



ORIGINAL ARTICLE

The association of serum lipids, lipoproteins and apolipoproteins with selected trace elements and minerals in phenylketonuric patients on diet

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Summary Objective: Classical phenylketonuria (PKU) is an inborn error of metabolism characterized by high Phenylalanine (Phe) levels in blood and treated with a special low Phe diet which can be defined as "nonatherogenic". Since coronary heart disease (CHD) was reported to be a disease of zinc and copper imbalance, we aimed indirectly to evaluate the effect of the special diet on the size of LDL particles and to investigate whether some minerals and trace elements are involved in their lipoprotein metabolism.

Methods: Eighty-six ($N = 86$) PKU patients were divided into two groups. Group A ($N = 44$) on a strict diet and group B ($N = 42$) who did not adhere to their treatment. Healthy children ($N = 98$) were the controls. Serum total cholesterol (t-Chol), triacylglycerol, High-density lipoprotein (HDL) and t-Chol in very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were measured with enzymatic methods, whereas Apolipoprotein AI (Apo AI), Apolipoprotein AII (Apo AII) and Apolipoprotein B (Apo B) were determined by nephelometric techniques. LDL/Apo B positively correlated with LDL size. Magnesium (Mg), calcium (Ca), copper (Cu) and zinc (Zn) measurements were performed by atomic absorption spectrometry.

Results: t-Chol, LDL, VLDL, Apo B, the ratio t-Chol/HDL, Apo AI/Apo B and LDL/Apo B as well as copper levels and the ratio Zn/Cu in group A statistically significantly differed as compared to those of group B and Controls. Positive correlations were found between Mg and HDL and Apo AI in all the groups whereas the mineral correlated with t-Chol, Apo B and the ratio LDL/Apo B only in the group A of patients. Copper negatively correlated with triacylglycerol, LDL, and Apo B and positively with t-Chol in group A. Zinc showed negative relationships in HDL and Apo A in all the studied groups. The ratio Zn/Cu negatively correlated with triacylglycerol and LDL in all the groups and positively with the ratios Apo AI/Apo B and LDL/Apo B in group A.

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Conclusion: Some of the minerals and trace elements were correlated with the lipids and lipoproteins and were also involved in the size of LDL particles in PKU patients on strict diet. Larger and less atherogenic LDL particles were associated with a high Zn/Cu ratio.

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Introduction

Classical phenylketonuria (PKU) is an inborn error of metabolism characterized by the deficiency of the enzyme phenylalanine hydroxylase. Consequences of this deficiency are the elevated blood concentrations of the amino acid phenylalanine (Phe) and the low, almost undetectable, concentration of tyrosine. Mental retardation and eczema are the main clinical findings in untreated patients.^{1,2} PKU is currently treated by a special diet to avoid elevated blood levels of the amino acid. Because of the severe restriction of conventional foods, supplements of amino acids (other than Phe), protein free energy sources, and macro and trace minerals are necessary to ensure the adequacy of the diet. The quantity of natural protein intake (Phe intake) is individualized and depends on the residual activity of the enzyme phenylalanine hydroxylase. These protein requirements are achieved generally with vegetable proteins. Such a diet can be defined as "nonatherogenic" because of the reductions of animal lipids, cholesterol, saturated fatty acids and increased intake of dietary fiber. All these factors have well-known effects on lipid control.³⁻⁵

In our previous study⁶ blood cholesterol levels were found low in PKU patients on diet as compared to those of healthy children of the same age. On the contrary, their triacylglycerol levels were higher as a result of their special diet containing a large amount of carbohydrates. In a recent study⁷ a relationship between high plasma Phe levels and an inhibition of cholesterologenesis was reported, although the low cholesterol intake of the special diets could also decrease serum cholesterol values.

Dreon et al.⁸ found that low fat, high-carbohydrate diets caused a subset of men with pattern A to convert to pattern B LDL particles. Additionally in an other report⁹ they found smaller dense LDL particles in premenopausal women, a group with low expression of pattern B LDL, when consuming a low fat diet (20% of energy). The same authors reported that a very low fat (10% of energy), high-carbohydrate diet can induce expression of LDL-subclass pattern B.¹⁰

Furthermore, the low-density lipoprotein/apolipoprotein B (LDL/Apo B) ratio correlates well with LDL size and had been used as a marker of atherogenicity of LDL.¹¹ Additionally variations in high-density lipoprotein (HDL) levels are partially due to differences in fractional catabolic rates. Large HDL particles with high HDL-cholesterol/HDL-Apo A1 plus ApoAII ratio exhibit low catabolic rates.¹²

