# Clinical & Medical Biochemistry

**Research Article** **Open Access**

# Low Serum Cholesterol Concentration in Adult Patients with Phenylketonuria- One Centre Experience

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## Abstract

**Introduction:** Phenyloketonuria is a rare metabolic disorder caused by a deficiency of the phenylalanine (Phe) hydroxylase enzyme. A reduced intake of natural proteins helps optimise plasma Phe concentration. A relationship between high plasma Phe level and the inhibition of cholesterol synthesis was previously observed but the mechanisms are unclear. Low LDL-cholesterol concentrations were observed in children and adolescent PKU patients, but not in adults. This is the first paper to present lipid profile in adult patients with this condition.

**Methods:** Lipid profile was analysed in adult patients with PKU. We examined associations between Phe and four outcomes: total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. Confounding factors (predictors) were taken into account: body mass index (BMI), age and gender. The statistical analysis was performed using multiple linear regression.

**Results:** Among 176 adult patients 91 were females (52%). The mean age was 32 ± 10.7 years. 82 patients (46%) were on strict PKU diet. Mean Phe was 1017 ± 440 µmol/L. Mean total cholesterol was 4.33 ± 0.94 mmol/L, LDL-cholesterol 2.48 ± 0.8 mmol/L, HDL-cholesterol 1.2 ± 0.34 mmol/L, triglycerides 1.6 ± 0.9 mmol/L. There was no correlation between Phe concentration and lipid profile in our cohort of adult patients with PKU. No cardiovascular events were documented in our cohort.

**Conclusions:** In conclusion, the outcomes of the study demonstrate that mean total cholesterol, LDL-cholesterol and triglycerides concentrations were substantially lower as compared to healthy population, which may confer their reduced cardiovascular risk. Lipid profile remained low irrespectively of Phe concentration.

**Keywords:** PKU; Phenyloketonuria; Cardiovascular risk; Cholesterol; Lipids; BMI

**Abbreviations:** PKU: Phenyloketonuria; Phe: Phenylalanine; BMI: Body Mass Index; LDL-cholesterol: Low density lipoprotein cholesterol; HDL-Cholesterol: High density lipoprotein cholesterol.

## Introduction

Phenyloketonuria (OMIM 261600) is a rare autosomal recessive metabolic disorder caused by a deficiency of the phenylalanine (Phe) hydroxylase enzyme (EC 1.14.16.1). Treatment consists of a reduced intake of natural proteins [1]. Animal protein is replaced by amino-acid formulas that consist of Phe-free amino acid mixtures supplemented with tyrosine, vitamins and other micronutrients [1]. As a result of reduced animal lipids consumption, plasma cholesterol concentration is low [2,3]. A relationship between high plasma Phe level and the inhibition of cholesterol synthesis has been previously described [4-7]. It has been hypothesized that two main regulatory enzymes in the cholesterol synthesis, i.e., 3-hydroxy-3-methylglutaryl-CoA-reductase and mevalonate-5-pyrophosphate decarboxylase in brain and liver, are suppressed by high plasma Phe [4,6].

An inverse association between raised Phe and plasma cholesterol has been reported previously in children with PKU [2,3,8-12]. In adult patients, however, low-normal cholesterol concentration with no correlation between Phe and plasma cholesterol levels has been observed [13]. The controversy exists as to whether the hypocholesterolaemia is due to a disruption to cholesterol biosynthesis or the low protein diet. Most studies attribute low cholesterol levels in treated PKU patients to their vegetarian diet [8,10]. However, serum cholesterol concentrations depend on other regulatory factors such as the cholesterol synthesis, comorbidities such as diabetes or familial hyperlipidaemia, obesity, alcohol consumption. Obesity, which leads to metabolic syndrome,

