A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.

Gemma Ornosa Martín

UNIVERSITAT ROVIRA i VIRGILI

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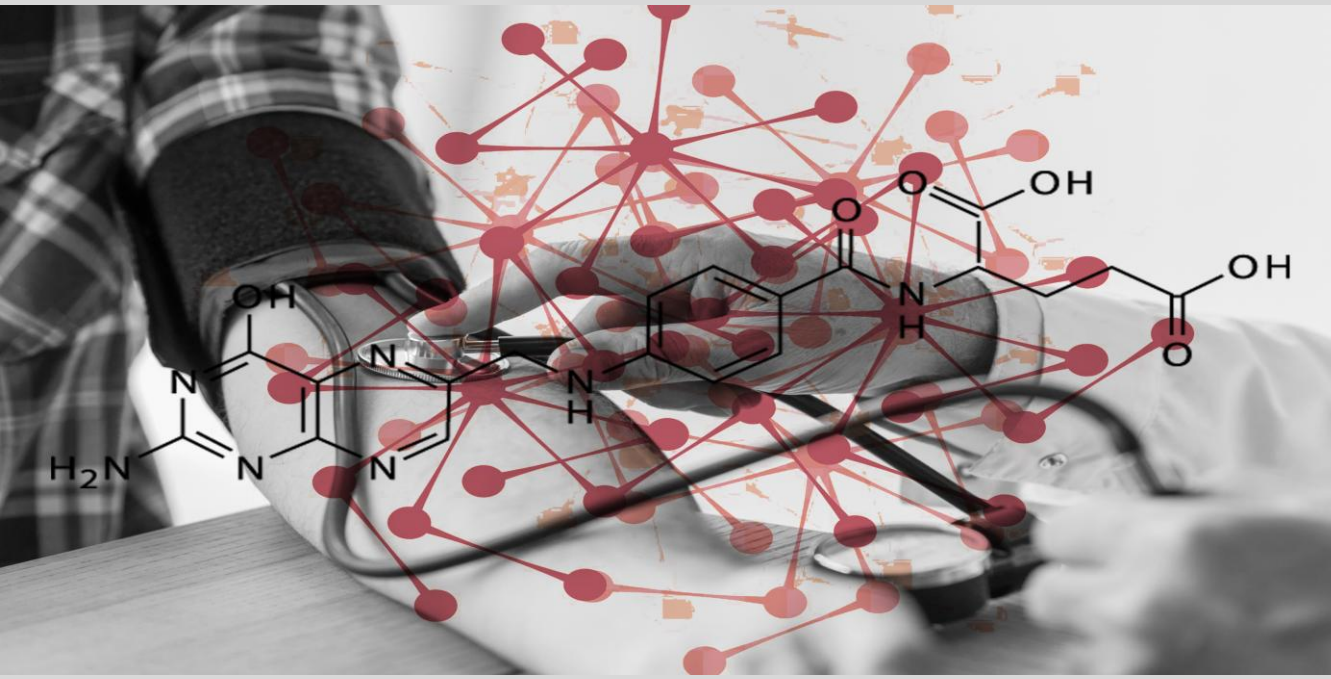

DOCTORAL THESIS
2019

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A study of the association between one carbon metabolism and blood pressure in adults and transgenerationally between pregnant women and their children

Doctoral thesis

Thesis supervised by Dr. Michelle Murphy

Department of Basic Medical Sciences

UNIVERSITAT ROVIRA i VIRGILI

Reus

2019

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FAIG CONSTAR que aquest treball, titulat "A study of the association between one carbon metabolism and blood pressure in adults and transgenerationally between pregnant women and their children", que presenta la **Gemma Ornosa Martin** per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament de **Ciències Mèdiques Bàsiques** d'aquesta universitat.

HAGO CONSTAR que el presente trabajo, titulado "A study of the association between one carbon metabolism and blood pressure in adults and transgenerationally between pregnant women and their children", que presenta **Gemma Ornosa Martin** para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de **Ciencias Médicas Básicas** de esta universidad.

