U.PORTO

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Terapêutica nutricional em casos de fenilcetonúria materna no CHP: estudo retrospetivo e evolução dos descendentes

Nutritional management of maternal PKU in CHP (NMMPKU): retrospective study and offspring outcome

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Porto, 2018

Resumo

Introdução: Uma terapêutica nutricional adequada durante a gravidez é essencial para prevenir a síndrome de fenilcetonúria materna. O objetivo deste estudo é descrever as práticas nutricionais e avaliar a evolução da descendência, em 7 casos de fenilcetonúria materna seguidos no Centro Hospital do Porto.

Metodologia: Foram recolhidos dados de sete gravidezes relativos ao controlo metabólico, ingestão nutricional, antropometria e marcadores bioquímicos. Foi estudado um total de 6 mulheres, visto que uma mulher esteve grávida duas vezes. Os dados foram analisados em 3 momentos diferentes: antes, durante e após a gravidez. Foi avaliada a composição corporal antes e após a gestação e a antropometria neonatal.

Resultados: Antes da gravidez, a mediana de [Fen] sanguínea variou entre 2.6-13.0 mg. Duas gravidezes foram planeadas. Durante a gravidez, a mediana de [Fen] sanguínea diminuiu para 2.4 - 7.1, 1.2 - 5.9 e 1.0 - 5.3 mg/d, no final de cada trimestre, respetivamente. Após a gravidez, a mediana [Fen] sanguínea foi 6.7 - 20.2 mg/dL Todas as mulheres tiveram uma dieta restrita em Fen suplementada com substitutos proteicos. O aporte de Fen antes da gravidez foi 414 - 2966 mg/dia e no final de cada trimestre foi 460 - 1393, 469 - 12899 e 1336 - 1899 mg/dia, respetivamente. O ganho de peso na gravidez variou entre 5 - 21 Kg. Cinco partos foram eutócicos e dois distócicos. A antropometria neonatal variou entre 2420 - 3410 g (peso), 45 - 49 cm (comprimento) e 31.5 - 35 cm (perímetro cefálico).

Conclusão: Apesar de apenas duas mulheres terem iniciado a dieta antes da conceção, todas atingiram um bom controlo metabólico durante a gravidez, permitindo uma evolução normal da descendência.

Palavras Chave: Fenilcetonúria materna; Terapêutica Nutricional; Recomendações

Abstract

Background: Optimal nutritional management during pregnancy is essential to prevent maternal phenylketonuria (MPKU) syndrome. This study aims to describe the nutritional management practices compared with infant outcome in a series of MPKU case reports followed at Centro Hospitalar do Porto (CHP).

Methods: Data on metabolic control, nutritional intake, anthropometry and biochemical markers of 7 pregnancies, 6 single and 1 multiple, from 2007 to 2018 was collected. A total of 6 PKU women, 1 was pregnant twice, was studied. Data was collected before (BP), during (DP) and post pregnancy (PP). Body composition BP and PP was interpreted. Neonatal anthropometry was recorded for the 8 offspring.

Results: BP median blood [Phe] was 2.6 - 13.0 mg. Two pregnancies were planned. DP median blood [Phe] range reduced into 2.4 - 7.1, 1.2 - 5.9 and 1.0 - 5.3 mg/dL by the end of trimester 1, 2 and 3, respectively. PP median blood [Phe] range increased to 6.7 - 20.2 mg/dL. All women were treated with a Phe-restricted diet supplemented with protein substitute. Phe intake BP was between 414 - 2966 mg/day and, by the end of trimester 1, 2 and 3, was 460 - 1393, 469 - 12899 and 1336 - 1899 mg/d, respectively. Maternal weight gain was 5 - 21 Kg. Five were eutocic births with two distocic. Infants born at 36 - 40 weeks, with 2420 - 3410 g (weight), 45 - 49 cm (length) and 31.5 - 35 cm (head circumference). Two women stopped diet PP.

Discussion: Although sub-optimal metabolic control BP, good blood Phe control was achieved throughout all pregnancies, allowing normal neonatal outcomes.

