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Increased plasma adiponectin concentrations in poorly controlled patients with phenylketonuria normalize with a strict diet: evidence for catecholamine-mediated adiponectin regulation and a complex effect of phenylketonuria diet on atherogenesis risk factors

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Abstract

Adiponectin (Adpn), an adipose tissue–derived hormone, prevents endothelial inflammation and early atherogenesis. Classic phenylketonuria (PKU), an inborn error of phenylalanine (Phe) metabolism, results in a reduction of catecholamine biosynthesis and requires treatment with lifelong low-Phe diet to prevent mental dysfunction and allow proper intellectual development. In this study, we evaluated the effect of the quality of PKU diet on plasma Adpn concentrations and related biochemical indices of endothelial dysfunction and atherogenesis. Patients with PKU were divided into groups A (n = 18), who were on a strict diet, and B (n = 18), who were on a loose diet, after evaluation of their 30-day dietetic diaries and measurement of Phe blood concentrations. Twenty healthy children of similar ages and body mass indexes served as controls (group C). Group A patients had normal circulating catecholamines and Adpn and decreased tumor necrosis factor α concentrations and low-density lipoprotein cholesterol/apolipoprotein B ratio compared with groups B and C. Despite these favorable parameters, however, homocysteine concentration was twice as high in group A compared with groups B and C. Interestingly, group B patients under loose dietary control had significantly elevated Adpn concentrations compared with groups A and C and increased tumor necrosis factor α and an unfavorable lipid profile, but normal levels of homocysteine. These data support the hypothesis that catecholamines inhibit Adpn secretion and that the elevated Adpn of the poorly controlled patients might moderate their risk for endothelial dysfunction and atherogenesis. Homocysteine production appears to be unfavorably affected by a strict PKU diet, diverging from the rest of the atherogenesis risk factors, which were improved in the well-controlled patients.

1. Introduction

Classic phenylketonuria (PKU), an inborn error of phenylalanine (Phe) metabolism resulting in a reduction of catecholamine biosynthesis, is treated with a low-Phe diet started as soon as possible, in the first few days of life [1,2]. The natural protein intake (Phe intake) of these patients must be individualized and depends on their residual activity of the enzyme phenylalanine hydroxylase. The dietary requirements of PKU patients are achieved mainly with vegetable proteins. Such a diet is characterized by a reduction of animal cholesterol and saturated fatty acids and an increase of dietary fiber intake and is in effect "vegetarian" [3,4].

In PKU patients who are on a strict diet, the serum concentrations of total cholesterol (TC), low-density lipoprotein

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cholesterol (LDL-C), and apolipoprotein (apo) B are decreased, although their LDL particles are larger than those of patients on a "loose diet" and controls [4]. Paradoxically, homocysteine (Hcy), a sulfur-containing amino acid that has been associated with an increased risk for atherosclerosis and premature occlusive vascular disease, is elevated in the circulation of diet-controlled PKU patients [5]. Furthermore, the plasma leptin, the adipose tissue–derived hormone that inhibits food intake and stimulates basal metabolic rate, is normal in PKU patients who are controlled by a strict diet, but significantly elevated in poorly controlled patients. This finding is probably due to the diminished concentrations of catecholamines in poorly controlled patients allowing disinhibition of leptin secretion [6].

Adipose tissue, which usually accounts for more than 10% of body weight, is not only a reservoir for energy storage, but also an active endocrine tissue manufacturing and secreting several hormones and cytokines called adipokines, and leptin is but one of them [7,8]. Adiponectin (Adpn) was identified recently as one of the adipokines with important metabolic and immunoregulatory effects [9]. This protein is produced only by adipose tissue and is abundantly present in the circulation. Its concentrations are higher than those of all known inflammatory cytokines, including those of tumor necrosis factor α (TNF-α) or interleukin 6. Physiological concentrations of Adpn inhibit TNF-α–induced expression of endothelial-leukocyte adhesion molecule 1 and decrease monocyte/macrophage adhesion to endothelium [10]. Delporte et al [11] recently reported that catecholamines inhibit Adpn production in vitro and in vivo in humans. Adiponectin levels correlated strongly and negatively with body mass index (BMI), and a low Adpn concentration in plasma might be a useful early marker of insulin resistance and a risk factor for atherosclerosis [9].