has been previously shown to be a common finding among children and adolescents with PKU [14,15] irrespective of their compliance with low-protein diet [15]. Adolescent patients with PKU have been shown to have significantly low LDL-cholesterol compared to healthy controls [15,16]. HDL-cholesterol concentration that confers a low cardioprotective risk has not been different from healthy controls [15] or has been low in young patients compliant to low-protein diet compared with healthy controls and non-compliant patients [16]. In addition, raised homocysteine concentration and platelets counts were high in adolescent PKU patients that might increase the risk of atherosclerosis in this population [16]. However, Htun et al. have demonstrated that, despite low HDL-cholesterol concentrations and high triglycerides concentrations, adolescents with well-controlled PKU have normal carotid intima thickness and B-stiffness index [17]. In addition, platelet activation has not been enhanced meaning early atherosclerotic changes have not been induced [17]. In the present retrospective case notes review, we have explored the relationship between plasma Phe, diet, body mass index (BMI), age, gender and cholesterol in adult patients with PKU attending our Metabolic Clinic.

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## Methods

### Study design

It was a retrospective observational study and a review of our clinical practice. All patients have their blood tests (lipid profile) requested as part of their routine care when attend our Metabolic Clinics appointments every 6 months. We follow our local protocol that was implemented as our clinical guidelines after input from the paediatric metabolic team who previously cared for the majority of these patients.

### Patients

Demographic data for 176 adult PKU patients were extracted from the Electronic Patient Record and included in the analysis. Data included: BMI (kg/m²), age, gender and adherence to PKU diet. Pregnant PKU patients were excluded from the study.

### Biochemical tests

Lipid profile included total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were analysed using enzymatic method on Siemens Advia 2400 automated analyser in Clinical Biochemistry Department. Plasma Phe samples was collected on dry blood spot cards and was detected by multiple reaction monitoring mode, on a Waters Acquity-TQD tandem mass spectrometer in the Willink Biochemical Genetics Laboratory.

### Statistical analysis

The statistical analysis was performed using multiple linear regression (Stats Direct 3 Statistical Software). We fitted four models, with each of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides as outcome variables. Phe was centred by subtracting the mean value, and was entered as a continuous predictor variable. We included interactions with Phe x gender, to allow for the possibility that the relationship between Phe might vary according to this variable. We additionally controlled for age and BMI as potential confounding variables. The variables to be included were selected on a priori grounds to answer the research question of the present study. We did not perform any automated variable selection, as this is known to be a poor procedure that produces spurious (due to multiple testing) and non-generalizable (due to overfitting) results [18]. Results were presented as regression coefficients and 95% CIs, which give ranges of values for the effects that are consistent with the study data.

## Results

### Baseline characteristics

**Patients:** Among 176 patients 91 were females (52%). All patients were adult (>16 years) and the mean age was 32 ± 10.7 years (range 17-64 years). 82 patients (46%) were on strict PKU diet; no meat and no fish (Table 1). Patients often on vegetarian diet and had 2-3 amino acid supplements per day. None of our patients had documented cardiovascular disease such as myocardial infarction, stroke or angina.

**Biochemical tests:** Lipid profile and Phe results were expressed as mean (± SD) and are summarized in (Table 1). Mean total cholesterol was comparable with the target (<5 mmol/L) determined for non-metabolic patients [13]. Mean LDL-cholesterol was within the target (<3 mmol/L) for healthy non-metabolic population.

**Estimated associations:** There was no evidence of an association between plasma Phe and four fractions of lipids profile after confounding factors such as BMI, age and gender were taken into account. The Phe x gender interaction (1=male, 0=female) did not show any relationship between these predictors and lipid profile. Phe x SD refers to the change in the outcome for a 1SD increase in Phe and showed no significant correlation with four lipid fractions. We found there was a mild positive correlation between BMI and total cholesterol, LDL-cholesterol and triglycerides. There was a negative correlation between BMI and HDL-cholesterol, the finding also observed in a non-PKU population (Table 2).

## Discussion

Our findings support the concept that there was no association between plasma Phe concentration and lipid profile in a large cohort of adult patients with PKU. In this paper we discuss the effect these individual factors had on cholesterol concentrations.

This study was the first to examine the effect of Phe on lipid profile in such a large cohort of adult patients with PKU. Consideration of several confounding factors in the analysis was one of key advantages of the methodology (Table 2). Apart from examining the effect of gender on Phe concentrations, we also assessed the effect of the interaction Phe x gender on four lipid fractions. The aim was to allow for the possibility that the relationship between Phe might vary according to these variables.