Keywords: Maternal Phenylketonuria; Nutritional Management; Guidelines

List of abbreviations

PKU - Phenylketonuria

Phe - Phenylalanine

Tyr - Tyrosine

NBS - Newborn screening

SLPF – Special Low Protein Foods

MPKU - Maternal phenylketonuria

CHP - Centro Hospitalar do Porto

NP - Natural protein

PE - Protein equivalent

TP - Total protein

BMI - Body mass index

WHO - World Health Organization

T1 - Trimester 1

T2 - Trimester 2

T3 - Trimester 3

AAM - Phe-free L-amino acids mixtures

GMP - Glycomacropeptide

Índex

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1. Introduction

Phenylketonuria (PKU) is an inborn error of protein metabolism characterized by an increased blood phenylalanine (Phe) concentration. In the great majority of the affected individuals, PKU results from a deficiency of the enzyme phenylalanine hydroxylase responsible for the conversion of Phe into tyrosine (Tyr) [1]. Diagnosis is made nowadays by newborn screening (NBS) in most of the developed countries [2, 3]. In Portugal, NBS for PKU was introduced in 1979 [4]. This allows early diagnosis and prompt treatment initiation in order to prevent the typical phenotype observed in untreated individuals, which results from the high Phe levels that accumulate in the blood and are responsible for neurotoxicity [5]. PKU requires a lifelong dietary treatment consisting on a Phe and natural protein restricted diet, supplemented with Phe-free L-amino acids and special low protein foods (SLPF), aiming to keep metabolic control within target range [6].

A good metabolic control throughout life is advocated and it gains even more importance in women at pre-conception stage and during pregnancy [7]. The teratogenic effects of high maternal blood Phe levels were first described by Charles Dent in 1956 [8, 9]. Microcephaly, congenital heart defects, low birth weight, intrauterine growth and mental retardation are common consequences of fetus exposure to high maternal blood [Phe] [10, 11]. Lenke and Levy reported that 12% of offspring exposed to blood Phe levels >20 mg/dL, had congenital heart disease [11]. In Maternal Phenylketonuria Collaborative Study, 73% of offspring exposed to a maternal blood Phe level > 20 mg/dL had microcephaly and 92% suffered from mental retardation [12].

Considering the above mentioned literature, maintenance of treatment is crucial to prevent maternal PKU (MPKU) syndrome [11]. In the past, a stricter target blood Phe control (1-4 mg/dL) was recommended in pregnant PKU women [13]. This was mainly justified by the mechanism of Phe active transport through the placenta, that usually results in higher fetal concentrations compared to maternal blood Phe levels [14]. To achieve the best pregnancy outcome, a restricted low Phe diet before conception should be implemented. Before and during pregnancy, metabolic control levels should be within target range: 2-6 mg/dL [7]. Diet compliance is challenging and PKU women must be fully aware of the importance of the dietary treatment, especially before and during pregnancy, in order to prevent the mentioned complications.

Apart from blood Phe control, weight gain, general nutritional status, adequate nutritional intake and prevention of nutritional deficits should be recognized as important targets to monitoring during any pregnancy. In MPKU, monitoring all these parameters gains even more importance to prevent several offspring complications [15, 16].

2. Objectives

This study aims a) to describe the nutritional management practices before, during and after pregnancy in a series of women under follow-up at Centro Hospitalar do Porto (CHP) - Reference Treatment Centre of Metabolic Disorders in Portugal, and b) to match the metabolic control of women during the observation period with offspring outcome.

3. Methods

3.1. Participants

A sample of 6 PKU women who maintained follow-up at CHP before, during and after pregnancy and their children were studied (Table I). Disease severity was classified according to the Portuguese Nutritional Consensus, considering NBS blood [PHE], as follows: hyperphenylalaninemia (blood [Phe] < 6 mg/dL), mild PKU (blood [Phe] ≥ 6 mg/dL and ≤ 20 mg/dL) and classical PKU (blood [Phe] > 20 mg/dL) [(4)]. A total of 7 pregnancies (one woman was pregnant twice) that occurred between 2007 and 2018 are reported. There were 6 single and 1 multiple pregnancies with a total outcome of 8 children (3 females and 5 males).

Table I. Patients' neonatal blood Phe, disease severity and genotype.

Patient	**A**	**B**	**C**	**D**	**E**	**F**
Neonatal blood Phe (mg/dL)	80	18	21	10	12	15
Disease severity	Classical	Mild	Classical	Mild	Mild	Mild
Genotype	R261Q	R261Q	P281L	I65T	I65T	R270K
	V388M	P281L	P281L	R261Q	I65T	F410C

3.2. Study design

This was an observational, longitudinal and retrospective study. All data was collected retrospectively from patients' clinical records available at CHP. Data on metabolic control, nutritional intake, anthropometry and biochemical markers was considered on three different time periods: before, during and after pregnancy. Body composition pre and post-pregnancy was also collected.

Intrauterine development, obtained from the CHP's Obstetrician clinical records, was recorded for the offspring. Neonatal anthropometry (weight, length and head

circumference) was collected from the child health bulletin. In order to assess infant progress, psychological reports of children were collected from the Psychologists clinical records. Study design is shown in Annex A.

