Pathology (February 2015) **47(2)**, pp. 134–137

CHEMICAL PATHOLOGY

Plasma cholesterol in adults with phenylketonuria

ROBIN A. WILLIAMS[1], AMANDA J. HOOPER[1,2,3], DAMON A. BELL[1,2,4], CYRIL D. S. MAMOTTE[5] AND JOHN R. BURNETT[1,2]

[1]Department of Clinical Biochemistry, PathWest Laboratory Medicine, Royal Perth Hospital, Perth, [2]School of Medicine and Pharmacology, University of Western Australia, Perth, [3]School of Pathology and Laboratory Medicine, University of Western Australia, Perth, [4]Department of Internal Medicine, Royal Perth Hospital, Perth, and [5]School of Biomedical Science and CHIRI Biosciences Research Precinct, Curtin University, Bentley, WA, Australia

Summary

Phenylketonuria (PKU) is an autosomal recessive disorder of phenylalanine (Phe) catabolism resulting from a deficiency of L-phenylalanine hydroxylase (PAH). An association between hyperphenylalaninaemia (HPA) and hypocholesterolaemia has been reported in children. However, controversy exists as to whether this is due to the low protein diet or to a disruption to cholesterol biosynthesis inherent to those with PKU. We investigated the relationship between blood Phe and plasma cholesterol in 41 apparently healthy adults with PKU (26 female, 15 male, age 18–57 years, median age 26 years) attending a PKU outpatient clinic at an adult tertiary care hospital. Of these patients, 33 (80%) were compliant with a Phe-restricted diet with amino acid supplementation, whereas eight (20%) were not. The PKU subjects had a mean body mass index (BMI) of 30.3 ± 1.8 kg/m^2; 72% were obese, 14% overweight, with only 14% having normal BMI. The mean blood Phe was 1194 ± 522 μmol/L with plasma total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and apolipoprotein (apo) B concentrations of 4.3 ± 0.8 mmol/L, 1.6 ± 0.8 mmol/L, 1.2 ± 0.3 mmol/L, 2.3 ± 0.8 mmol/L, and 0.83 ± 0.21 g/L, respectively. The mean LDL-cholesterol was 19% lower in PKU females than that of 8944 age-matched females from a community population (2.5 ± 0.8 mmol/L vs 3.1 ± 0.9 mmol/L, $p < 0.001$). Similarly, the mean LDL-cholesterol was 32% lower in PKU males than 3786 age-matched males (2.1 ± 0.7 mmol/L vs 3.1 ± 1.0 mmol/L, $p < 0.0001$). No correlations were observed between Phe and total cholesterol, LDL-cholesterol or apoB in the PKU cohort. Adults with PKU had low-normal cholesterol concentrations, with no correlation observed between Phe and cholesterol levels. Our findings support the concept that the HPA found in PKU, rather than an effect of a low-protein diet, leads to hypocholesterolaemia. Studies are required to determine whether this cholesterol-lowering effect confers cardioprotection.

Key words: Cholesterol, LDL, phenylalanine, phenylketonuria, PKU.

Received 15 August, revised 18 September, accepted 23 September 2014

INTRODUCTION

Phenylketonuria (PKU; OMIM 261600), is an autosomal recessive inborn error of phenylalanine (Phe) catabolism caused by a deficiency of L-phenylalanine hydroxylase (PAH; EC 1.14.16.1), which results in increased L-Phe levels in the blood.[1,2]

In humans, mutations in the *PAH* gene on chromosome 12q23.2 cause PKU. PKU is typically treated using a Phe-restricted, low protein diet with amino acid supplementation to prevent the development of metabolic and pathological sequelae of hyperphenylalaninaemia (HPA), including intellectual impairment.[3]

An inverse association between HPA and cholesterol has been reported in children with PKU.[4–10] However, controversy exists as to whether the hypocholesterolaemia is due to the low protein diet or to a disruption to cholesterol biosynthesis inherent to those with PKU. It is plausible that the hypocholesterolaemia found in PKU might confer a cardioprotective effect.

In the present study, we have investigated the relationship between plasma Phe and cholesterol in adults attending a PKU outpatient clinic.

MATERIALS AND METHODS

Participants

The subjects consisted of 41 apparently healthy PKU patients (26 female, 15 male, age 18–57 years, median age 26 years, mean age 31 years) attending a PKU outpatient clinic at an adult tertiary care hospital. Of these patients, 33 (80%) were compliant with Phe-restricted diet with amino acid supplementation, as assessed by plasma Phe measurements and, in a subset ($n = 8$), a 3-day standard food record, whereas eight (20%) were not.