I STATE that the present study, entitled "A study of the association between one carbon metabolism and blood pressure in adults and transgenerationally between pregnant women and their children", presented by **Gemma Ornosa Martin** for the award of the degree of Doctor, has been carried out under my supervision at the Department of **Basic Medical Sciences** of this university.

Reus, 17 de juliol de 2019
Reus, 17 de julio de 2019
Reus, 17th July 2019

El/s director/s de la tesi doctoral
El/los director/es de la tesis doctoral
Doctoral Thesis Supervisor/s

Dr. Michelle Murphy

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A vosaltres, Mami, Papi i Albi
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i no deixar mai de confiar amb mi.

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espero que sempre caminem junts en la mateixa direcció,
et vull sempre amb mi.
.

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Table of contents

UNIVERSITAT ROVIRA I VIRGILI
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Abstract

One carbon (1C) metabolism nutrients and the *MTHFR* 677 C>T polymorphism have been found to be associated with blood pressure and hypertension. An adequate status of 1C metabolism nutrients has been proven to be beneficial for some health outcomes in adults and also in pregnancy, from *in utero* development until later in life including childhood and adulthood. Mandatory folic acid fortification of flour has been demonstrated to reduce the incidence of neural tube defects (NTDs) and to lower fasting plasma total homocysteine (tHcy). There is increasing interest in the association between 1C metabolism and blood pressure although there is still some inconsistency in the evidence and the underlying mechanism is unknown. Foetal programming might be involved.

The aim of this thesis is to 1) explore the associations between fasting total plasma homocysteine (as a marker of 1C metabolism nutrient and metabolite status), the *MTHFR* 677C>T polymorphism and hypertension in an adult population unexposed to mandatory folic acid fortification or B vitamin supplement use and 2) investigate whether the *MTHFR* 677C>T polymorphism is associated with blood pressure in very early life in the Reus-Tarragona Birth Cohort study from early pregnancy until 7.5 years of age in the children.

Seven hundred and eighty eight participants randomly selected from the population town halls' registers, stratified by age and sex, participated in the population study. This population was unexposed to mandatory folic acid fortification and B vitamin supplement use. Both women and men in the 3rd tHcy tertile had lower folate and cobalamin status, and more of them had suboptimal riboflavin status (based on Erythrocyte Glutathionine Reductase Activation Coefficient category) compared to the other tertiles. Being in the

3rd tertile of tHcy compared to the 1st increased 1.8 times [OR=1.8 (1.0, 3.3)] the risk of having hypertension in the total population. Stratifying by age group, showed that the tHcy-hypertension association is driven by that observed in the >50 years group [OR= 2.5 (1.2, 5.4)]. Having the *MTHFR* 677TT genotype increased the probability of having hypertension compared to the *MTHFR* 677CC genotype, and the association was maintained after adjusting for confounding variables, in people aged ≤ 50 years [OR= 8.2 (1.3, 53.9)].

In the Reus-Tarragona Birth Cohort two hundred and twelve mother-child dyads were followed up from < 12 gestational weeks (GW) of pregnancy and the children at 7.5 years. Clinical, obstetrical, 1C metabolic and lifestyle data was collected from pregnant women throughout pregnancy and anthropometrical, 1C metabolic, blood pressure, clinical and lifestyle data from the children. Biochemical data was obtained from fasting blood samples collected at <12 GW, 24-27 GW, 34 GW and from the children at 7.5 years. Non-fasting blood samples were also collected at labour and from the cord, before expulsion of the placenta. Blood pressure measurements were taken at the child check-up visit ("office blood pressure measurement") and then over a 24h period with Ambulatory Blood Pressure Monitoring (ABPM). Maternal and child plasma and red blood cell (RBC) folate, plasma cobalamin, fasting plasma total homocysteine (tHcy), methylmalonic acid (MMA), maternal holotranscobalamin (holoTC) as well as Erythrocyte Glutathione Reductase Activation assay (EGRAC), in the mothers, were determined. The *MTHFR* 677 C>T polymorphism was also determined from leukocyte DNA. Mean systolic (SBP) and diastolic (DBP) blood pressure measurements at check-up were 103.8 and 64.1 mmHg, respectively. Differences between girls and boys were found only in DBP. The prevalence of child hypertension was