3.3. Data Collection

3.3.1. Metabolic control

Tandem mass spectrometry was used to measure blood Phe concentration [Phe] from blood spots which were collected at fasting state. The number of dried blood samples, median of blood [Phe] and % of blood [Phe] within the therapeutic range were assessed for each study period. During pregnancy, satisfactory metabolic control was defined as blood [Phe] between 2 and 6 mg/dL, while blood [Phe] < 8 mg/dL was defined as a target before and after gestation [4]. Metabolic control from blood spots was analysed one year before conception and during pregnancy. At the moment of data collection, 1year post-pregnancy metabolic control was only available for 4 women. Although all patients were already under obstetrician follow-up, metabolic control under target range was used to define fully planned pregnancy.

3.3.2. Nutritional intake

Nutritional intake was collected from the diet history reported in nutrition appointments. Nutritional intake before pregnancy was recorded from the last nutrition appointment of each woman before conception. Nutritional intake during pregnancy was collected from the first nutrition appointment post-conception and also every last one in each trimester. After pregnancy it was considered the first post-partum nutrition appointment. The following intakes were recorded: natural protein (NP, g/kg/day), protein equivalent from protein substitute (PE, g/kg/day), total protein (TP, g/kg/day), phenylalanine (Phe, mg/day), total tyrosine from protein

substitute (Tyr, g/Kg/day). The type and intake of protein substitute (Phe-free L-amino acids mixtures or glycomacropeptide-based protein substitute) was registered.

3.3.3. Anthropometry

Weight and height were measured using a Seca® mechanic scale and a stadiometer, with a precision level of 0,5 Kg and 1 mm, respectively. Height and weight were measured wearing lightweight clothes and without shoes or adornments. Body mass index (BMI) was calculated according World Health Organization (WHO): weight (Kg)/height^2 (m^2) [17]. Maternal weight gain was calculated based on the weight from the last nutrition appointment before conception and at the day of delivery, and interpreted according to published recommendations [18].

3.3.4. Body composition analysis

Information relative to body composition before and after pregnancy was collected. Body composition analysis was assessed using a single frequency (50Hz) bioelectrical impedance analyser, Akern Quantum/S, according to previously described standards [19]. Resistance (Rz) and reactance (Xc) were used to estimate the percentage (%) of body fat and lean mass.

3.3.5. Biochemical markers

Biochemical monitoring was performed at fasting state. The following analytical data was collected before, during and after pregnancy: glucose (mg/dL), uric acid (mg/dL), creatinine (mg/dL), urea (mg/dL), haemoglobin A1c (%), triglycerides (mg/dL), total cholesterol (mg/dL), HDL cholesterol (mg/dL), LDL cholesterol

(mg/dL), apolipoprotein A1 (mg/dL), apolipoprotein B (mg/dL), iron (µg/dL), transferrin (mg/dL), ferritin (ng/mL), albumin (g/dL), prealbumin (mg/dL), C-reactive protein (mg/dL), insulin (µU/mL), homocysteine (µmol/L), vitamin B12 (pg/mL), vitamin D (nmol/L), folic acid (ng/mL), calcium (mmol/L), phosphorus (mmol/L), plasma selenium (mmol/L) and plasma zinc (µmol/L).

3.3.6. Intrauterine development

Records of several ultrasounds from all pregnancies were used in order to assess intrauterine development.

3.3.7. Offspring anthropometry and progress

Information regarding infant's weight, length and head circumference at birth and APGAR score was collected from CHP clinical records or child health bulletin.

Offspring development was assessed at the psychology appointments. The Griffiths Mental Development Scales [20] for testing babies and young children (from birth to seven years of age) was used. For children older than 7 years the Wechsler Intelligence Scale for Children – Third Edition (WISC-III) was used [21] .

3.3.8. Ethical Statement

This study protocol was approved by the Ethics Committee of Centro Hospitalar do Porto, on the 12 th of June 2018, with the reference 2018.117(102-DEFI/101-CES) (Annex B). Written informed consent was obtained from each patient or caregiver.

3.3.9. Statistical analysis

Due to the nature of the study, the statistical analysis was essentially descriptive, based on tabular and graphical analysis.

4. Results

Age at conception varied between 25 and 31 years and only 2 women were formally on pre-conception diet (case 3 and 5). (Table II).

Table II. Age at conception, year of delivery, length of gestation, type of pregnancy and baby gender.