Because the PKU special diet is a lifelong affair, we evaluated the effect of the quality of this diet on the plasma concentrations of Adpn and of other biochemical parameters related to endothelial dysfunction and atherogenesis in well-controlled or poorly controlled PKU patients and appropriately matched controls.

2. Subjects and methods

2.1. Patients and controls

The study was approved by the "Aghia Sophia" Institutional Review Board. All patients underwent standard laboratory tests before entering the study. All patients were initially diagnosed by neonatal screening and placed on a special diet after a tetrahydrobiopterin loading test and dihydropteridine reductase evaluation. Their daily protein intake was largely replaced by PKU_2 (Milupa AG, Friedorf, Germany), which is a Phe-free mixture of amino acids. Phenylketonuria is treated by a special diet to avoid elevated blood levels of the amino acid Phe. Because of the severe restriction of conventional foods, supplements of amino acids (other than Phe), protein-free energy sources, and macrominerals and trace minerals are necessary to ensure adequacy of the diet. The quantity of natural protein intake is individualized and depends on the residual activity of the enzyme phenylalanine hydroxylase. Dietetic diary was kept for each PKU patient and controls for 30 consecutive days. Nutrition tables [12] were used for the calculation of dietary parameters. All patients and controls were prepubertal. The study population consisted of 36 PKU patients who were divided into 2 groups, A and B, according to their diet and their mean annual morning preprandial blood Phe (Phe mean) concentrations and 20 age-, sex-, and BMI-matched control children (group C) (Table 1); group A (n = 18) included patients who adhered strictly to their diet (mean annual Phe, 200 ± 105 μmol/L), whereas group B included 18 PKU patients who were on a loose diet, and they did not follow their special individualized diet as shown by the grossly elevated mean annual Phe levels (619 ± 155 μmol/L).

Height in centimeters was measured on a portable stadiometer calibrated with a machine meter rod, and weight in kilograms was measured with an electronic scale. Genital or breast development was graded according to Tanner.

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 30 days before the study. Most of these subjects took part in previous studies [4-6].

3. Methods

Blood (6.0 mL) was drawn from each member of the 3 groups after an 8- to 10-hour fasting at the same time of the day ≅9:00 AM for the evaluation of Phe Inst, Hcy, TNF-α, Adpn, TC, triacylglycerol (TG), high-density lipoprotein (HDL), LDL, apo A, apo B, apo A-I, apo A-II, and catecholamine (noradrenaline [NA], adrenaline [A], and dopamine [DA]) concentrations. All blood samples were

Table 1
Clinical characteristics of PKU patients (group A, group B) and controls (group C)

	Age (y)	Sex (M/F)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Group A (n = 18)	6.4 ± 1.2	10/8	112 ± 4.0	20.0 ± 3.8	18.0 ± 7.0
Group B (n = 18)	7.0 ± 1.0	9/9	115 ± 3.0	19.8 ± 3.0	19.0 ± 6.5
Group C (n = 18)	7.2 ± 1.0	11/9	116 ± 3.0	19.0 ± 4.0	18.5 ± 7.0

Data are expressed as mean ± SD. M indicates male; F, female.

Table 2
Estimated 30-day nutrient intake in PKU patients and controls

	Group A (n = 18)	Group B (n = 18)	Group C (n = 20)	Differences (*P*)		
				A vs C	B vs C	A vs B
Energy (kJ)	8390 ± 1384	8625 ± 2010	8709 ± 1675	–	–	–
Total protein (g)	70 ± 13	72 ± 14	74 ± 15	–	–	–
Natural protein (g)	6 ± 1.2	30 ± 1.6	74 ± 15	.0001	.001	.001
Carbohydrate (g)	230 ± 63	232 ± 30	245 ± 40	–	–	–
Fiber (g)	33 ± 7	21 ± 8	25 ± 9	.001	–	.01
Total fat (g)	75 ± 25	88 ± 4	106 ± 8	.01	.03	.001
Saturated fat (g)	28 ± 11	47 ± 11	58 ± 14	.001	.002	.05
Monounsaturated (g)	23 ± 7.7	32 ± 9	34 ± 12	.01	–	.001
Polyunsaturated (g)	24 ± 7.2	10 ± 4	13 ± 5	.001	.05	.01
Polysaturated (ratio)	0.9 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	.01	–	.001
Cholesterol (mg) (RNI 180-200)	310 ± 112	340 ± 120	345 ± 110	.05	–	.01
Vit B_1 (mg) (RNI 1.2-10)	1.2 ± 0.2	1.8 ± 0.3	1.9 ± 0.4	–	–	–
Vit B_6 (mg) (RNI 1.6-14)	1.3 ± 0.2	2.8 ± 0.3	2.9 ± 0.3	.05	–	.03
Vit B_{12} (μg) (RNI 1.4-14)	1.7 ± 0.1	3.2 ± 0.1	3.3 ± 0.2	.01	–	.01
Folate (μg) (RNI 200-1000)	138 ± 25	298 ± 38	318 ± 48	.001	–	.001

