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Arterial stiffness assessment in patients with phenylketonuria

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Abstract

In patients with phenylketonuria (PKU) compliant to diet greater tendency to overweight and higher inflammatory biomarkers levels than controls were reported. Although this could lead to atherogenesis, the elastic properties of large arteries in PKU patients have never been assessed. The aim of this study was to assess arterial stiffness measured by applanation tonometry in PKU patients compared to healthy controls.

We carried out a cross-sectional study in 41 PKU patients (range age: 6–50 years old) and 41 age- and gender-matched healthy controls. Evaluated data included pharmacological treatment with sapropterin, clinical, and biochemical parameters. Aortic stiffness was assessed noninvasively by applanation tonometry measuring central blood pressure, aortic augmentation index (Aix@HR75), augmentation pressure (AP), and pulse wave velocity (PWV).

We found higher PWV in classic PKU patients (6.60 m/second vs 5.26 m/second; *P:* .044). Percentage of PKU patients with PWV above 90 percentile was higher than controls (14.63% vs 2.32%; *P*: .048). A positive relationship was observed between the annual Phe median and PWV (r: 0.496; *P*: .012). PKU subjects with lower Phe tolerance showed more body weight (67.6 kg vs 56.8 kg; *P*: .012) and more PWV than those with higher Phe tolerance (6.55 m/second vs 5.42 m/second; *P*: .044).

Our data show increased aortic stiffness in PKU patients, measured by applanation tonometry, when compared to healthy controls. Higher Phe levels are associated with a bigger PWV increase, which is not present in those subjects compliant to diet or under sapropterin treatment. These results could have marked effects in both research and clinical daily practice for a proper evaluation of cardiovascular risk in PKU subjects.

Abbreviations: AC = mid-upper arm circumference, Aix@HR75 = augmentation index, AP = augmentation pressure, BH4 = tetrahydrobiopterin, BMI = body mass index, BP = blood pressure, cDBP = central diastolic BP, CPKU = classic PKU, cPP = central pulse pressure, cSBP = central systolic BP, DBP = diastolic BP, ESC = European Society of Cardiology, ESH = European Society of Hypertension, HDL-C = high-density lipoprotein cholesterol, HPA = hyperphenylalaninemia, LDL-C = low density lipoprotein-cholesterol, MPKU = mild–moderate PKU, Phe = phenylalanine, PKU = phenylketonuria, PWV = pulse wave velocity, SBP = systolic BP, TC = total cholesterol, WC = waist circumference.

Keywords: arterial stiffness, cardiovascular risk, phenylketonuria, pulse wave analysis, pulse wave velocity, sapropterin

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1. Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive disorder of phenylalanine (Phe) metabolism, caused in most cases (98%) by deficient activity of the hepatic enzyme L-phenylalanine-4-hydroxylase (PAH; EC 1.14.16.1), due to mutations in the *PAH* gene (NM 000277.1). Loss of PAH activity results in increased blood Phe concentrations (hyperphenylalaninemia, HPA) and in other tissues including brain, causing irreversible neurological damage if left untreated. The prevalence in Caucasians is of 1 in 10,000 individuals.[1] It is usually detected by newborn screening, that is routinely performed in Spain since 1980,[2] enabling an early diagnosis and treatment. The recommended targets for plasma Phe concentrations at different ages are not standardized and vary between countries.[3,4]

There is a general agreement that PKU patients need long-term dietary counseling which is based on low Phe intake. This diet consists of a restriction of natural protein and supplementation of essential amino acids with a Phe-free amino acid mixture.[5] The long-term safety of this dietary treatment and its potential impact on later noncommunicable diseases risk are needed to be evaluated.[4]

A negative correlation has been observed between plasma cholesterol and phenylalanine concentrations, but cardiovascular risk may be increased in PKU patients due to higher prevalence of overweight/obesity and higher systolic blood pressure (BP).[6]

A decline in elastic properties of the aorta and other large capacitance arteries associates with cardiovascular morbidity and mortality. It has been supported by the European Society of Cardiology (ESC) guidelines, to use increased arterial stiffness when possible, as a further measure of cardiovascular risk.[7] As it has been recently reviewed, applanation tonometry can be used to measure pulse wave velocity (PWV) and pulse wave analysis (PWA) as systemic arterial stiffness markers and both are predictors of adverse cardiovascular events.[8]With this method is possible to derive the augmentation index (Aix@HR75), which expresses the percentage of central pulse pressure (cPP) caused by reflections. The Aix@HR75 correlates with other measures of arterial stiffness such as PWV[9] and also predicts cardiovascular outcome.[10] Normal PWV and Aix@HR75 values for the pediatric and adult population have been previously assessed.[11,12]