Pregnancy case	1 (patient A)	2 (patient A)	3 (patient B)	4 (patient C)	5 (patient D)	6 (patient E)	7 (patient F)
Age at conception (years)	25	27	29	31	27	29	26
Year of delivery	2008	2011	2011	2016	2017	2018	2018
Length of gestation	38 weeks 2 days	36 weeks 6 days	39 weeks 5 days	39 weeks 2 days	38 weeks 2 days	39 weeks 5 days	40 weeks 4 days
Type of pregnancy	Unplanned	Unplanned	Planned	Unplanned	Planned	Unplanned	Unplanned
	Single	Multiple	Single	Single	Single	Single	Single
Baby gender	Male	Male Male	Male	Female	Female	Male	Female

Before pregnancy median blood [Phe] ranged between 2.6-13.0 mg/dL (20-95% of the Phe values within target range). During pregnancy median blood [Phe] range reduced into 2.4-7.1, 1.2-5.9 and 1.0-5.3 mg/dL by the end of trimester 1 (T1), 2 (T2) and 3 (T3), respectively. Post-pregnancy median blood [Phe] range increased to 6.7-20.2 mg/dL (Table III). The evolution of median blood [Phe] 1 year before pregnancy, throughout pregnancy and after pregnancy is illustrated in Figure I.

Table III. Metabolic control before, during and after pregnancy.

	Case	Before Pregnancy	Trimester 1	Trimester 2	Trimester 3	After pregnancy
Number of blood spots	1	4	15	26	29	22
	2	5	19	29	27	13
	3	64	23	20	24	31
	4	79	24	25	28	2
	5	44	21	26	24	7
	6	25	19	7	28	3
	7	5	4	10	8	n.a.
Median blood [Phe] range (mg/dL)	1	11.4	2.9	1.8	1.0	6.7
	2	10.1	2.4	1.2	1.1	10.4
	3	2.6	3.6	1.6	2.5	8.7
	4	8.1	5.5	3.7	4.0	20.2
	5	8.0	4.0	3.6	2.5	8.4
	6	13.0	4.0	5.2	4.1	9.6
	7	8.0	7.1	5.9	5.3	n.a.
% of [Phe] measurements within the therapeutic range	1	25	80	38	0	55
	2	20	58	24	4	15
	3	95	83	30	71	95
	4	46	63	88	75	0
	5	50	90	96	83	29
	6	4	84	86	86	0
	7	40	0	50	75	n.a.

n.a.*- not available

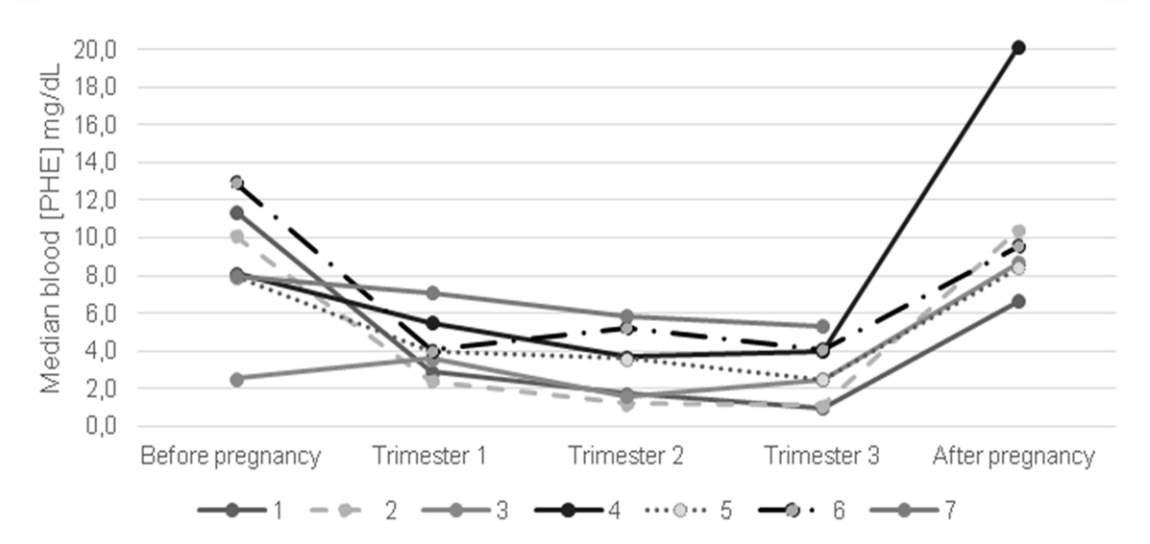

Figure I. Metabolic control before, during and after pregnancy.