Statistics: analysis of variance followed by Tukey test. Data are expressed as mean ± SD. RNI indicates reference nutrient intake (Garrow JS, James WP, Ralf A. Human Nutrition and Dietetics. 10th ed. Churchill-Livingstom; 2000. p. 427-48).

placed on ice during collection, aliquoted after centrifugation, and immediately frozen (−70°C) until analysis.

4. Analytical procedures

Phenylalanine (Phe Inst) was measured simultaneously with the other parameters with a standardized enzymatic assay in dried blood samples on filter paper (2992, Schleicher and Schull, USA) with the MMR 2000 (R and D Diagnostics Co, Athens, Greece) [13]. Serum TC, TG, HDL-cholesterol (HDL-C), and LDL-C were measured using the ADVIA-1650 Chemistry System (Bayer Corporation, Tarrytown, NY), whereas apo A-I and apo B were determined by latex particle–enhanced immunonephelometric assays on the BN ProSpec nephelometer (Dade Behring, Liederbach, Germany) [4]. Interassay coefficients of variation (CVs) for TC, TG, HDL-C, apo A, apo A-I, and apo B ranged between 3.5% and 5.1%. Plasma total Hcy was measured by reverse-phase high-performance liquid chromatography with fluorometric detection according to Refsum et al [14]. Plasma NA, A, and DA levels were measured by reverse-phase high-performance liquid chromatography with electrochemical detection. The interassay CVs for NA, A, and DA were 3.2%, 2.9%, and 3.4%, respectively [15].

Tumor necrosis factor α concentrations were measured in plasma using an Amersham Pharmacia (Aylesbury, UK) RIA kit with interassay and intra-assay variations of 4.5% and 4.8%, respectively. Adiponectin concentrations were measured in plasma using Chemicon (CHEMICON International, Inc, Temecula, Calif) Sandwich ELISA Kit [8]. Interassay and intra-assay CVs were 9.8% and 8.3%, respectively.

Table 3
Biochemical findings in PKU patients (group A, group B) and controls (group C)

	Group A (n = 18)	Group B (n = 18)	Controls (n = 20)	Differences (P)		
				A vs B	A vs C	B vs C
Phe Inst (μmol/L)	195 ± 105	719 ± 155	90 ± 90	.0001	.001	.0001
Tyr (μmol/L)	78 ± 3.2	26 ± 1.5	80 ± 2.5	.001	NS	.001
TC (mmol/L)	3.2 ± 0.2	4.2 ± 0.3	3.9 ± 0.3	.001	.002	.04
TG (mmol/L)	0.97 ± 0.4	1.2 ± 0.5	1.03 ± 0.3	NS	NS	NS
HDL (mmol/L)	1.14 ± 0.2	1.15 ± 0.2	1.07 ± 0.2	NS	NS	NS
LDL (mmol/L)	1.6 ± 0.2	2.4 ± 0.2	2.3 ± 0.3	.001	.001	NS
Apo A-I (g/L)	1.53 ± 0.2	1.54 ± 0.2	1.45 ± 0.2	NS	NS	NS
Apo A-II (g/L)	0.35 ± 0.1	0.38 ± 0.1	0.33 ± 0.1	NS	NS	NS
Apo B (g/L)	0.46 ± 0.1	0.84 ± 0.2	0.76 ± 0.1	.001	.001	NS
LDL/apo B	1.42 ± 0.2	1.15 ± 0.1	1.14 ± 0.1	.001	.002	NS
Hcy (μmol/L)	15.7 ± 3.9	9.11 ± 2.2	9.0 ± 1.7	.002	.002	NS
DA (pmol/L)	180 ± 9.6	39 ± 3.0	196 ± 7.6	.001	NS	.001
A (pmol/L)	705.0 ± 115	144 ± 30.0	637 ± 80.0	.001	.05	.001
NA (nmol/L)	2.2 ± 0.5	1.1 ± 0.02	2.5 ± 0.06	.001	NS	.001
TNF-α (pg/mL)	12.7 ± 2.8	17.5 ± 3.2	16.9 ± 2.6	.003	.004	NS
Adpn (μg/mL)	11.2 ± 1.5	16.9 ± 2.1	8.6 ± 3.4	.001	.001	.008