Abstract

6.2%. Mean SBP and DBP measured by ABPM were 103.1 and 61.5 mmHg, respectively. Girls' systolic ABPM mean was lower than boys'. Diastolic ABPM mean was similar between sexes. Using multiple logistic regression analysis we observed that child *MTHFR* 677 CT genotype was associated with increased probability of having prehypertension or hypertension [OR=6.5 (1.0, 43.5)] in the office BP model. Maternal *MTHFR* 677 CT genotype was also associated with increased probability of having prehypertension or hypertension [OR= 7.4 (1.0, 55.0)].

We found that the *MTHFR* 677 C>T polymorphism is associated with increased risk of hypertension both in the adult and in early life.

Keywords: folate – homocysteine – *MTHFR* 677 C>T polymorphism – pregnancy – foetal programming – child – blood pressure– hypertension

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Abbreviations

Abbreviations	Definition
1C	One carbon
ABPM	Ambulatory blood pressure monitoring
ACEIs	Angiotensin converting enzyme inhibitors
AHA	American Heart Association
AMP	Adenosine monophosphate
ARBs	Angiotensin receptor blockers
BBs	Betablockers
BMI	Body mass index
BP	Blood pressure
CCBs	Calcium channel blockers
CI	Confidence interval
CIA	Confidence interval analysis
CVD	Cardiovascular disease
DASH	Dietary approach to stop hypertension
DBP	Diastolic blood pressure
DFE	Dietary folate equivalent
DHF	Dihidrofolate
dUMP	Deoxyridine monophosphate
dTMP	Deoxythymidylate monophosphate
EASTAC	Erythrocyte aspartate aminotransferase activation coefficient
EGRAC	Erythrocyte glutathione reductase activation coefficient
eNOS	Endothelial nitric oxid synthase
ESC	European Society of Cardiology
ESH	European Society of Hypertension
FAD	Flavin adenine dinucleotide
FMN	Flavin mononucleotide
GSH	Glutathione
GSSG	Glutathione disulfide
GR	Glutathione reductase
GW	Gestational weeks
GWAS	Genome-wide association studies
holoTC	Holotranscobalamin
IOM	Institute of Medicine
JNC	Joint National Committee
MMA	Methylmalonic acid

Abbreviations

NAD	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NCDs	Non-communicable diseases
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Clinical Excellence
NO	Nitric oxide
NTDs	Neural tube defects
PBS	Phosphate buffered solution
PCFT	Proton coupled folate transporter
RBC	Red blood cell
RDA	Recommended Dietary Allowance
RTBC	Reus-Tarragona Birth Cohort
SAM	S-adenosylmethionine
SBP	Systolic blood pressure
tHcy	Fasting plasma total homocysteine
THF	Tetrahydrofolate
WHO	World Health Organization

Enzyme nomenclature

Abbreviation	International nomenclature	Definition
BHMT	EC 2.1.1.5	Betaine-homocysteine s-methyltransferase
DHFR	EC 1.5.1.3	Dihydrofolate reductase
eNOS	EC 1.14.13.39	Endothelial nitric oxide synthase
FPGS	EC 6.3.2.17	Folylpoly-gamma-glutamate synthetase
FOLH1	EC 3.4.17.21	Folate hydrolase
MTHFD1	EC 1.5.1.5	Methylenetetrahydrofolate dehydrogenase
MTHFR	EC 1.5.1.20	Methylenetetrahydrofolate reductase
MTR	EC 2.1.1.13	Methionine synthase
MTRR	EC 2.1.1.13	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
TYMS	EC 2.1.1.45	Thymidylate Synthase

Genetic polymorphism nomenclature

Abbreviation	Reference SNP	Definition
MTHFR 677C>T	rs1801133	Methylenetetrahydrofolate reductase 677C>T

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Gemma Ornosa Martín

List of figures

UNIVERSITAT ROVIRA I VIRGILI
A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.
Gemma Ornosa Martín

List of tables

INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
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1. One carbon metabolism

One carbon metabolism (1C) is a complex metabolic network that consists of the transfer of C units between different components of the folate and methionine cycles, among others. It is involved in a lot of different reactions and processes such as methionine synthesis, purine synthesis or glycine and serine metabolism. This network is very important for cell growth, differentiation and development.