Satisfactory metabolic control before and after pregnancy: blood [Phe] < 8 mg/dL
Satisfactory metabolic control during pregnancy: blood [Phe] 2 - 6 mg/dL

All women were treated with a Phe-restricted diet. In 4 cases (no. 1, 2, 3 and 5) they took only Phe-free L-amino acids mixtures (AAM) (65-84 g/d). In 3 pregnancies GMP was used. In two cases a combination of GMP (30g/d) and AAM (28-56g/d) was used. In the last case report (no. 7), GMP was used as the unique protein substitute (45-60g/d) (Table IV).

Table IV. Protein substitutes used during trimester 1, 2 and 3 and protein equivalent intake.

Case	**Trimester 1**		**Trimester 2**		**Trimester 3**	
	AAM* (g of PE**/day)	GMP*** (g of PE**/day)	AAM* (g of PE/day)	GMP*** (g of PE**/day)	AAM* (g of PE**/day)	GMP*** (g of PE**/day)
1	73.5	–	77	–	84	–
2	72.5	–	83	–	83	–
3	65	–	65	–	65	–
4	28	30	56	30	56	30
5	65	–	68.5	–	75.5	–
6	50	30	50	30	50	30
7	–	60	–	45	–	45

***AAM - Phe-free L-amino acids mixtures**
****PE - protein equivalent**
*****GMP - glycomacropeptide**

Protein equivalent intake during pregnancy was 0.4-1.3 g/kg/day (Table V). Phe intake before pregnancy was between 414-2966 mg/day and, by the end of T1, T2 and T3 was 460-1393, 469-1899 and 861-1899 mg/day, respectively (Table VI). Two women went off diet post pregnancy.

T**able V.** Protein intake.

	Case	Before Pregnancy	At conception	Trimester 1	Trimester 2	Trimester 3	After pregnancy
Natural protein intake g/Kg/day	**1**	0.22	0.18	0.19	0.18	0.40	0.20
	2	0.57	0.33	0.20	0.20	0.42	0.19
	3	0.24	0.24	0.24	0.43	0.43	0.43
	4	0.24	0.14	0.18	0.23	0.38	1.39
	5	0.45	0.47	0.46	0.50	0.55	0.62
	6	0.20	0.21	0.21	0.21	0.22	0.21
	7	0.68	0.37	0.38	0.47	0.41	0.42
Protein equivalent g/Kg/day	**1**	1.04	0.94	1.17	1.16	1.13	1.07
	2	0.76	0.79	1.20	1.31	1.10	0.98
	3	0.31	0.62	1.13	0.94	0.86	0.86
	4	0.94	0.91	0.88	1.21	1.12	0.00
	5	1.17	1.22	1.34	1.25	1.24	1.40
	6	1.00	1.01	1.03	1.02	0.94	1.00
	7	0.34	0.92	0.69	0.50	0.44	0.45
Total Protein g/Kg/day	**1**	1.30	1.16	1.39	1.37	1.56	1.31
	2	1.37	1.15	1.45	1.56	1.53	1.19
	3	0.63	0.94	1.39	1.38	1.31	1.30
	4	1.20	1.08	1.12	1.48	1.52	1.39
	5	1.62	1.69	1.82	1.75	1.79	2.02
	6	1.21	1.22	1.25	1.25	1.18	1.23
	7	1.02	1.30	1.07	0.97	0.85	0.87
Total Protein g/day	**1**	74.27	72.79	87.36	91.17	115.89	77.04
	2	75.78	61.68	87.07	99.35	115.50	74.70
	3	36.91	55.24	79.84	96.06	98.58	98.15
	4	74.46	69.03	73.62	105.03	117.08	93.74
	5	80.41	80.41	88.17	96.15	109.20	109.10
	6	96.80	96.88	96.88	97.74	100.17	98.52
	7	75.47	113.96	92.96	88.00	88.00	88.00

Table VII. Tyrosine from protein substitute and total diet Phe intake.

	Case	Pre Pregnancy	At conception	Trimester 1	Trimester 2	Trimester 3	Post pregnancy
Tyrosine from protein substitute (g/day)	**1**	4.8	4.8	5.9	6.2	6.7	5.0
	2	3.4	3.4	6.6	7.4	9.4	5.7
	3	1.0	1.9	6.4	7.4	8.4	8.4
	4	4.9	4.9	4.9	7.2	7.2	0.0
	5	5.8	5.8	6.4	6.6	7.2	7.2
	6	8.2	8.2	8.2	8.2	8.2	8.2
	7	2.7	7.0	5.4	4.0	4.0	4.0
Phe intake (mg/day)	**1**	414	444	460	469	1336	465
	2	1415	737	476	521	1343	436
	3	593	593	554	1259	1367	1342
	4	658	458	643	808	1420	4517
	5	990	990	1018	1219	1520	1516
	6	713	716	716	742	861	775
	7	2966	1393	1393	1899	1899	1899

Maternal weight gain was between 5 - 21 Kg. For the first 5 cases, body fat % increased from 25.8 - 32.7% before pregnancy to 30.9 - 37.7% post pregnancy. In the last 2 case reports, body composition analysis post-pregnancy was not yet available at the moment of this study (Table VII).