Data are expressed as mean ± SD.

5. Statistical analyses

Data were expressed as mean ± SD and were evaluated by analysis of variance followed by the Tukey post hoc test. Correlation coefficients between several parameters were computed using the Spearman rank test because the data were not normally distributed. All statistical procedures were performed using the Statgraphics plus version 5.1 for Windows (Graphic Software System, Statistical Graphic Corp, USA), whereas box plots were prepared using the Sigma Plot software version 8.0 program (Sigma Aldrich Chemical Co, USA).

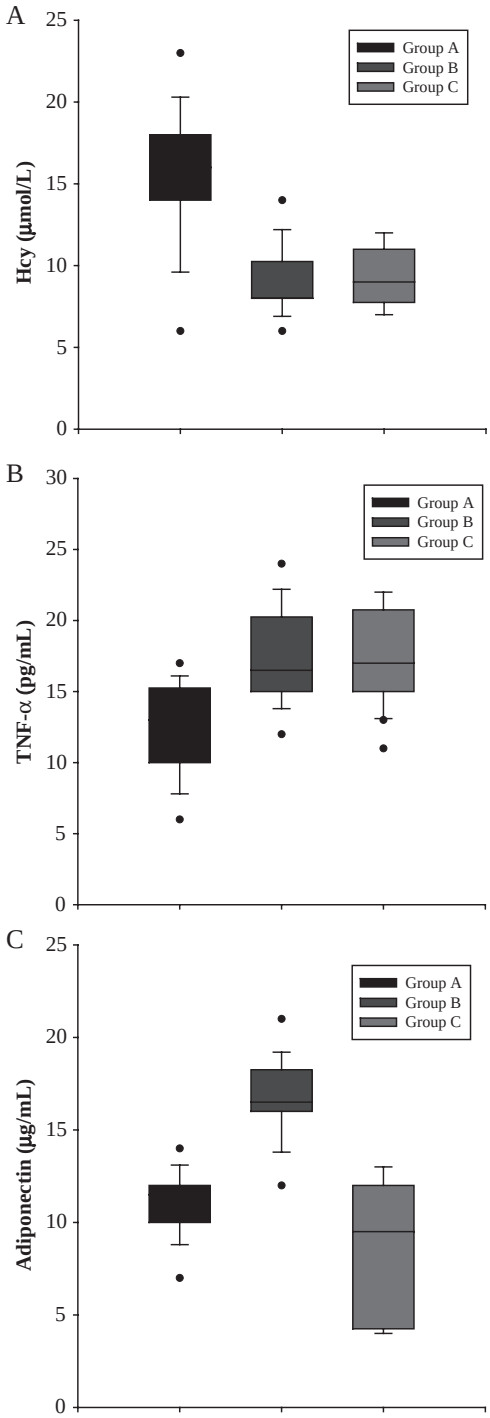

Fig. 1. Box-plot presentation of Hcy (A), TNF-α (B), and Adpn (C) levels in PKU patients and controls. Data are shown as median and outliers.