Previously, Klevay¹³ hypothesized that coronary heart disease (CHD) is predominantly a disease of zinc and copper metabolic imbalance principally due to the copper deficiency. In fact, copper deficiency increases serum cholesterol levels through modulating the cholesterol biosynthesis¹⁴ and both metals regulate the enzyme copper/zinc superoxide dismutase. Copper and iron have been shown to be pro-oxidants and play an important part in lipoprotein peroxidation.^{15,16}

The aim of this study was to evaluate the effect of PKU special life-long diet on the size of LDL particles and to investigate whether some mineral and trace elements are involved in the PKU patients lipoprotein metabolism.

Patients and methods

The study was approved by the Greek Ethical Committee.

Patients

Eighty-six ($N = 86$) patients with PKU were classified into two groups according to their mean annual Phe (Phe mean) concentrations. All PKU patients were discovered by "screening" and were put on the special diet after tetrahydrobiopterin (BH4) loading test and dihydropteridine reductase evaluation. Their daily protein intake was largely replaced by PKU2 (Milupa AG) which is a Phe-free mixture of amino acids enriched by vitamins and trace elements. The product contains no fat of any kind. Olive oil was the main source of their fat intake. Group A consisted of 44 patients, mean age 6.78 ± 1.5 years (mean Phe levels $492 \pm 100 \mu\text{mol/l}$), who had good compliance with the special diet

as shown by their monthly Phe blood evaluation. Group B included 42 patients (mean age 8.0 ± 3.2 years, who did not strictly adhere to the diet resulting in high Phe blood levels (mean Phe levels $1763 \pm 160 \mu\text{mol/l}$). Normal children of comparable age ($N = 98$) mean age 7.68 ± 2.3 years and sex, were used as a control group. Regular consumers of any medication or vitamin supplements were excluded from the Control group, whereas PKU patients were requested to discontinue their vitamin supplementation for the last 30 days.

Additionally, dietetic diary was kept for each PKU patient and controls for 30 successive days. Nutrition tables¹⁷ were used for the calculation of their dietary records.

Methods

Blood (6.0 ml) was drawn from each member of the three groups after 8–10 h fasting for the evaluation of Phe, Cholesterol (Chol). Triacylglycerol, high-density lipoprotein-cholesterol (HDL), low-density lipoprotein-cholesterol (LDL), very-low-density lipoprotein-cholesterol (VLDL), Apolipoprotein B (Apo B), Apolipoprotein AI (Apo AI), Apolipoprotein AII (Apo AII) selected trace elements Copper (Cu), Zinc (Zn) and minerals Magnesium (Mg), Calcium (Ca). Phe was measured with a standardized enzymatic assay in dried blood samples on filter paper (2992 Schleicher and Schull) with R and D Diagnostics MMR 2000.¹⁸

Analytical procedure

Serum total cholesterol (t-Chol), triacylglycerol, HDL-cholesterol and cholesterol in VLDL and LDL were measured using the ADVIA-1650 Chemistry System (Bayer Corporation, Tarrytown, NY, USA), while Apo AI and Apo B were determined by latex-particle-enhanced immunonephelometric assays on the BN ProSpec nephelometer (Dade Behring, Liederbach, Germany). Quality control has previously been indicated.¹⁹ Interassay variation coefficients for t-Chol, Triacylglycerol, HDL-cholesterol, Apo AI, and Apo B were 3.5%, 3.7%, 5.1%, 5.0% and 4.5%, respectively.

Duplicate serum samples were analyzed for calcium (Ca), magnesium (Mg), copper (Cu) and zinc (Zn) by atomic absorption spectrometry using the Perkin Elmer Analyst 800 atomic absorption spectrometer. Lanthanum chloride (Merck Darmstadt, Germany) was added to avoid interferences. Aliquots of human serum were used as an internal control to assess precision in the mineral determinations. The interassay relative standard deviation

was 2.1%, 4.5%, 4.0%, 5.0% for Ca, Mg, Cu and Zn, respectively. Certified reference materials (CRM 63, CRM 185, Community Bureau of Reference Brussels) were used to assess accuracy. All laboratory equipment employed in the trace elements analysis was washed with nitric acid to avoid contamination. Distilled-deionized water (AAS grade) was used for the preparation of dilutions (1:50 for Ca and Mg, 1:5 for Zn and 1:2 for Cu) and standards of the mineral/trace element analysis.