Five deliveries were eutocic and 2 distocic. Infants born at 36 - 40 weeks, with weight between 2420 - 3410 g, length between 45 - 49 cm and head circumference between 31.5 - 35 cm (Table VIII). All newborn had an APGAR at 1st minute=9; 5th minute=10 and 10th minute=10. All women were able to breastfed until 1 - 7 months (Table IX). Data regarding infant psychological development of case 6 and 7 is not available, since they were too young at the time of data collection to be evaluated (Tables X and XI).

Table VII. Maternal weight gain, BMI, % Body Fat Mass and % Body Lean Mass before and after pregnancy.

Case	Weight gain (Kg)	BMI (Kg/m^2)		% Body Fat Mass		% Body Lean Mass	
		BP*	AP**	BP	AP	BP	AP
1	13.5	21,66	22,48	31.7	31.6	68.3	68.4
2	19.5	21,15	22,48	25.8	32.6	74.2	67.4
3	21	22,62	23,10	31.3	30.9	68.7	69.1
4	15	23,92	27,01	32.7	37.7	67.3	62.3
5	13.5	21,71	23,24	31.3	33.1	68.7	66.9
6	5	36,40	n.a.***	49.8	n.a.	50.2	n.a.
7	14	32,89	n.a.	47.8	n.a.	52.2	n.a.

***BP - Before pregnancy**
****AP - After pregnancy**
*****n.a. – not available**

Table VIII. Neonatal measurements of the offspring.

Case	Weight (g)	Length (cm)	Head circumference (cm)
1	2970	48.0	34.0
2	2450	46.5	33.0
	2420	46.0	33.5
3	2570	47.50	31.5
4	3410	49.0	33.5
5	2660	45.0	32.0
6	2560	49.0	34.5
7	3.170	47.0	35.0

Table IX. Breastfeeding, length of breastfeeding and reason to stop breastfeeding.

Pregnancy case	1	2	3	4	5	6	7
Breastfeeding	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Length of breastfeeding	6 months	1 month	7 months	1 month	1 month	n.a.*	n.a.
Reason to stop breastfeeding	Increased stress	Low milk production.	Baby refuse breast milk	Low milk production.	Low milk production.	n.a.	n.a.

n.a.*- not available

Table X. Offspring development - The Griffiths Mental Development Scales for Children.

Case	Age	Global development age	Global Developmental Quotient
1	13 months	15.1 months	112
4	15 months	13.6 months	91
5	7 months	8.0 months	113

Table XI. Offspring development - Wechsler Intelligence Scale for Children –WISC-III.

Case	Age	Global Intelligence Quotient
2	7 years 2 months	101
	7 years 2 months	85
3	7 years	95

5. Discussion

The main result of this retrospective study is that although only sub-optimal metabolic control was achieved before pregnancy, an excellent blood Phe control was registered throughout all pregnancies, allowing normal neonatal outcomes in all 8 newborns. In order to prevent maternal PKU syndrome, a Phe-restricted diet should be implemented before conception and maintained throughout pregnancy in all PKU females planning to conceive. It is recommended that blood [Phe] is kept between 2 and 6 mg/dL[(7)]. According to our knowledge, this is the first study systematically describing nutritional management practices in maternal PKU case reports in follow-up at Portuguese Metabolic reference treatment centres.

Case 1 was the first pregnancy to be followed in CHP centre, with a median of blood [Phe] lower than all others cases. During the last trimester all blood [Phe] measurements were below the lower limit of the reference range, with a median of blood [Phe] of 1.0 mg/dL (Table III). In addition, considering this was the first case at CHP centre, an excessive Phe restriction may have been proposed. Finally, we cannot rule out the possibility that patients themselves over-restrict their Phe intake considering the concern of the Phe teratogenicity. It is important to underline that Phe is an essential amino acid, so a chronic excessive Phe restriction may result in suboptimal intrauterine growth and development [22].

A total protein intake (natural protein and protein equivalent) ≥ 70 g is recommended in the treatment of MPKU [7]. With the exception of the first nutrition appointment of cases 2, 3 and 4, all women met recommendations throughout gestation (Table V). Diet compliance is challenging, even for those who have had a Phe and natural protein restrictive diet since infancy. In the post-natal period, cases 4 and 7 were unable to continue the Phe-restricted diet.

