P < 0.01$), and body mass index ($r = 0.42$, $P < 0.01$). All 26 subjects with coronary heart disease had highly elevated homocysteine levels, 958 ± 84 μmol/l [6], but only 21 of these subjects had elevated total cholesterol, > 6.72 mmol/l (260 mg/dl) and/or elevated triglyceride levels, > 2.08 mmol/l (184 mg/dl). All 26 subjects without heart disease had low plasma homocysteine levels, 38 ± 11 μmol/l [6], and none had elevated lipid levels.

In a group of patients with coronary heart disease treated with moderate doses of pyridoxine, folate, cobalamin, choline, riboflavin, and troxerutin, the plasma concentrations of homocysteine, α-amino adipic acid, total cholesterol, triglycerides, and LDL apo B were found to be reduced following a 21-day period of treatment (Table 1). The homocysteine and α-amino adipic acid levels declined to 68% ($P < 0.001$) and 57% ($P < 0.001$) of the pretreatment values, respectively, and the cholesterol, triglycerides, and LDL apo B levels declined to 79% ($P < 0.001$), 68% ($P < 0.01$), and 63% ($P < 0.001$) of the pretreatment values, respectively. There was no significant change in the HDL-cholesterol level during the treatment period. In the untreated group there was no change in these amino acid and lipid concentrations during the 21-day period of observation. The mean body mass decreased from 80.6 ± 10.6 to 78.6 ± 10.0 kg in the treated group and from 82.1 ± 8.6 to 79.2 ± 6.9 kg in the untreated group. There was no significant difference in weight loss between the treated and untreated groups.

TABLE 1

EFFECT OF PYRIDOXINE, FOLATE, COBALAMIN, CHOLINE, RIBOFLAVIN, AND TROXERUTIN ON PLASMA HOMOCYSTEINE, α-AMINO ADIPIC ACID, CHOLESTEROL, TRIGLYCERIDES, AND LIPOPROTEINS IN ATHEROSCLEROSIS

The results are given as mean ± SD.

Analysis	Units	Treated (n = 12)		Untreated (n = 10)	
		Before	After 21 days	Before	After 21 days
Homocysteine	μmol/l	792 ±258	539 ±249 *	816 ±237	849 ±286
α-Amino adipic acid	μmol/l	308 ±190	175 ±116 *	265 ±128	275 ±103
Cholesterol	mmol/l	6.80± 0.83	5.35± 0.83 *	6.62± 1.24	6.72± 0.72
Triglycerides	mmol/l	2.53± 1.01	1.72± 0.51 **	2.33± 0.98	3.08± 1.25
LDL apo B	mmol/l	3.56± 0.58	2.24± 0.59 *	3.69± 0.83	3.50± 0.69
HDL-cholesterol	mmol/l	1.19± 0.24	1.14± 0.32	1.06± 0.15	1.22± 0.14

* $P < 0.001$; ** $P < 0.01$.

Discussion

The results show that the elevated plasma homocysteine level observed in atherosclerosis (792 μmol/l) was lowered by approximately 32% through the use of nutrients which were chosen to prevent homocysteine accumulation and its atherogenic effect. Pyridoxine is a precursor of pyridoxal phosphate, the coenzyme which is necessary for catabolism of homocysteine to cystathionine, cysteine, cysteine sulfinic acid and taurine [19]. Folic acid is a precursor of methyltetrahydrofolate, the coenzyme which transfers its methyl group to cobalamin to form methylcobalamin. Subsequently, the excess homocysteine is methylated by methylcobalamin to form methionine, which is non-atherogenic [8]. Riboflavin is required in formation of methylenetetrahydrofolate reductase, the flavoprotein which converts methylenetetrahydrofolate to methyltetrahydrofolate. Flavins are also needed to reduce the cobalt atom of cobalamin to the active form which accepts methyl groups to form methylcobalamin [12]. Choline is another important source of methyl groups which facilitate conversion of homocysteine to methionine by transmethylation. Troxerutin is an antioxidant flavonoid which scavenges free radical substances such as oxygen radicals and semidehydroascorbate [13], the radical form of the coenzyme which is involved in conversion of homocysteine to homocysteic acid, the atherogenic sulfonyl form of homocysteine [15]. The simultaneous use of the 6 administered nutrients in the study is believed to lower plasma homocysteine levels by promoting catabolism of homocysteine to cystathionine and subsequently to taurine (pyridoxine), increased methylation of homocysteine to methionine (folate, cobalamin, choline, and riboflavin), and inhibition of homocysteine oxidation (troxerutin). These nutrients were used together to insure a biochemical response of individuals who may have had an isolated deficiency of one or another of the substances. Further investigation is needed to determine whether these nutrients, given singly or in lesser combinations, might affect homocysteine and lipid levels in individuals with different deficiencies.