Statistical analysis

Data were evaluated by analysis of variance (ANOVA) and Student's *t*-test, adjusted for multiple comparison. *P* values <0.05 were considered statistically significant. Correlations were determined by Pearson's test.

Results

As shown in Table 1, energy and total protein intake did not differ between the groups, but natural protein fiber and saturated fat differed greatly. Significant differences were also found in the intake of total fat, saturated, monounsaturated and polyunsaturated fat, and cholesterol between group A and controls as well as between the two groups of patients. The intake of copper was decreased in the group of patients with the good compliance with their diet whereas the intake of the other trace elements and minerals did not differ, except zinc in group A, which was found increased.

As shown in Table 2, t-Chol, VLDL-Chol, LDL-Chol, Apo B, the ratios t-Chol/HDL-Chol, Apo A-1/Apo B and LDL-Chol/Apo B as well as copper levels and the ratio zinc/copper in group A statistically significantly differed in group A as compared to those of group B and Controls. The other biochemical parameters, lipids, lipoproteins, apolipoproteins, mineral and zinc were similar among the groups.

Table 3 gives the existence of positive correlations between magnesium and HDL-Chol and Apo A-1 in all the groups whereas the mineral correlated with t-Chol, ApoB and the ratio LDL-Chol/Apo B only in group A of PKU patients. The trace element copper showed negative relationships in triacylglycerol, LDL-Chol and Apo B and positive in t-Chol in group A. Zinc negatively correlated with HDL-Chol and Apo A-1 in all the studied groups. The ratio Zn/Cu negatively correlated with triacylglycerol and

Table 1 Estimated 30 d nutrient intakes for the two groups of PKU-patients and controls.

Nutrients (per 100 g)	Nutrient intake (mean±SD)			Significance of differences (P)		
	Group A (N = 44)	Group B (N = 42)	Controls (N = 98)	A vs. Control	B vs. Control	A vs. B
Energy (kcal)	2020±400	2038±450	2078±470	—	—	—
Carbohydrate (g)	268.2±18	232.0±20	230±17	<0.05	—	<0.05
t-Protein (g)	70±18	72.0±20	73.0±17	—	—	—
Natur. protein (g)	9±1.2	40±20	73.0±17	<0.001	<0.001	<0.001
Fibre (g)	30±8.0	21±8.0	16.6±8.0	<0.001	<0.01	<0.001
Total fat (g)	80±5.0	97±4.0	99.0±5.0	<0.01	—	<0.01
Saturated fat (g)	29±10	45.0±11	56±11	<0.001	<0.001	<0.0001
Monounsaturat fat (g)	25±8	42±9	46±8	<0.007	—	<0.05
Polyunsaturat fat (g)	26±7	11.7±4	11.0±3.5	<0.001	—	<0.001
Polyunsaturat: Satur	0.90±0.2	0.3±0.1	0.2±0.1	<0.001	—	<0.0001
Cholesterol (mg)	302±114	410±112	455±110	<0.01	<0.05	<0.05
Calcium (mg)	1380±140	1320±120	1300±140	—	—	—
Copper (µg)	1116±60	1590±78	1510±80	<0.001	—	<0.001
Magnesium (mg)	196±38	205±40	201±58	—	—	—
Zinc (mg)	12±2.2	9±2.0	9±2.19	<0.001	—	<0.001

Values are expressed: mean±SD.

Table 2 Lipids, lipoproteins, apolipoproteins (Apo) some minerals and trace elements in the serum of PKU-patients vs. Controls.