In view of the broad spread of Phe ranges (130-1906 µmol/L), Phe results were presented as 1SD to express the change in the outcome for a 1SD increase in Phe.

### Cardiovascular risk

We demonstrated that irrespective of plasma Phe concentrations, serum total cholesterol and LDL-cholesterol concentrations were decreased in our patients with PKU. These findings were comparable with recent studies on adult patients with PKU [19,20]. Artuch et al. [5] has previously shown that the levels of their lipids, lipoproteins and apolipoproteins indicated a less atherogenic profile. LDL-cholesterol levels were previously shown to be significantly lower in patients with PKU compared to healthy population [5,15,16]. Additionally, LDL-cholesterol/ApoB ratio was higher in PKU patients who had good dietary compliance when compared to those PKU patients who had a relaxed diet. High LDL-cholesterol/ApoB ratio usually correlates with the presence of larger and less atherogenic LDL particles, which are less susceptible to oxidative damage than small LDL particles [21].

In our study the mean total cholesterol was significantly lower in adult PKU patients when compared to cholesterol targets outlined for normal population. The mean serum LDL-cholesterol was 2.5 mmol/L and was well within the target of 3 mmol/L for healthy non-PKU

| Baseline characteristics (n=176) | Mean ± SD |
|---|---|
| Total cholesterol | 4.33 ± 0.94 |
| LDL-cholesterol | 2.48 ± 0.8 |
| LDL-cholesterol in 'PKU diet group' | 2.37 ± 0.75 |
| LDL-cholesterol in 'off PKU diet group' | 2.6 ± 2.8 |
| HDL-cholesterol | 1.2 ± 0.34 |
| Triglycerides | 1.6 ± 0.96 |
| Gender (F/M) | 91 F/ 85 M |
| Age | 32.5 ± 10.75 |
| BMI (kg/m²) | 28.6 ± 7.1 |
| Phe (± SD) | 1017 ± 440.7 |
| PKU diet | 82 (46%) |
| Off PKU diet | 94 (54%) |

**Table 1:** Baseline characteristics.

population [13]. Further studies are required to determine whether this effect confers the reduced cardiovascular risk. Nevertheless no evidence of ischemic disease or past medical history of cardiovascular events was observed in our patients and hypertension was not a problem either.

### Diet

The outcomes of our study fully support the findings by Williams et al. [22], who demonstrated that compliance with dietary restriction did not appear to impact on plasma cholesterol concentrations [22]. We showed that our PKU patients, who had good adherence to their diet, were not at high risk of developing atherosclerosis because their mean LDL-cholesterol was low at 2.38 mmol/L. Although the difference was not statistically significant (p=0.15), the value was lower from the mean LDL-cholesterol (2.6 mmol/L) found in patients who were not following PKU diet. These results contradict the previously described findings by Artuch et al. [5] and suggested that protein-restricted diet and Phe restriction, associated with a generally healthy lifestyle, may reduce serum LDL-cholesterol [5].

Importantly, the mean triglycerides concentration was 1.6 mmol/L (± 0.9). Higher triglyceride values in young patients with PKU were reported previously and were believed to be secondary to the significantly high calories consumption from carbohydrates [23]. It should be emphasized that serum cholesterol concentrations depend on a vegetarian diet consisting of olive oil and cream cheese. Notably, the increased total percentage of calories from fat in the form of monounsaturated fatty acids in the diet, and as a result lowering the polysaturated fatty acids and carbohydrates, may have a more beneficial effect on all fractions of lipid profile including serum HDL-cholesterol and triglycerides [24]. Lifelong protein restriction may lead to chronic deficit of omega-3 and omega-6 fatty acids with the risk of early atherosclerosis. Htun et al. [17], however, have shown that despite low HDL-cholesterol concentration and normal carotid intimal thickness adolescent PKU patients who avoid fish in their diet have low risk atherosclerotic risk [17].