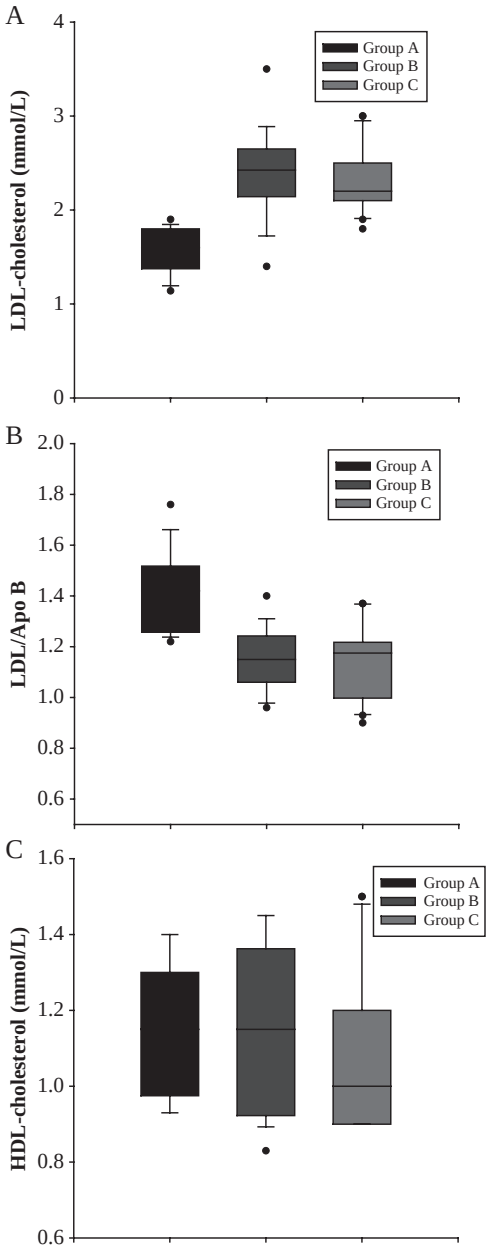

Fig. 2. Box-plot presentation of LDL-C (A), LDL-C/apo B (B), and HDL-C (C) levels in PKU patients and controls. Data are shown as median and outliers.

6. Results

Age, height, weight, and BMI did not differ among the 3 groups studied (Table 1). Similarly, energy and total protein intake did not differ among the groups; however, as expected, fiber and saturated fat intake differed greatly (Table 2). In addition, differences were noted in the intake of

total fat, monounsaturated and polyunsaturated fat, and cholesterol between group A and controls, as well as between the 2 groups of patients. The intake of vitamin B_6, vitamin B_{12}, and folate was significantly decreased in the group of patients on the strict diet after a month of discontinuation of vitamin supplements.

As expected, Phe Inst was significantly higher in the blood of group B patients, whereas tyrosine (Tyr) levels were significantly lower in the same group of patients than those of group A and controls (Table 3). Triacylglycerol, HDL, apo A-I, and apo A-II did not differ among the 3 groups of study. However, LDL, apo B, and TNF-α levels were significantly lower in group A than those of group B and controls, whereas TC concentrations were significantly lower in group A than in group B patients (Figs. 1 and 2). On the contrary, the LDL/apo B ratio, which represents the size of the LDL particles, and Hcy concentrations were increased in group A. Adiponectin concentrations were almost 2-fold higher in the patients on a loose diet than those of controls and 50% higher than those on a strict diet.

Plasma catecholamine concentrations were slightly but not significantly reduced in group A, whereas A, NA, and DA concentrations were significantly lower in poorly controlled patients (group B) than those of the other 2 groups.

Inverse correlation coefficients were observed between TNF-α and HDL and apo A in group A only, and very strong negative coefficients were observed between TNF-α and LDL/apo A in all groups studied (Table 4). Positive correlations were found between TNF-α and Hcy. The latter correlated negatively with LDL/apo B in all 3 groups and with HDL in group A only. In addition, positive correlation coefficients were found between Hcy and apo B in all 3 groups of the study, whereas Adpn correlated inversely with both TNF-α and Hcy. Furthermore, weak positive correlation coefficients were observed between Adpn and HDL or apo A in group A, whereas very strong positive correlation coefficients were found between Adpn and the size of LDL particles. Adiponectin correlated inversely with BMI in controls only.