Both PWV and Aix@HR75 increase with increasing arterial stiffness and vascular damage. The SphygmoCor system has been the most commonly used and best-validated device for PWV and Aix@HR75 evaluation even in children and adolescents.[13,14]

The aim of the present study was to define the influence that HPA has on systemic arterial stiffness (PWA and PWV) measured by applanation tonometry.

2. Subjects and methods

2.1. Study population

This cross-sectional study was performed at Superior Center of Reference Unit (CSUR) Statewide to the Diagnosis and Treatment of Congenital Metabolic Diseases Unit of the University Clinical Hospital of Santiago-Galicia between October 2015 and July 2016. The study protocol was approved by the Research Ethics Committee of Santiago-Lugo (2015/393). The treatment of their clinical data for research purposes at the beginning of the study and the study protocol complies with the Helsinki Declaration of 1964, as revised in October 2013 in Fortaleza, Brazil. Both cases and controls (or their parents if children were under 16 years) were properly informed and signed an informed consent to enter the study. PKU patients and controls were matched by age and gender. Patients were followed in their center from the date of diagnosis up to now. We included both patients diagnosed by newborn screening as well as those later diagnosed. Exclusion criteria were: those who underwent sporadic monitoring, changes in amino acid mixture in the month before inclusion, previous cardiovascular disease (coronary heart disease or stroke or peripheral revascularization) and pregnancy.

The clinical variables collected for each patient were: age, sex, phenotype (classic PKU (CPKU) with $>1200\,\mu$mol/L of serum Phe concentration at diagnosis and mild–moderate PKU (MPKU) with 360–1200 μmol/L), Phe tolerance (low <500 mg/day, high >500 mg/day), annual median blood Phe levels (blood Phe levels considered adequate for children under 6 years of age were $<360\,\mu$mol/L; $<480\,\mu$mol/L between 6 and 10 years and $\leq 600\,\mu$mol/L in older patients), anthropometric measurements (weight, height, body mass index (BMI)), waist circumference (WC), and mid-upper arm circumference (AC), systolic and diastolic brachial BP. Data from PWA and carotid-femoral PWV were also collected.

Adherence to treatment in patients with PKU was established according to their metabolic control by annual median blood Phe levels and the preestablished "safe" thresholds for each age (as above mentioned).

Definition and staging of high BP in children and adolescents were performed as the US National High Blood Pressure Education Program report based on BP at presentation.[14] High BP was defined as systolic BP (SBP) and/or diastolic BP (DBP) >95th percentile for sex, age, and height; and prehypertension was SBP or DBP between the 90th to 95th percentile on repeated measurements. Diagnosis of essential hypertension in adults was established according to the definitions provided by the ESC/European Society of Hypertension (ESH) guidelines.[7] No patients in the reference cohort were receiving antihypertensive medications or statins at baseline.

2.2. Arterial tonometry

Pulse wave analysis was performed noninvasively with the SphygmoCor device (AtCor Medical, Sydney, Australia). Right radial artery applanation tonometry after resting supine for 5 minutes was used to derive the central pressure waveform obtained through a validated transfer function.[15] This allows the calculation of the central systolic and diastolic BP (cSBP and cDBP), cPP and the difference between the first and the second systolic peaks (augmentation pressure, AP). These data were expressed as mm Hg. Aix or the AP/cPP ratio was also calculated and normalized for a heart rate of 75 beats per minute expressed as a percentage (Aix@HR75). Carotid-radial PWV was also measured as an index of aortic stiffness and expressed as meters per second. The references values for Arterial Stiffness Collaboration were used to establish normal values and above 90 percentile.[16] Subjects less than 18 years old were classified according to references values previously reported by Reusz et al.[11]

The quality of the pulse waves captured was assessed by the clinician both visually (on the PC screen) and numerically using the SCOR-Px built-in quality index score, QI% (derived from pulse height, pulse height variation, and diastolic variation). Only high-quality recordings, defined as an in-device quality index of at least 80% (derived from an algorithm including average pulse

height, pulse height variation, diastolic variation, and the maximum rate of rise of the peripheral waveform), were included into the analysis.