	Groups			P		
	A (N = 44)	B (N = 42)	C (N = 98)	A vs. B	A vs. C	B vs. C
Phe (µmol/L)	492±100	1763±160	250±90	<0.001	<0.001	<0.01
Triacylglycerol (mmol/l)	1.09±0.72	1.15±0.36	1.10±0.4	NS	NS	NS
t-Cholester (mmol/l)	3.23±0.24	4.15±0.42	4.19±0.50	<0.001	<0.001	NS
LDL-chol(mmol/l)	1.59±0.27	2.39±0.54	2.41±0.5	<0.001	<0.001	NS
HDL-chol(mmol/l)	1.13±0.16	1.15±0.16	1.20±0.18	NS	NS	NS
VLDL-C	0.49±0.3	0.59±0.26	0.61±0.3	<0.01	<0.001	NS
t-Chol/HDL-chol	2.87±0.46	3.67±0.52	3.56±0.5	<0.001	<0.001	NS
Apo AI (g/l)	1.53±0.20	1.55±0.25	1.50±0.3	NS	NS	NS
Apo B (g/l)	0.46±0.12	0.77±0.18	0.80±0.20	<0.001	<0.001	NS
Apo All (g/l)	0.35±0.07	0.38±0.05	0.39±0.05	NS	NS	NS
Apo AI/Apo B	3.58±0.9	2.30±0.90	2.28±0.8	<0.001	<0.001	NS
HDL-chol/Apo AI	0.29±0.03	0.28±0.04	0.28±0.05	NS	NS	NS
LDL-chol/Apo B	1.37±0.16	1.26±0.14	1.28±0.15	<0.01	<0.01	NS
Ca (mmol/l)	2.30±0.13	2.32±0.15	2.30±0.17	NS	NS	NS
Mg (mmol/l)	0.78±0.9	0.80±0.8	0.82±0.08	NS	NS	NS
Cu (µmol/l)	14.4±2.1	18.3±2.9	17.9±2.90	<0.001	<0.001	NS
Zinc (µmol/l)	11.98±0.98	12.30±1.30	12.8±1.50	NS	NS	NS
Ca/Mg	2.97±0.27	2.95±0.26	2.96±0.3	NS	NS	NS
Zn/Cu	0.85±0.15	0.69±0.13	0.72±0.15	<0.001	<0.001	NS

Values are expressed: mean±SD.

NS = not stat. significant.

LDL-Chol in all the groups. On the contrary, the ratio of trace elements positively correlated with the ratios Apo A-1/Apo B and LDL/Apo B in the groups of patients who strictly adhered to their diet.

Discussion

Plasma lipoprotein abnormalities underlie and may even be essential for the common occurrence of atherosclerotic vascular diseases.

Table 3 Pearson correlations (*r*) between lipids, lipoproteins (Apo) and minerals and trace elements in serum of PKU patients (*N* = 86) and Controls (*N* = 98).

Groups	Ca	Mg	Ca/Mg	Cu	Zn	Zn/Cu
Triacylglycerol	A			-0.392*		-0.439**
	B					-0.41**
	C					-0.38*
t-Cholesterol	A	-0.401**	-0.396*	0.49**		0.49***
	B					
	C					
LDL-Chol	A			-0.48 [†]		0.51***
	B					
	C					
HDL-Chol	A	0.424**			-0.39*	
	B	0.394*			-0.41**	
	C	0.411**			-0.38*	
T Chol/HDL	A	-0.410**				
	B					
	C					
Apo AI	A	0.434**			-0.46**	
	B	0.414**			-0.38*	
	C	0.398*			-0.42**	
Apo B	A	0.410**		-0.51***		
	B					
	C					
Apo AI/Apo B	A					0.516***
	B					
	C					
HDL-Chol/Apo AI	A					
	B					
	C					
LDL-Chol/Apo B	A	0.49**				0.59***
	B					
	C					

P* < 0.05; *P* < 0.01; ****P* < .001.

The abnormalities include elevated concentrations of LDLs and VLDLs and reduced concentrations of HDLs, as generally estimated from measurements of plasma cholesterol, triacylglycerol and HDL-Chol. Other studies, however, indicate that the most pronounced lipoprotein abnormalities in patients with early onset CHD are high triacylglycerol and low HDL-Chol with lesser elevations of LDL-Chol. In these individuals, the LDL particles are somewhat smaller and more dense than in those with a more favorable lipoprotein profile.²⁰

PKU patients in group A showed remarkably lower concentrations of t-Chol, LDL-Chol and Apo B as compared to those of group B and Controls (Table 2). Additionally, the ratio t-Chol/HDL-Chol was lower in the same group of patients whereas the ratios Apo A-1/Apo B, LDL-Chol/Apo B were statistically significantly increased as compared to group B and controls. This could be due to their vegan-vegetarian diet^{5,6,21} in which the only sources of lipid intake were olive oil and cream cheese. Other food containing saturated fat, such as eggs, meat, etc. are contraindicated. The data

presented in Table 1 indicate that the intake of these nutrients were remarkably decreased (low saturated fat intake) in the compliant group (group A) as compared to the patients of the noncompliant group (higher saturated fat intake) (group B) as well as to the controls who consumed adequate natural protein and lipids. In our previous study, no correlations were found between LDL levels and Homocysteine concentrations in PKU patients.²² According to Campos et al.¹¹ a high LDL-Chol/Apo B ratio is related to the presence of larger and less atherogenic LDL particles, as we found in the group A of our patients. As mentioned, large LDL particles are less susceptible to oxidative damage than small LDL particles.²³