Table 4
Significant correlation coefficients between circulating concentrations of lipoproteins and apolipoproteins or BMI with plasma levels of TNF-α, homocysteine, and Adpn

	Groups	TNF-α	Hcy	Adpn
HDL	A	−0.59*	−0.53*	0.56*
	B	–	–	0.46*
	C	–	–	
Apo A	A	−0.49*	–	0.48*
	B	–	–	
	C	−0.80***	–	
Apo B	A	0.50*	−0.48*	−0.50*
	B	–	−0.95***	–
	C	–	–	–
LDL/apo B	A	−0.92***	−0.92***	0.67**
	B	−0.94***	−0.94***	0.70**
	C	−0.95***	−0.81**	0.88***
TNF-α	A	–	0.95***	−0.60**
	B	–	0.94***	−0.67**
	C	–	0.80***	−0.94***
Hcy	A	0.95***	–	−0.67**
	B	0.94***	–	−0.60**
	C	0.83***	–	−0.80***
BMI	A	–	–	–
	B	–	–	–
	C	–	–	−0.68**

* $P < .05$.
** $P < .01$.
*** $P < .0001$.

7. Discussion

Proinflammatory cytokines such as TNF-α and interleukin 6 synthesized by fat cells and elsewhere play pathogenetic roles in atherogenesis. Adiponectin, on the other hand, appears to be protective in an experimental model of vascular injury [16], perhaps because it suppresses the attachment of monocytes/macrophages to endothelial cells, a fundamental early step in the atherosclerotic process. Elevated plasma Hcy concentrations are associated with an increased risk for premature occlusive vascular disease, including coronary artery disease [17-19]. Inadvertently, PKU patients on a strict diet (group A) have moderate hyperhomocysteinemia, which might produce coronary and peripheral artery disease through endothelial activation [17-19]. The reduced bioavailability of certain nutrients in the synthetic special diet of these patients, in combination with their relatively low intake of vitamins B_6, B_{12}, and folate, might have resulted in their undesired hyperhomocysteinemia [5].

In baboons, Hcy infused for 3 months caused patched endothelial aortic denudation of intimal proliferating smooth cells and increased platelet consumption [20]. Direct chemical injury to vascular human endothelial cells by increased concentrations of Hcy was also demonstrated in vitro [17]. In addition, Hcy oxidized LDL in the presence of redox metals possibly exerting further unfavorable effects on the endothelium via the oxidized molecules [20]. Lipids and especially LDL are significantly reduced in patients with good compliance to their diet, and the LDL/apo B ratio, which indirectly shows the size of LDL particles, is elevated in these patients, a favorable effect decreasing atherogenicity [4]. In our study, Hcy correlated negatively with Adpn and positively with TFN-α concentrations. Tumor necrosis factor α inversely correlated with Adpn in all 3 groups of children studied, as earlier reported in adult patients [21,22]. In addition, Adpn correlated positively with HDL, apo A, and the size of LDL particles (LDL/apo B), all favorable parameters.

Interestingly, patients on a loose diet had very low concentrations of the catecholamines NA, A, and DA and very high Adpn concentrations in their plasma. We may suggest but we cannot yet prove that the diminished concentration of these biogenic amines might have disinhibited Adpn production and/or secretion, as we previously demonstrated with leptin [6], and might have, hence, increased the plasma Adpn concentrations through such a mechanism [11,23]. Increased Phe concentrations, as we

found in the poorly controlled patients (group B), decrease the availability of the catecholamine precursor Tyr for catecholamine biosynthesis and might be the primary cause of the catecholamine depletion in the central nervous system and periphery of these patients [6,24,25]. In group B patients, the large excess of Phe may saturate the blood brain barrier and, thus, prevent other amino acids such as Tyr from entering the brain and be available for the biosynthesis of catecholamines and other neurotransmitters, leading to major brain dysfunction and decreased peripheral activity of their sympathetic nervous system [6,24,25].

We suggest that the observed increased Adpn levels in PKU patients on a loose diet (group B) might be due to decreased inhibition by their low DA, NA, or A plasma and/or nerve terminal levels, directly acting on adipocyte Adpn production and/or release in the their blood stream [11,23,26].Alternatively but not mutually exclusively, the high Phe levels of these patients might have increased Adpn production by fat because of active uptake of this aromatic amino acid [27].

In conclusion, the normal or increased Adpn concentrations, respectively, in well-controlled or poorly controlled PKU patients might moderate the inflammatory response of endothelial cells induced by the hyperhomocysteinemia observed in PKU patients who are well controlled on a strict diet and the high concentrations of TNF-α, TC, and LDL-C observed in poorly controlled patients.

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