BP was measured using an oscillometric device (Omron IT-750; Omron Healthcare, Tokyo, Japan) and a cuff of the appropriate size applied after 5 minutes of rest in the supine position. Subjects were asked not to practice physical activity and avoid caffeinated beverages before visits. Simultaneous supine measurements at both arms were taken, and shortly afterward 3 supine measurements at the arm with the higher BP. The latter 2 were averaged and used for data analysis.

2.3. Data collection

Standing height was measured with a wall-mounted stadiometer and body weight, with digital scales. A trained nurse measured anthropometric characteristics. The BMI was weight in kilograms divided by height in squared meters. Patients were weighted barefoot and after overnight fasting. The nutritional status was performed by calculating the formula BMI = weight (kg)/height^2 (m^2). Patients older than 18 years old (1995/1998) were classified as underweight (BMI <18.5), normal weight (BMI 18.5–24.99), overweight (BMI 25–29.99), and obese (BMI ≥30) according to the WHO criteria. Subjects less than 18 years old were classified according to BMI by using The WHO Child Growth Standards (underweight: BMI percentile <15; normal: BMI percentile 15–85, overweight: BMI percentile 85–95, obese: BMI percentile >95).[17]

WC was measured at a level midway between the lower rib margin and the iliac crest and AC was measured at a point half way between the elbow and the shoulder. The results of these measurements, expressed in cm, were stratified regarding to the sex and age according to the Galinut's references in children.[18] For adults, it was used specific values of International Diabetes Federation[19] for the waist.

Biological assessments were performed in order to detect triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C). All biochemical measurements were obtained from fasting morning plasma samples at the same hour (8 hours), and the patients were free of acute infection or medication (except patients who were treated with sapropterin therapy). Tandem mass-spectrometry was used for measuring the Phe levels from dried blood spots and plasma. TC, HDL-C, and triglycerides concentrations were determined by standard procedures with the Advia 2400 Analyser (Siemens Diagnostic Systems, Munich Germany), LDL-C is estimated from the Friedewald formula (total cholesterol – triglycerides/5-HDL-C) when the triglycerides is less than 350 mg/dL. If the triglyceride concentration is >350 mg/dL, LDL-C is estimated by a direct method based on cholesterol oxidase, esterase, and peroxidase, after removal of lipoprotein cholesterol other than LDL (Advia 2400, Siemens). B12 is determined with an Advia Centaur XP Analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany).

High concentrations of TC > 200 mg/dL, LDL-C > 130 mg/dL, and triglycerides > 100 mg/dL in patients between 0 and 9 years old and >130 mg/dL in patients older than 10 years old. Low concentrations of triglycerides if <30 mg/dL and of HDL-C <40 mg/dL.

2.4. Statistical test

In order to determine significant associations and/or differences between the variables, the following methods were applied: if one of the variables was quantitative and the other qualitative, we used the Student *t* test if the quantitative variable followed a normal distribution and the Wilcoxon rank-sum test otherwise. If both variables were quantitative, we used the Pearson correlation if they came from a bivariate normal distribution and the Spearman rho statistic if not. If both variables were qualitative, the Fisher exact test was applied. In the case of multigroup comparison, we used the ANOVA or the Kruskal–Wallis test according to whether the quantitative variable was normal or not.

The Shapiro–Wilk test was used to determine if the quantitative variables followed a normal distribution, and all the *P* values were adjusted using the Benjamini–Hochberg method. Only *P* values lower than .05 were considered significant. Statistical analysis was performed using R Core Team,[20] version 3.2.3.

3. Results

During the study period we evaluated 82 subjects, 60 (73.8%) females; range age: 6 to 50 years old; mean age: 21.75 ± 11.85 years. There were 41 patients (50.0%) who were diagnosed with PKU22 (53.65%) were diagnosed with CPKU and 19 patients (46.34%) with MPKU. Regarding to pharmacological treatment, 7 of PKU patients (17.07%) were treated with sapropterin therapy in addition to dietary treatment. As it was previously designed, there were no statistical significant differences between PKU patients and their matched controls regarding age or gender (Table 1 and Additional file, http://links.lww.com/MD/C35).

During the last year, adequate annual median blood Phe levels were observed in 34 (82.9%) of 41 PKU patients, all of them under 18 years old. None of the patients treated with tetrahydrobiopterin (BH4) therapy had Phe levels above the recommended limit indicated for their age.