Tyrosine is a conditionally essential amino acid in PKU with particular relevance during pregnancy, so its ingestion should be > 6 g/day [7]. At the first nutrition appointment of the pregnancy, only cases 6 and 7 met the recommendations. Case 7 was the only above the recommendations at the beginning of gestation (Table VI). Non-satisfactory tyrosine intake during pregnancy in PKU has been associated with fetal damage [23, 24]. Careful monitoring of tyrosine intake during pregnancy is recommended, although in case 4 recommendations were only met from 16 weeks onwards. We should also consider the protein substitute source, and hypothetical

different tyrosine bioavailability in different protein substitutes as previously speculated [25].

In all pregnancies, Phe intake increased during T2 and T3 (Table VI). According to American College of Medical Genetic and Genomics practice guidelines, Phe intake during pregnancy should be 265-770 mg/day, 400-1650 mg/day and 700-2275 mg/day in T1, T2 and T3, respectively [26]. It has been reported that a slight increase in Phe tolerance in the last trimester may indicate the non-PKU affected fetus [27]. This was later confirmed by the NBS screening results in all offspring from this study. Case 3 and 7 had a weight gain during pregnancy higher than the recommendations (Table VII). It has been reported that an adequate maternal weight gain decreases the risk of microcephaly [18, 28]. Therefore, monitoring weight during gestation is important, also preventing excessive weight gain. Number of nutrition appointments during pregnancy ranged from 8-14 (*data not shown*), allowing immediate dietary advice towards weight control.

In all pregnancies a combined risk assessment was performed. Although case 4 had a positive result for trisomy 18 and 13, and case 7 for Down's Syndrome, none of these were later confirmed. The risk for chromosome abnormalities development is not studied in PKU pregnancies, so it is assumed that this follows that of the general population [29].

An increase of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides and C-reactive protein concentrations was observed in the majority of cases (*data not shown*). A decrease of zinc and selenium plasma concentrations was also observed in some cases (*data not shown*). All these biochemical imbalances may be due to the expected physiological adaptations during this life-cycle period [30, 31].

All nutritional management practices in maternal PKU help to optimize metabolic control and all the variables discussed in this work, but the most important objective is to guarantee that offspring outcome is not affected by the metabolic control or its treatment. Although nutritional management of these 7 pregnancies did not fully match the recommendations, the 6 children that were already psychologically evaluated had a developmental quotient within the reference population norm (≥ 85).

This study has several limitations. One is the fact that nutritional intake was based on food history, which may not completely reflect real ingestion. Moreover, the deliveries of pregnancy 6 and 7 occurred recently, between April and May 2018. For this reason, there is lack of post-pregnancy information for these two cases, regarding metabolic control and body composition. Also, blood [TYR] were only available for the most recent pregnancies. Body composition assessment was not done with the same time interval in relation to the beginning and end of the pregnancy of each case, which may difficult a deeper interpretation.

6. Conclusion

Diet compliance is changeling, especially in MPKU. Nutritional monitoring during pregnancy is essential. Pregnancy planning is critical for outcome, allowing a favourable metabolic control before conception. Although only 2 women formally started a diet in the pre-conception period, all cases were able to achieve a good metabolic during pregnancy and managed to have healthy offspring who apparently were not exposed to MPKU syndrome.

7. Acknowledgments

Special thanks to my supervisor, Manuela Ferreira de Almeida and to Júlio César Rocha. Thanks to Prof. Anita Macdonald who also contributed for this work.

Thanks to Dr. Carla Carmona and Dr. Ana Cunha who helped me during data collection.