As we can see in Figure 1, this metabolic network consists of different substrates, amino acids, enzymes and cofactors that interact all together. B-vitamins such as folate, cobalamin, riboflavin and pyridoxine act as co-factors of the different reactions of one carbon metabolism. The availability of these vitamins affects the outcome of these reactions. An imbalance in status in any of these vitamins leads to an increase in fasting total plasma homocysteine concentration.

Two inter-dependent cycles are part of this network: the folate cycle and the methionine cycle. The folate cycle is the donor and receptor of C units converting dietary folate into active forms and also transporting methyl groups to methylation reactions involved in genes expression. Homocysteine is remethylated to methionine in the methionine cycle (1). Depending on the step of the cycle, a different form of folate is used.

The folate and methionine cycles intersect at the reaction in which methionine synthase (MTR) catalyses the conversion of homocysteine to methionine. The methyl group for this reaction is provided by the conversion of 5-methyltetrahydrofolate (5-CH_3THF) to tetrahydrofolate (THF). Cobalamin is a coenzyme for MTR and is essential for the transfer of the said methyl

group, provided by the folate cycle, for the remethylation of homocysteine to methionine.

Homocysteine is a biomarker of the 1C metabolism network status and it increases when there is low status of folate and other B-vitamins. Homocysteine is a precursor for the formation of S-adenosylmethionine (SAM), a methyl donor for some methylation reactions. SAM can be produced endogenously in the methionine cycle or from dietary methionine. SAM is involved in the post-translational modifications of proteins that can occur in histones and non-histones proteins. Homocysteine is also involved in the transulfuration pathway in which cystathione and then cysteine is produced.

In the folate cycle purines are formed from 10-formylTHF. 5,10-methylenetetrahydrofolate (5,10-CH_2THF) is converted to dihydrofolate (DHF) with the help of thymidylate synthase (TYMS) transferring 1C units to the reaction in which deoxyuridine monophosphate (dUMP) is converted to deoxythymidylate monophosphate (dTMP). These reactions are involved in the stabilisation of DNA (2).

Flavin adenine dinucleotide (FAD) is a cofactor for the enzyme methylene tetrahydrofolate reductase (MTHFR), responsible for catalysing the conversion of 5,10-CH_2THF to 5-methyltetrahydrofolate (5-methylTHF).

Impaired 1C metabolism has been associated with foetal developmental problems like neural tube defects (NTDs) (3,4), pregnancy complications like preeclampsia and adverse pregnancy outcomes (5,6), as well as cancer (7,8) and cardiovascular diseases (9–11), among others.