Using controls versus PKU categories as a grouping variable, without regard for individuals' phenotype, children and adults patients with PKU had a similar office systolic and diastolic BP than controls as well as heart rate. The same happened to cSBP and cDBP. However, in the group of PKU patients, both arterial stiffness markers as AP and Aix@HR75 were found to be increased among PKU patients when compared to controls (5.09 ± 4.76 mm Hg vs 2.74 ± 4.11 mm Hg and 12.15 ± 13.28% vs 8.84 ± 12.52%, respectively); although these differences did not reach statistical significance. Percentage of PKU patients with PWV above 90 percentile was higher than controls (14.63% vs 2.32%; *P* = .048).

With regard to PKU phenotype, we did not find differences on cSBP, AP, Aix@HR75, or PWV between controls and MPKU. We found higher PWV in CPKU patients than in controls (6.60 m/second vs 5.26 m/second; *P*: .044) (Fig. 1). Linear regression analysis showed a significant relationship between age and PWV (r = 0.853, *P* = 5.96 e-12), cDBP (r = 0.597, *P* = .0002), or Aix75 (r = 0.495, *P* = .004). Multiple linear regression comparing age to PWV showed a significant effect of age (beta = 0.813, *P* = 4.83e-07) and cDBP (beta = 0.813, *P* = 3.30e-05). Using a multiple regression analysis, we found that the most strong predictors of PWV in PKU patients were age and cDBP (adjusted $R^2 = 0.8138$, *P* = 7.912e-13; age *P* = 4.83e-07, cDBP *P* = 3.30e-05).

In PKU patients was observed a linear relationship between the annual Phe median and the PWV (r = .496; *P* = .012) or the cSBP (r = .510; *P* = .017) (Fig. 2). Regarding BH4 treatment, it is remarkable to note that PWV was higher in those PKU subjects without treatment (6.23 m/second vs 4.85 m/second) although

Table 1

Clinical characteristics in 82 subjects and differences between groups regarding PKU condition.

	PKU subjects (n = 41)		Controls (n = 41)		
	Mean	SD	Mean	SD	*P*
Age, y	23.18	12.88	20.33	10.69	.437
Sex, % male	26.83	—	26.83	—	1.000
Weight, kg	60.45	19.16	55.43	23.51	.501
Height, cm	156.0	11.09	155.0	16.00	.826
Waist, cm	76.51	18.19	72.41	15.72	.470
BMI, kg/m^2	24.38	6.22	22.28	7.08	.387
Heart rate, bpm	68.98	9.14	73.62	11.90	.663
oSBP, mm Hg	114.24	13.38	113.00	16.73	.857
oDBP, mm Hg	69.71	11.45	67.20	10.01	.445
TC, mg/dL	160.50	35.62	174.31	33.39	.468
LDL-C, mg/dL	89.02	27.89	96.97	27.85	.377
HDL-C, mg/dL	53.37	13.54	57.53	16.74	.476
TG, mg/dL	91.98	52.54	86.73	46.34	.978
cSBP, mm Hg	101.04	14.61	100.42	10.99	.754
cDBP, mm Hg	70.83	11.54	77.13	10.59	.128
cPP, mm Hg	31.56	10.64	28.82	7.57	.3775
AP, mm Hg	5.09	4.76	2.74	4.11	.139
Aix@HR75, %	12.15	13.28	8.84	12.52	.426
PWV, m/s	6.00	1.70	5.26	0.95	.150

Aix@HR75 = augmentation index, AP = augmentation pressure, BMI = body mass index, cDBP = central diastolic blood pressure, cPP = central blood pressure, cSBP = central systolic pressure, HDL-C = high density lipoprotein, LDL-C = low density lipoprotein-cholesterol, oDBP = office diastolic blood pressure, oSBP = office systolic blood pressure, PKU = phenylketonuria, PWV = carotid-femoral pulse wave velocity, SD = standard deviation, TC = total cholesterol, TG = triglycerides.

these differences did not reach statistical significance once adjusted.