Biochemical analyses

Plasma total cholesterol, triglyceride, and HDL-cholesterol were measured on venous blood samples, after an overnight fast, using Roche reagents on a Hitachi 917 analyser. LDL-cholesterol concentrations were calculated by difference using the Friedewald equation.[11] Apolipoprotein (apo) B was measured by immunonephelometry using Behring reagents on a Behring BN-II. Phe was measured by liquid chromatography tandem mass spectrometry.

Mean gender-specific plasma lipid concentrations were compared with local normative data from the literature.[12,13] LDL-cholesterol concentrations were compared with an age- (years) and gender-matched subset drawn from a recent community laboratory population from Perth, Western Australia.[12]

Genetic studies

Forward and reverse primers were designed for the PCR amplification of all 13 exons of the *PAH* gene including intron-exon boundaries. Genomic DNA isolated from peripheral blood leucocytes was PCR-amplified and then sequenced using Big Dye Terminator v3.1 chemistry (Applied Biosystems, USA). The resulting chromatograms were visually checked and aligned to the

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: 10.1097/PAT.0000000000000210

PAH reference sequence (NC_000012.10) using CLC Gene Workbench (CLC Bio, Denmark). In two patients in whom either or both *PAH* mutations could not be detected, multiplex ligation-dependent probe amplification (MLPA) was performed according to the manufacturer's instructions (P055 PAH, MRC-Holland, The Netherlands). *APOE* genotypes were determined as previously described.[14]

Ethics

The study was approved by the Royal Perth Hospital Human Research Ethics Committee.

Statistical analyses

Values are expressed as the mean ± the standard deviation (SD). A *t*-test was used to compare differences with a $p < 0.05$ considered to be statistically significant. Pearson correlation and linear regression analysis was performed using Analyse-it.

RESULTS

The PKU subjects had a mean body mass index (BMI) of $30.3 \pm 1.8\,kg/m^2$; 72% were obese, 14% overweight, with only 14% having normal BMI. The mean blood Phe was $1194 \pm 522\,\mu mol/L$ (reference interval 35–85) with plasma total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and apoB concentrations of 4.3 ± 0.8 mmol/L, 1.6 ± 0.8 mmol/L, 1.2 ± 0.3 mmol/L, 2.3 ± 0.8 mmol/L, and 0.83 ± 0.21 g/L, respectively (Table 1).

The mean plasma lipid concentrations for males showed a total cholesterol of 4.1 ± 0.7 mmol/L, triglyceride 1.9 ± 0.9 mmol/L, HDL-cholesterol 1.1 ± 0.2 mmol/L, and LDL-cholesterol 2.1 ± 0.7 mmol/L. The mean plasma lipid concentrations for females showed a total cholesterol of 4.4 ± 0.9 mmol/L, triglyceride 1.3 ± 0.7 mmol/L, HDL-cholesterol 1.3 ± 0.3 mmol/L, and LDL-cholesterol 2.5 ± 0.8 mmol/L. Males had significantly higher triglyceride and lower HDL-cholesterol levels than females ($p < 0.05$ for both).

The total cholesterol (males 5.6 ± 1.1 vs 4.1 ± 0.7 mmol/L, $p < 0.0001$; females 5.2 ± 1.2 vs 4.4 ± 0.9 mmol/L, $p < 0.01$) and HDL-cholesterol (males 1.4 ± 0.3 vs 1.1 ± 0.2 mmol/L, $p < 0.001$; females 1.6 ± 0.5 vs 1.3 ± 0.3 mmol/L, $p < 0.01$) were lower, whereas the triglyceride (males 1.2 ± 1.0 vs 1.9 ± 0.9 mmol/L, $p < 0.01$; females 0.8 ± 0.5 vs

Table 1 Phenylalanine, lipid and apolipoprotein B concentrations, and *PAH* mutation findings in 41 adults with PKU