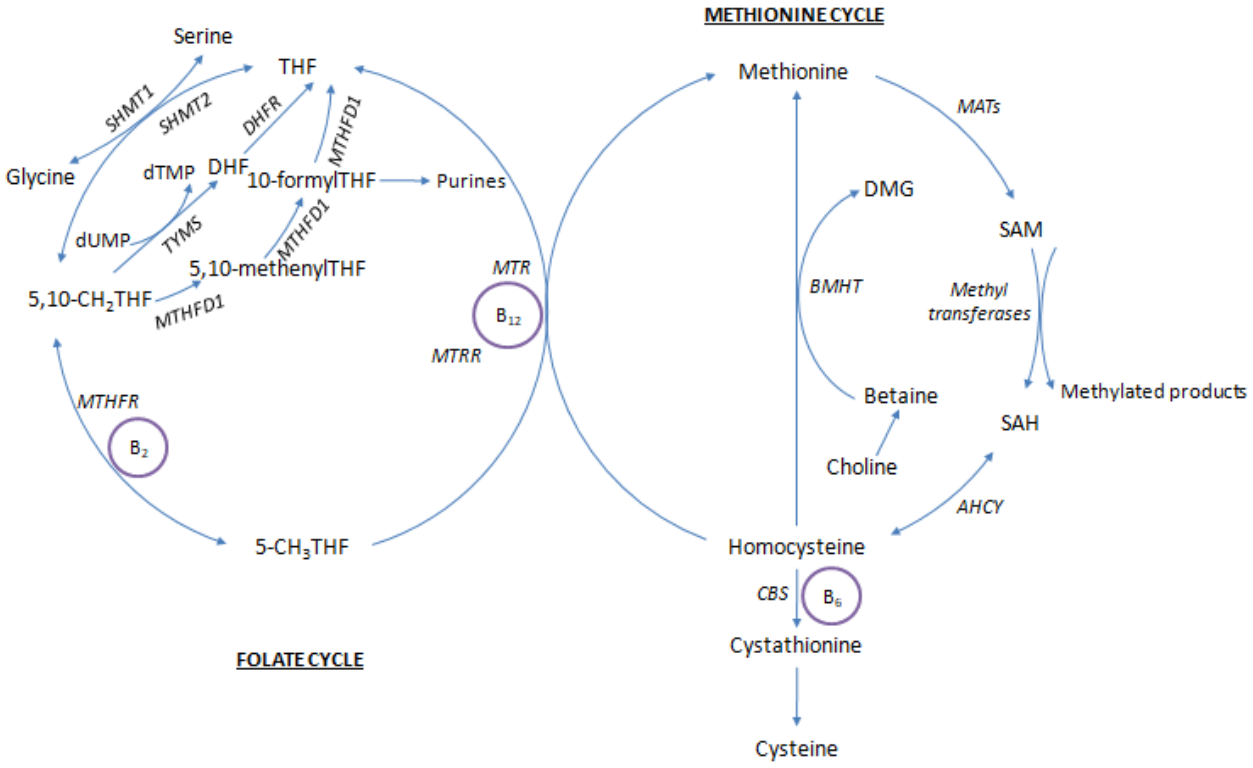

Figure 1. Folate and methionine cycles.
Abbreviations: AHCY, adenosylhomocysteinase; BHMT, betaine-homocysteine s-methyltransferase; DHFR, dihydrofolate reductase; DMG, dimethylglycine; MAT I & III, methionine adenosylmethyltransferases I & III; Met, methionine; MTHFD1, methylenetetrahydrofolate dehydrogenase; MTR, methionine synthase; MTRR, 5-methyltetrahydrfolate-homocysteine methyltransferase reductase; SAH, s-adenosylhomocysteine; SHMT, serine hydroxymethyltransferase. Adapted from (12).

2. Folate

Structure, characteristics and functions

Folate (also known as vitamin B_9) is a water-soluble vitamin from the B-vitamins group. It is composed of a pteridine ring attached to a p-aminobenzoic acid and an L-glutamic residue. The different compounds of the folate family vary in the length of L-glutamic residue, the oxidation of the molecule and number of carbons linked.

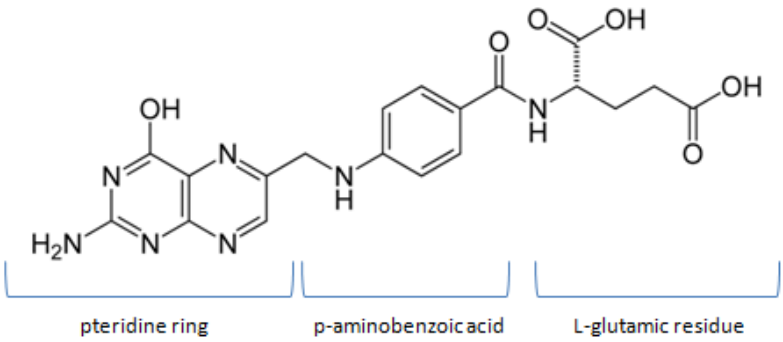

Figure 2. Structure of folic acid.
Adapted from (13).