Over the observation period, inadequate z-BMI was observed in 18 (43.9%) of PKU patients (61.1% with overweight and 38.9% with obesity). The percentage of controls with increased z-score of BMI was slightly lower, with 17 (39.53%), 52.94% of them with overweight and 47.06% with obesity. Nevertheless, a higher amount of PKU patients showed increased waist perimeter (WC was above the recommended limit threshold in 19 (46.34%) patients vs 12 (27.9%) in controls, *P*: .074). It is worth mentioning that a statistically significant linear correlation was observed between PWV and weight (r = .765; $P < .001$), waist perimeter (r = .684; $P < .001$), and BMI (r = 0.688; $P < .001$) in PKU subjects (Fig. 3). Those PKU patients under sapropterin treatment showed lower BMI than those without BH4 therapy (20.43 kg/m^2 vs 25.18 kg/m^2; $P = .037$). PKU subjects with lower Phe tolerance (n: 21, 51.2%) showed more body weight (67.6 kg vs 56.8 kg; $P = .012$) and more PWV than those with higher Phe tolerance (6.55 m/second vs 5.42 m/second; $P = .044$).

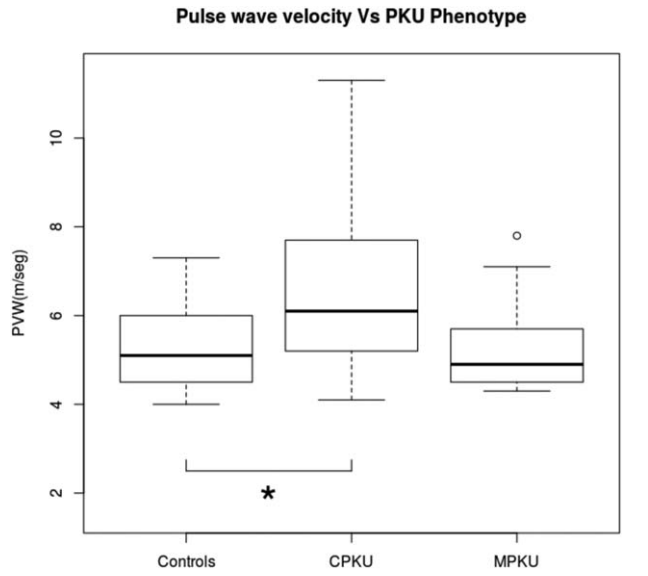

Figure 1. Box plot of pulse wave velocity according to phenylketonuria phenotype. The X-axis indicates the controls, mild–moderate PKU, and classic PKU groups. Box plots show mean values (solid horizontal line); 25th and 75th percentiles (box); minimum and maximum values (bars); outliers (open circles). The * indicates the statistical significant differences: $P < .05$, using the statistical tests described in the Subjects and Methods Section.

Regarding blood lipid profile, TC, HDL-C, and LDL-C levels were found to be lower in PKU patients than in controls although the differences were not significant. No differences were found when assessing triglycerides concentrations.

4. Discussion

In children and young adults, PWV is regarded as the most widely used and accepted method for assessment of vascular stiffness as stated by American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young.[21] The present study is the first showing higher PWV in CPKU patients than controls, independently of the office peripheral BP level. So we found stiffer arterial walls between PKU patients. As arterial stiffening causes early return of reflected arterial pressure waves from periphery, augmentation of these waves on incident aortic pressure wave can be measured as a stiffness marker (AP). Aix@HR75 is the difference between the second and the first systolic peaks of the aortic waveforms. Both arterial stiffness markers were found to be increased among our PKU patients.

PWV has been recently involved as target-organ damage when faster than 10 m/second, in the ESC/ESH Hypertension Management Guidelines.[7] Applanation tonometry can be used to detect PWV (carotid-femoral or carotid-radial arteries). For each 1