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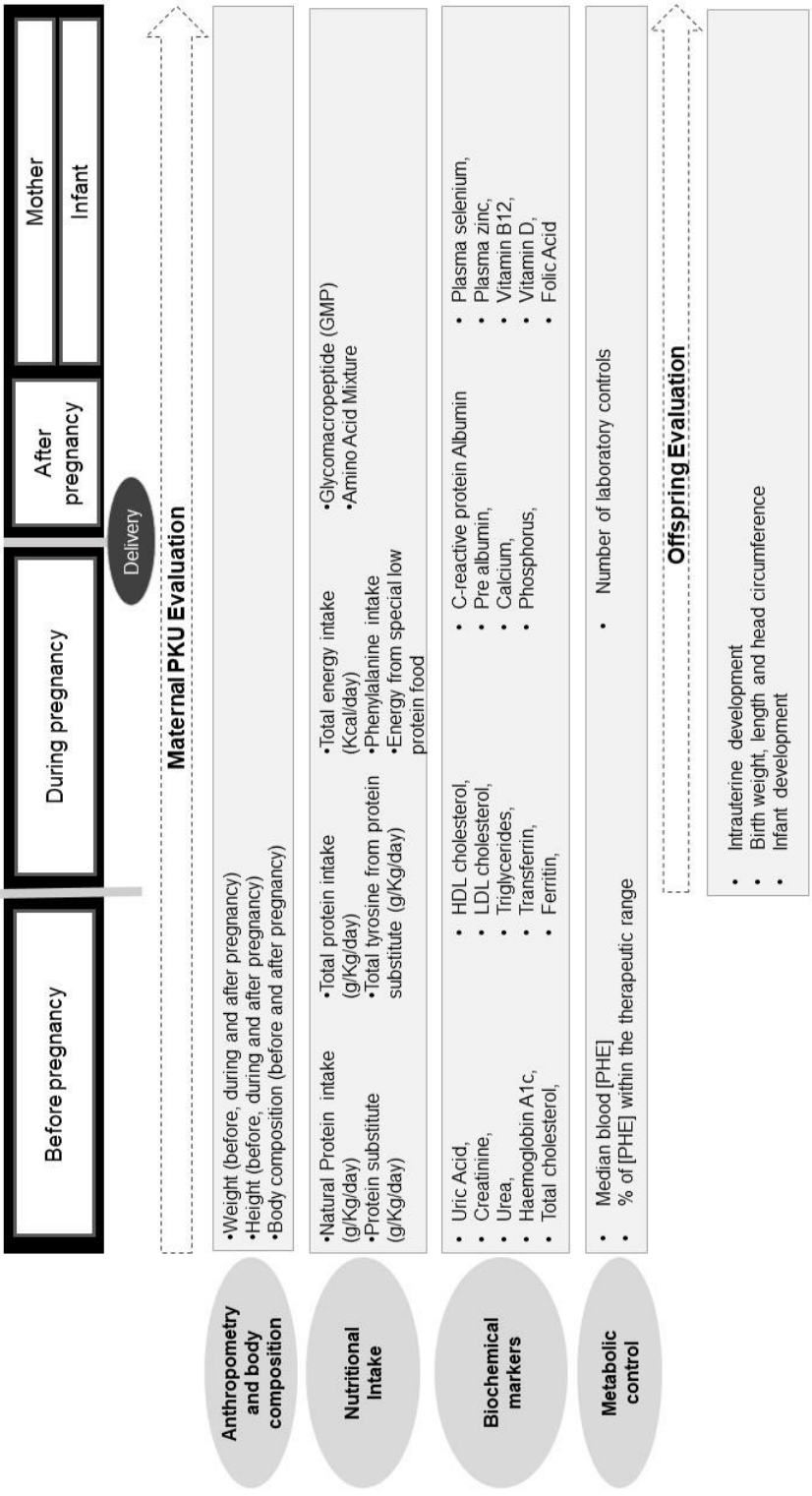
Annexes

Annex A – Study design



Annex B - Ethical Statement

REPÚBLICA PORTUGUESA
SAÚDE

SNS SERVIÇO NACIONAL DE SAÚDE

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ASSUNTO: Trabalho Académico - Licenciatura - **"Nutritional management of maternal PKU (NMMPKU) in CHP: Retrospective study and offspring outcome "** – N/ REF.ª 2018.117(102-DEFI/101-CES)

O Conselho de Administração do CHP autoriza a realização do estudo acima mencionado, a realizar no Centro de Genética Médica desta Instituição e tendo como Investigador Principal Élia Pinto, aluna da Faculdade de Ciências da Nutrição e Alimentação da Universidade do Porto.
O estudo foi previamente analisado pela Comissão de Ética para a Saúde, pelo Gabinete Coordenador de Investigação, pela Direção do Departamento de Ensino, Formação e Investigação do CHP e pelo Presidente do Conselho de Administração, tendo obtido parecer favorável.

Cumprimentos,

CONSELHO DE ADMINISTRAÇÃO
13/06/2018
Dr. PAULO BARBOSA — Presidente
Dr.ª ÉLIA GOMES — Vogal Executivo
Prof. Doutor JOSÉ BARROS — Diretor Clínico
Dr. RUI PEDROSO — Vogal Executivo
Enf.º EDUARDO ALVES — Enfermeiro Diretor

* Em todas as eventuais comunicações posteriores sobre este estudo é indispensável indicar a nossa ref.ª.