### Age and gender

It was previously demonstrated that with age, patients find it difficult to comply with low-protein diet that may impact on their serum lipid profile [10] but the correlation between serum cholesterol and age, plasma Phe and has never been proven [8]. Our study did not show any considerable association between age and four lipid profiles (Table 2).

Although we did not observe a correlation of any statistical significance between gender and four fractions of the lipid profile, we noted that total cholesterol, LDL-cholesterol and HDL-cholesterol may be increased in male patients by 0.03, 0.023 and 0.007 mmol/L, respectively, levels which are not clinically significant. The Phe x gender interaction did not show any association between gender and total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides (Table 2).

### BMI

Artuch et al. [5] showed that a high percentage of patients with low Phe tolerance and good dietary compliance had raised BMI, the finding not perceived in our study [5]. Couce et al. [25] noticed that biochemical parameters such as total cholesterol, triglycerides, LDL-cholesterol were higher in overweight or obese patients with PKU [2]. We documented a positive correlation between BMI and total cholesterol, LDL-cholesterol and triglycerides and a negative correlation between BMI and HDL-cholesterol. Among our patients with PKU, the range of BMI ranged from 18.9 to 46.9 kg/m². A variety of other lifestyle modifying (lack of exercise and alcohol consumption) and genetic factors could be a potential explanation of our observations.

One of limitations of this study is the lack of evidence of possible genetic influence in cholesterol metabolism or underlying liver disease. They should be considered as important variables in interpreting lipids data from PKU patients [24]. Additionally, as we did not have any control group, we compared results to targets for total cholesterol and LDL-cholesterol for healthy population as outlined in guidelines.

In conclusion, the outcomes of the study demonstrate that adult PKU patients have low serum cholesterol concentration and low incidence of cardiovascular events irrespective of diet or their BMI. Lipid profile in adult PKU patients should be requested only if there is

| | Total Cholesterol Coefficient (95% CI) | HDL Cholesterol Coefficient (95% CI) | LDL Cholesterol Coefficient (95% CI) | Triglycerides Coefficient (95% CI) |
|---|---|---|---|---|
| **Intercept** | 2.517 (1.771 to 3.263) | 1.311 (1.026 to 1.595) | 0.905 (0.189 to 1.622) | 0.68 (-0.212 to 1.573) |
| **BMI** | 0.028 (0.009 to 0.047) | -0.007 (-0.014 to 0) | 0.017 (-0.001 to 0.035) | 0.032 (0.011 to 0.054) |
| **Phe** | 0 (0 to 0) | 0 (0 to 0) | 0 (0 to 0.001) | 0 (-0.001 to 0) |
| **Phe × SD\*** | 0.003 (-0.179 to 0.185) | -0.019 (-0.088 to 0.051) | 0.139 (-0.033 to 0.312) | -0.129 (-0.346 to 0.088) |
| **Gender** | 0.03 (0.018 to 0.042) | 0.007 (0.003 to 0.012) | 0.023 (0.012 to 0.035) | 0.002 (-0.012 to 0.017) |
| **Age** | 0.224 (-0.503 to 0.951) | -0.113 (-0.39 to 0.165) | 0.453 (-0.225 to 1.131) | 0.228 (-0.613 to 1.068) |
| **Gender × Phe** | 0 (-0.001 to 0.001) | 0 (0 to 0) | 0 (-0.001 to 0) | 0 (-0.001 to 0.001) |

*Phenylalanine results refer to the change in the outcome for a 1 SD increase in Phenylalanine

**Table 2:** The relationship between predictors and four outcomes (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides). Results were expressed as coefficient (± 95% Confidence Interval). The analysis was performed using multiple linear regression.

a strong suspicion of cardiovascular disease or a strong family history of lipid disorder. No observed correlation between Phe concentration, adherence to low-protein diet, BMI, age, gender and lipid profile in our cohort of adult patients with PKU, indicates that there are other factors affecting lipid profile in male and female patients with PKU. Further research is needed to investigate mechanisms of hypocholesterolaemia in this cohort of patients.

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