The folate family consists of two different molecules: folate and folic acid. Natural forms of folate come from the diet and are found in nature in different forms, normally in the reduced and polyglutamate form. The most common form is 5-methylTHF (14). Folic acid (Figure 2), the monoglutamated and totally oxidized form is found in supplements and fortified foods. Folic acid is converted to 5-methylTHF in the human body. Folates in general are sensitive to oxidation by light, heat and oxygen during food processing. Folic acid is more stable and resistant to thermal degradation compared to food folate (15,16).

The principal functions of folate are nucleotide and amino acid synthesis, being involved in DNA synthesis and reparation. It plays a role in one carbon metabolism transferring methyl groups when needed and participates in DNA methylation, important in gene expression (17,18). Folate status is an important determinant of fasting plasma total homocysteine concentration (tHcy) (19). Folate deficiency has been identified as a major cause of hyperhomocysteinemia, thus being a risk factor for cardiovascular disease (CVDs) (20,21). Supplementation with folic acid reduces the risk of stroke (11,22) and cognitive impairment (23,24) among others.

Sources, bioavailability and requirements

Folate cannot be synthesised by humans and other mammals so it must be obtained from a variety of dietary sources. Foods rich in folate are liver, yeast, green leafy vegetables, fruits, nuts and seeds (25). Bread, potatoes, and dairy products have a low folate content but as they are highly consumed in our population, they contribute to the total folate intake (26). Main dietary sources of folate intake in Spain are vegetables (21.7-24.9%) and cereals (10.7-11.2%) (27). Folic acid comes from supplements and fortified foods. Vegetables were the main source of folate in the USA before fortification but after fortification the situation changed and bread and crackers were the main source, contributing to 15.6% of total intake (28). Countries like Finland, Ireland, Germany, France, Italy, Spain and Greece were reported to have moderate folate intake (250-350 µg/day). Norway, Sweden, Denmark and The Netherlands had low intake (<250 µg/day) (29). These differences may be due to the different dietary habits between Northern European diets and Mediterranean diets. Dietary intake of total folate increased in all groups of the NHANES study except in females older than 60 years. Mean dietary total

folate increased from 275 µg/day to 351 µg/day comparing NHANES III, conducted during 1988 to 1994 (prior to folic acid fortification), and NHANES 1999-2000, after fortification (28).

Intestinal hydrolysis of polyglutamate residues, food folate conjugation, intestinal folate absorption, food matrix, instability of labile folates before ingestion or during digestion and genetic variants are influencing factors of folate absorption that can modify folate bioavailability (13). The RDA (Recommended Dietary Allowance) is based on the daily needed amount of folate according to the Institute of Medicine (IOM) (25). Dietary Folate Equivalent (DFE) is the unit of measurement that considers the variation of bioavailability of food folate and folic acid from supplements and fortified foods. DFE is calculated as the quantity of food folate, plus the quantity of folic acid in the diet multiplied by 1.7 (30). Food folate bioavailability is lower than folic acid's (31–33). Folic acid bioavailability is 85% and food folate bioavailability is about 50% (34), so the factor of 1.7 is derived from the 85:50 ratio (35). Folate bioavailability is a relevant topic in countries without folic acid fortification where food folates are an important source of the vitamin (36).

Adequate intake of folic acid before pregnancy and during early pregnancy protects against NTDs (3,4,37). Czeizel et al. demonstrated that all women planning a pregnancy should take a vitamin supplement with folic acid because multivitamin supplementation containing 0.8 mg of folic acid during periconceptional period could prevent NTDs in women with no previous history of NTD-affected pregnancies (3). A study carried out in China found that taking periconceptional 400 µg/day of folic acid reduced by 85% the prevalence of NTDs in pregnancies of at least 20 Gestational Weeks (GW) (4).

Mandatory folic acid fortification of flour and cereal grain products began in January 1998 in the USA (38) and November 1998 in Canada (39). The aim was to increase folic acid intake in women of childbearing age in order to reduce the risk of NTDs in pregnancies (40,41). This policy consists of fortifying all products made from cereal grain flours with 140 µg folic acid/100 g of flour (38). As we can see in Figure 3, nowadays more than 75 countries have implemented this policy and the amount of folic acid added varies by country (42). The current recommended daily intake of folic acid supplements for women of reproductive age for reducing the risk of NTDs are 400 µg/day (40). The amount of folic acid in the fortification policy in the USA has not been increased in order to exceed the RDA of of 1000 µg/day for adults (25).