Magnesium plays an important role on lipoprotein metabolism. Magnesium is essential for fatty acid and protein synthesis, and is critical in energy—requiring metabolic process as an adenosine triphosphate co-factor.²⁴ Some authors have also reported a positive correlation between magnesium and HDL-Chol in adults, in agreement with the present results.²⁵ These same authors also describe a negative relationship between serum magnesium and t-Chol in adults as we found in the group A of PKU patients with the good compliance to their diet. The calcium/magnesium ratio did not show any relationship in the lipids, lipoproteins and apolipoproteins. This may be due to the homeostatic control of calciotropic hormones, which permit little variation of serum calcium levels.²⁵

Lower zinc absorption has been reported in those consuming vegetarian diets than in those eating nonvegetarian diets.²⁶ Consequently, it has been suggested that the zinc requirement for those consuming a vegetarian diet, such as PKU (group A) is approximately two-fold greater than for those consuming a nonvegetarian diet. Although the zinc intake was statistically higher in the group A of patients, on a vegan-vegetarian diet, greatly supplemented with their Phe free formula, the trace element levels in their serum did not differ as compared to group B and controls. However a protective effect of Zn on the risk of cardiovascular disease and cancer has been reported.²⁷

Additionally, this study shows that copper levels were reduced in the group A of our patients probably due to the kind of their special diet (food rich in copper such as red meat and seafood are contraindicated). Copper deficiency has been related to high serum t-Chol, as this metal modulates the activity of the rate controlling enzyme of cholesterol biosynthesis 3-hydroxy-3-methylglutaryl-coenzyme A reductase.^{13,14} Additionally, as shown in experimental hyperphenylalaninemia the enzyme activity was found decreased.²⁸ According

to the latter experimental conclusion, PKU patients with high Phe levels (group B) might be hypocholesterolaemic. This hypothesis is in contrast with our previous^{6,22} and present findings in these patients in whom t-Chol levels were almost as those in controls. Furthermore, low copper level is implicated with increased t-Chol levels.¹⁴ This suggestion is opposite to the present results since PKU patients (group A) on strict diet could be characterized hypocholesterolaemic. With regards to the PKU patients, who strictly adhered to their diet and consequently their Phe blood levels were almost normal, we strongly suggest that their low t-Chol levels could be mostly related to their reduced saturated fat intake.^{5,6} Low copper level, evaluated in the same patients (group A), was found related to the presence of larger LDLs (LDL/Apo B), which are less susceptible to oxidative damage than smaller LDLs.²³ These findings are reinforced by the higher total antioxidant status, found in these patients who adhere to their diet.²⁹ Moreover, present data shows that the zinc/copper ratio clearly appeared to be related to some lipid and lipoprotein parameters. These results also suggest a different influence of this ratio on t-Chol metabolism in these PKU patients in whom t-Chol levels were found reduced, showing that their vegan-vegetarian diet can offer a protection. However, another interesting hypothesis can be drawn from this data, as the zinc/copper ratio correlates with the LDL-Chol/Apo B ratio, which was found higher in group A of PKU patients. As mentioned above, large LDL particles are less susceptible to oxidative damage than small LDL particles.²³ Finally, present study results confirm all these interesting relationships, as the zinc/copper ratio was positively correlated with t-Chol and LDL-Chol levels as well as to the LDL-Chol/Apo B ratio (LDL atherogenicity), but negatively correlated with triacylglycerol levels. Previous and recent studies^{6,22,29} in PKU patients demonstrated significant reductions in fatty acid levels as well as in long-chain polyunsaturated fatty acids (LCPUVA) including plasma docosahexaenoic (DHA) and arachidonic acid (AA), the latter implicated with brain and retina functions.³⁰

In conclusion, the data suggest that some of the minerals studied play an important part in the lipoprotein metabolism in patients with PKU on diet, being involved in the metabolism and especially in the size of LDL particles the latter indirectly evaluated for first time in these patients.

On this basis PKU-patient's who strictly adhered to their diet, are not at risk of developing atherosclerosis since their lipids, lipoproteins and apolipoproteins may be less atherogenic as compared

to those of group B and Controls. Larger and less atherogenic LDL particles were also associated with a high Zn/Cu in PKU patients on strict diet. Evaluation of minerals and trace elements should be incorporated in the routine laboratory tests of PKU patients on diet.

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