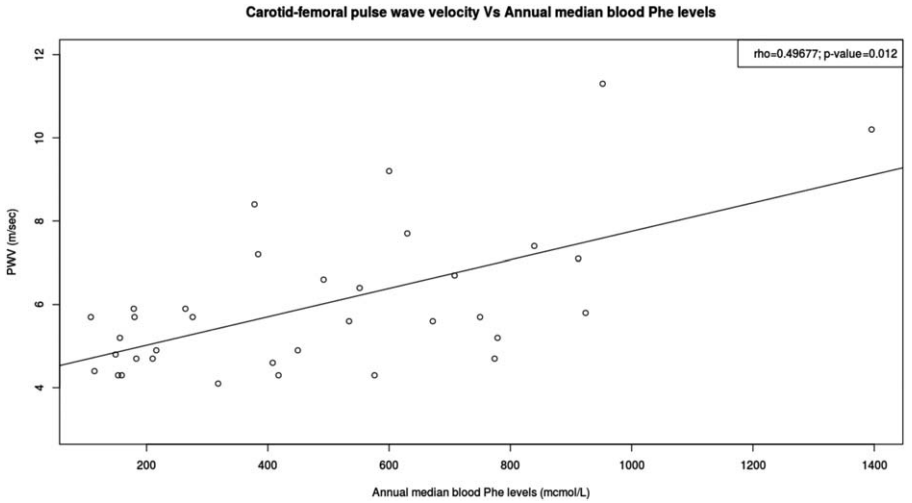

Figure 2. Linear correlation between annual median Phe levels and carotid-femoral PWVin patients with mild–moderate and classic phenylketonuria (PWV = 4.34 + 0.2 × annual Phe median).

standard deviation increment in PWV, the risk of a cardiovascular event (including coronary artery disease, stroke, or cardiovascular death) increased by 16% to 20% in the general population[22] and predicts cardiovascular outcome in hypertensive subjects.[23] Results from this study indicate that the Phe median is a significant correlate of carotid-femoral PWV in PKU subjects. The effect of increased PWV is additive to the effect of age per se, since the mean age of each group was essentially similar. Our findings suggest this relationship between Phe levels and arterial stiffness does not depend on age.

There are several publications that refer to the greater tendency to overweight and obesity in PKU patients.[24–26] In our cohort, we found this tendency although we did not find any statistical significant difference in BMI nor waist perimeter in the PKU patients; however, this may be related to our smaller study sample. We have to take into account that the greater weight gain of PKU patients may be related to the intake of protein substitute, which has a higher caloric content. Although discussion of whether or not PKU adults have a proven high incidence of cardiovascular events remains controversial, we have to take into