Elevation of plasma homocysteine was found to be correlated with elevated levels of cholesterol and triglycerides in our study population. In patients with coronary heart disease, the 6 nutrients which lowered plasma homocysteine levels also lowered the plasma levels of cholesterol, triglycerides and LDL apo B to a similar degree. The connection between homocysteine and lipid metabolism is incompletely understood, but certain homocysteine derivatives are known to influence lipid metabolism in several ways. Homocysteic acid has been shown to promote growth of hypophysectomized rats through the release of somatomedin, a mediator of the physiological action of growth hormone, which causes cellular hyperplasia and increased sulfation of cartilage matrix [20]. Growth hormone affects lipid metabolism by elevating the plasma concentration of free fatty acids. The end product of cholesterol metabolism, cholic acid, is excreted as the bile acid conjugate, taurocholic acid, and taurine is the end product of catabolism of homocysteine through cystathionine, cysteine, and cysteine sulfinic acid [19]. Certain hypocholesterolemic drugs, such as cholestyramine and its analogues, may also facilitate homocysteine catabolism through increased excretion of taurocholic acid by inhibition of bile acid reabsorption. Thus, increased formation and excretion of taurocholic acid may help to prevent homocysteine accumulation and simultaneously lower blood cholesterol, because of increased excretion of this bile acid conjugate formed from the end-products of homocysteine and cholesterol metabolism. Homocysteic acid is a precursor of phosphoadenosine phosphosulfate, the coenzyme which forms the sulfate esters of proteoglycosaminoglycans [15]. Excessive sulfation of proteoglycans, especially proteodermatan and proteochondroitin sulfates, causes aggregation, binding of lipoproteins, and their consequent deposition within atherosclerotic plaques [17]. The aggregates of lipoproteins and highly sulfated proteoglycosaminoglycans are sequestered from normal enzymatic degradation within the arterial wall. Lipoproteins also bind the sulfated proteoglycosaminoglycans of plasma [16]. Because the sulfate groups of these substances are derived in part from oxidized homocysteine, this phenomenon provides another connection between lipid and homocysteine metabolism. Finally, ad-

ministration of homocysteine thiolactone to rabbits causes elevation of cholesterol, triglycerides, phospholipids and LDL, directly demonstrating a relation between these areas of metabolism [7]. Therefore, the reduction of plasma levels of cholesterol, triglycerides, and LDL apo B observed in the treated group (Table 1) may be attributed to the lowered plasma level of homocysteine caused by the administered nutrients.

The present results also show a correlation between lowering of plasma homocysteine and lipids with lowering of plasma α-amino adipic acid. We previously showed that plasma α-amino adipic acid is also greatly increased in ischemic heart disease [6]. This substance is probably derived from allysine of α-amino adipic δ-semialdehyde residues of elastin which are released during acid hydrolysis of plasma. Accordingly, polymers derived from elastin are evidently present in increased quantities in plasma in coronary heart disease, indicating release of degraded elastin from the arterial wall [6]. Increased plasma homocysteine is believed to impair elastin polymerization by reaction with the aldehyde form of allysine [21,22]. Therefore, the reduced level of α-amino adipic acid observed in the treated group is probably a consequence of the reduced level of plasma homocysteine caused by the administered nutrients.

Previous work has shown that reductions of body mass of 5.3 kg in women and 6.2 kg in men, or greater, caused a significant decrease in plasma levels of cholesterol, triglycerides, and LDL apo B [23]. In the present study the smaller decreases of 2.0 and 2.9 kg in the treated and untreated groups, respectively, are unlikely to explain the observed changes in plasma amino acid and lipid levels because there was no significant difference in weight loss in the two groups.

Elevation of lipoproteins in type II lipoproteinemia is associated with enhanced aggregability and release reactions of platelets exposed to aggregating agents [24]. Recently, low concentrations of the free base form of homocysteine thiolactone, the cyclic anhydride of homocysteine, were found to cause platelet aggregation and release of thromboxane TBX_2 [25]. The reduction of levels of plasma homocysteine, cholesterol, triglycerides and LDL apo B demonstrated in the present study suggests that the increased susceptibility to platelet aggregation and thrombosis in atherosclerosis may also be controlled by nutrients which enhance homocysteine catabolism. Further study is needed to investigate this possibility.

The present work shows that a short period of treatment with moderate doses of naturally occurring, non-toxic nutrients reduces the accumulation of plasma homocysteine and plasma lipids in patients with atherosclerosis. Further work is needed with larger groups of patients treated for longer intervals to determine whether any reduction of risk of complications of atherosclerosis may accompany amelioration of the metabolic abnormalities characteristic of the disease.

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