#	Sex	Age (years)	Phe (μmol/L)	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-cholesterol (mmol/L)	LDL-cholesterol (mmol/L)	ApoB (g/L)	*PAH* mutation 1	*PAH* mutation 2
1	F	24	1285	4.0	0.5	1.4	2.4	0.70	c.194T>C (p.I65T)	c.1222C>T (p.R408W)
2	M	45	1735	3.6	0.8	0.9	2.3	0.84	c.194T>C (p.I65T)	c.896T>G (p.F299C)
3	F	24	1857	4.7	2.1	1.0	2.7	0.96	c.1066–11G>A	c.1222C>T (p.R408W)
4	M	25	1010	3.8	2.3	1.1	1.6	0.90	c.782G>A (p.R261Q)	c.1222C>T (p.R408W)
5	F	39	1035	4.1	1.5	1.2	2.2	0.84	NT	NT
6	F	57	1061	6.1	1.9	1.1	4.1	1.28	c.1222C>T (p.R408W)	c.1241A>G (p.Y414C)
7	F	41	1228	5.2	1.0	1.3	3.4	0.99	c.194T>C (p.I65T)	c.473G>A (p.R158Q)
8	F	20	792	5.5	2.3	1.2	3.2	1.17	c.464G>C (p.R155P)	c.1241A>G (p.Y414C)
9	F	25	1630	3.8	0.7	1.3	2.2	0.75	c.168+1G>A	**c.824+4A>G**
10	M	18	1170	4.1	1.8	1.0	2.3	0.72	c.653G>T (p.G218V)	c.829T>G (p.Y277D)
11	F	19	736	2.9	0.6	1.2	1.4	0.53	NT	NT
12	M	55	1672	4.7	2.9	1.3	2.1	0.86	c.473G>A (p.R158Q)	c.912+1G>A
13	F	51	297	4.2	1.1	1.1	2.6	1.01	c.473G>A (p.R158Q)	c.912+1G>A
14	F	49	1960	3.3	0.7	1.2	1.8	0.73	c.1068C>G (p.Y356*)	c.1222C>T (p.R408W)
15	M	18	701	4.9	3.5	1.0	2.3	0.95	c.1223G>A (p.R408Q)	?
16	F	44	1879	4.6	1.0	1.8	2.3	0.79	c.194T>C (p.I65T)	c.1315+1G>A
17	F	26	1150	5.8	1.1	1.6	3.7	1.01	c.117C>G (p.F39L)	c.1222C>T (p.R408W)
18	F	34	1221	4.0	1.5	1.2	2.1	0.98	c.814G>T (p.G272*)	c.814G>T (p.G272*)
19	F	25	1301	4.5	2.1	1.8	2.1	0.76	c.1222C>T (p.R408W)	c.1315+1G>A
20	M	21	1894	3.8	1.2	1.0	2.3	0.93	NT	NT
21	M	36	1654	3.9	2.3	1.3	1.5	0.62	NT	NT
22	M	46	1147	4.3	2.7	0.7	2.4	1.00	**c.168+1G>T**	c.331C>T (p.R111*)
23	F	31	717	4.7	3.1	1.1	2.2	1.04	NT	NT
24	F	33	650	5.5	1.5	1.2	3.6	1.26	c.727C>T (p.R243*)	c.1241A>G (p.Y414C)
25	M	46	991	3.4	3.3	1.1	0.8	0.69	NT	NT
26	F	28	1246	3.3	0.6	1.6	1.4	0.48	NT	NT
27	M	27	1949	3.6	0.6	1.4	1.9	0.69	c.1315+1G>A	c.1315+1G>A
28	F	35	266	4.4	1.6	1.4	2.3	0.74	c.442–5C>G	c.1066–11G>A
29	M	18	1670	2.8	1.2	1.1	1.1	0.59	c.165delT (p.F55Lfs*)	c.165delT (p.F55Lfs*)
30	F	18	1371	4.0	1.5	1.3	2.0	0.71	**c.492dupT (p.A165Cfs*)**	c.526C>T (p.R176*)
31	F	18	1828	5.1	0.6	1.4	3.4	0.97	c.526C>T (p.R176*)	c.1042C>G (p.L348V)
32	M	19	546	5.3	1.5	1.0	3.6	1.11	c.836C>T (p.P279L)	c.836C>T (p.P279L)
33	F	45	943	3.3	2.5	0.8	1.3	0.58	c.194T>C (p.I65T)	c.194T>C (p.I65T)
34	F	36	906	4.8	1.2	1.5	2.8	0.97	c.1222C>T (p.R408W)	c.1241A>G (p.Y414C)
35	M	22	1423	3.6	1.5	1.1	1.8	0.57	c.1315+1G>A	c.344_347del (p.K115Tfs*)
36	F	20	1506	3.9	1.6	1.1	2.1	0.65	c.194T>C (p.I65T)	c.1222C>T (p.R408W)
37	M	45	2242	5.2	1.4	1.4	3.2	0.98	?	?
38	F	19	418	4.6	0.6	1.7	2.6	0.76	c.728G>A (p.R243Q)	c.1169A>G (p.E390G)
39	F	20	185	3.0	0.4	1.5	1.3	0.35	c.638T>C (p.L213P)	c.1066–3C>T
40	M	19	1058	4.2	2.0	1.0	2.3	0.66	c.1222C>T (p.R408W)	c.1315+1G>A
41	F	18	608	5.6	1.6	1.8	3.1	0.83	c.117C>G (p.F39L)	c.117C>G (p.F39L)
Mean ± SD	31 ± 12	1194 ± 522	4.3 ± 0.8	1.6 ± 0.8	1.2 ± 0.3	2.3 ± 0.8	0.83 ± 0.21			

Novel mutations shown in bold.
?, mutation not detected; NT, not tested.