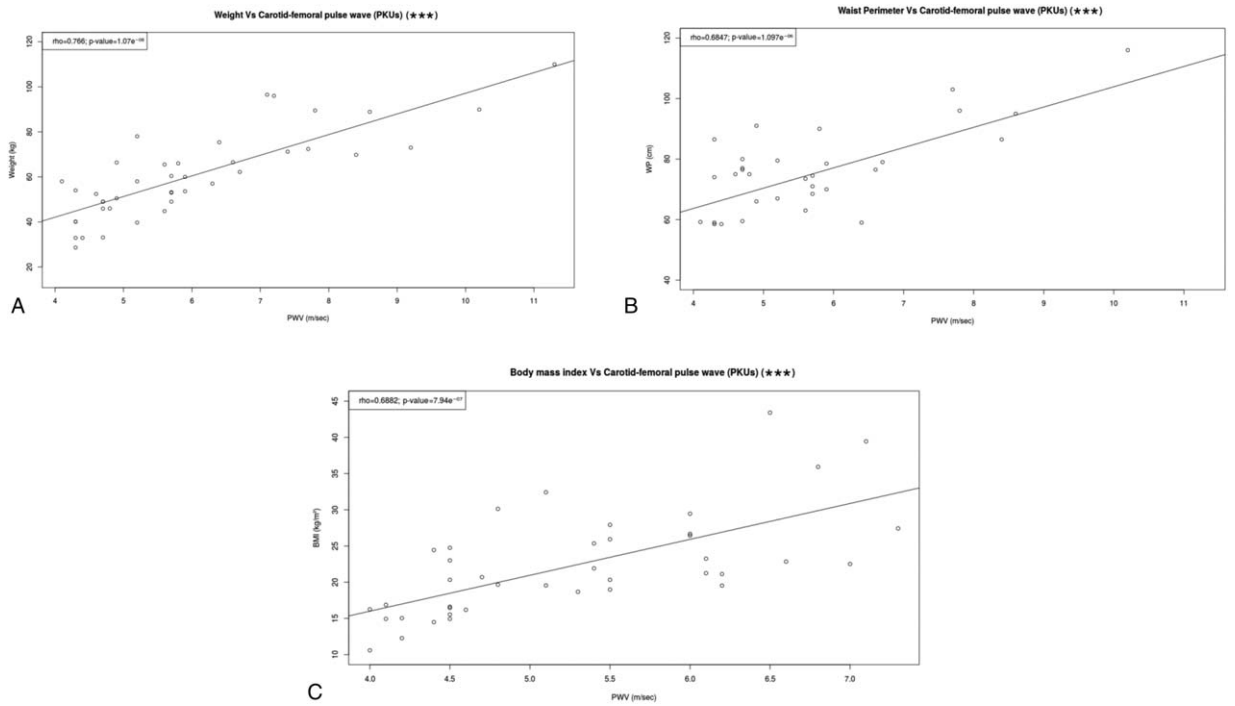

Figure 3. Correlations between anthropometric measurements and pulse wave velocity in patients with mild–moderate and classic phenylketonuria. Linear correlation between PWV and (A) weight (PWV = 1.62 + 0.07 × weight), (B) waist perimeter (PWV = 0.74 + 0.08 × waist perimeter), and (C) body mass index (PWV = 0.68 + 0.22 × body mass index).

account that BMI correlates with others cardiovascular risk factors such as increased levels of triglycerides, homocysteine, and BP. Increased adiponectin concentrations has also been reported in PKU subjects poorly controlled, which might moderate their risk for endothelial dysfunction and atherogenesis.[27] Moreover, we found a linear relationship between anthropometric measurements and PWV in PKU patients which may also underlie obesity-dependent cardiovascular risk.

It is observed that patients under BH4 and less severe vegan-like diet have tendencies to normalize these parameters. In our cohort, 85.72% of PKU subjects under sapropterin treatment had adequate z-BMI (vs 50% of those without treatment). Similarly, PKU patients with low Phe tolerance showed more body weight and hence increased arterial stiffness. Regarding BH4 treatment, PWV was lower in those PKU subjects under treatment suggesting improved endothelial function as previously reported in animal models when chronic oral administration of BH4 also increased vascular BH4 increased nitric oxide production from endothelial cells.[28]

The cholesterol concentration of PKU children has been analyzed in a limited number of studies. Compared to healthy controls, PKU children on diet used to exhibit lower cholesterol levels.[8,29] Experimental hyperphenylalaninemia has been demonstrated to inhibit two of the main regulatory enzymes (3-hydroxy-3-methylglutaryl-CoA reductase (EC: 1.1.1.98) and mevalonate-5-pyrophosphate decarboxylase (EC: 4.1.1.33)) of brain and liver cholesterogenesis.[30] In our study, plasma cholesterol concentrations, HDL-C and LDL-C were lower in PKU patients than in controls although it did not reach statistical significance. Serum triglycerides were no significant different in PKU than in healthy controls. It is interesting to note than in spite of less atherogenic profile in terms of cholesterol levels, arterial stiffness has shown to be increased in those PKU subjects, suggesting different etiopathogenic pathways. Maybe due to moderate hyperhomocysteinemia with low vitamin B12 and folate levels showed in PKU patients on a strict diet and low-quality protein intake.[31] It was also previously suggested elevated values of high-sensitivity C-reactive protein in adults PKU patients as an early marker of endothelial dysfunction.[4]

Certain factors should be considered in the interpretation of these results as limitations of the study. First, because of the design, we cannot determine the exact circumstances involved in increased arterial stiffness among PKU patients as none causality relationship may be established. Because of the number of patients evaluated, some differences in subgroup analysis may have not reached statistical significance. It is possible that other factors such as familiar inherited cardiometabolic disturbances, smoking habit or regular physical activity could have accounted for some of the differences in arterial stiffness. Conversely, this study had a series of strengths, including a large number of variables investigated to explore associations with arterial stiffness. Furthermore, the model utilized was robust as only high-quality recordings were included into the analysis.

The study has shown that applanation tonometry is a simple and noninvasive method that maybe used to screen for elevated aortic stiffness in PKU patients. Further longitudinal studies, with larger sample size, are warranted to investigate the impact of elevated aortic stiffness on cardiovascular prognosis in this group.

In conclusion, PKU patients have significantly increased aortic stiffness, measured noninvasively by carotid-femoral PWV when compared to healthy controls. Our findings showed that PKU patients, who strictly adhered to their diet and those under sapropterin treatment, are at lower risk of developing atherosclerosis since they showed lower PWV. In the future, applanation tonometry may be used for serial monitoring and risk stratification of PKU patients and as a potential guide for targeted therapy.

5. Authors' contribution

AH designed the study, supervised the analysis of the data, reviewed the publications included in the systematic review and drafted the manuscript. VC, RL, CC, and LC participated in its design and coordination of the study and helped draft the manuscript. VC, IR, and LC reviewed the publications included in the systematic review, analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

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