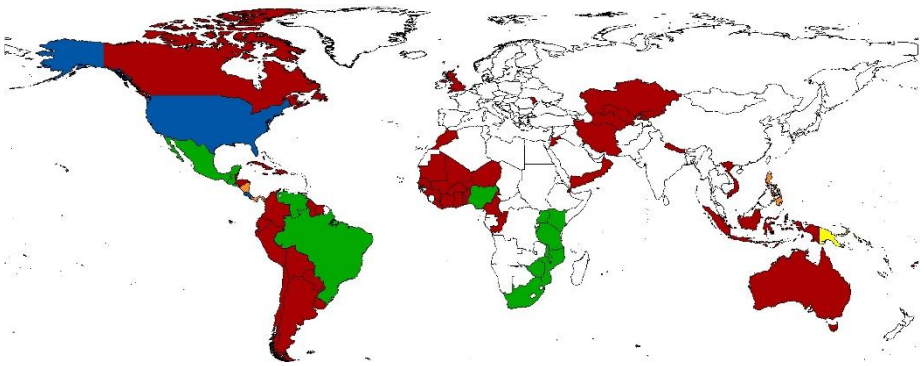

Figure 3. Countries with mandatory folic acid fortification.
Red countries have legislation for wheat flour alone, green countries for wheat and maize flour, orange countries have legislation for wheat flour and rice, blue countries for wheat and maize flour and rice (Costa Rica and the United States) and yellow country has legislation for rice alone (42).

Mandatory folic acid fortification increased serum and red blood cell (RBC) folate concentrations in the American population independently of age and sex. Serum folate increased from 11.4 nmol/L to 26.9 nmol/L, and mean RBC folate concentration increased from 375 nmol/L to 590 nmol/L. The optimal

RBC folate concentration to prevent NTDs is ≥906 nmol/L and only less than 10% of women of childbearing age achieved this value (28).

RDAs for folate during different life stages are shown in Table 1. For babies until 6 months of age and from 7-12 months the RDA is 65 and 80 µg/day respectively. For children from 1 to 3 years of age the recommendation is 150 µg/day DFE. From 4 to 8 years of age they need to increase the intake to 200 µg/day and from 9 to 13 years of age the RDA is 300 µg/day. In women and men over 14 years of age the RDA is 400 µg/day DFE (25). The IOM recommends that pregnant women from all ages should take 600 µg/day of DFE (25) but in Spain the RDA is 500 µg/day DFE (43) for the prevention of NTDs.

Table 1. Recommended Dietary Allowance of folate depending on life stage.

Life stage	**RDA (µg/day DFE)**
Babies until 6 months of age	65[1]
Babies from 7 to 12 months of age	80[1]
From 1 to 3 years old	150
From 4 to 8 years old	200
From 9 to 13 years old	300
> 14 years	400
Pregnant women	600
Women in lactation period	500

Abbreviations: DFE, Dietary Folate Equivalents; RDA, Recommended Dietary Allowance
[1]Adequate intake (25).

Absorption, transport and metabolism

Folate absorption and transport consists of different phases occurring in the intestine. As previously stated, mainly we find folates in the polyglutamated form but before being absorbed in the small intestine, polyglutamated forms of folate need to be deconjugated to monoglutamate forms by folate hydrolase 1 (FOLH1) in the intestinal brush border membrane (44). Only the monoglutamated form of folate can cross cell membranes. Folic acid is not capable of passing through biological membranes by simple diffusion, so there is a folate transport system for the regulation of normal folate homeostasis in the intestine. After this step they are ready to be transported to the circulation through the intestine. Monoglutamate folate can be absorbed through a saturated process in an acid pH or a nonsaturable absorption when folate concentrations are higher than 5-10 mmol/L in the intestine (45). The reduced folate carrier 1 (RFC1), also known as Solute Carrier family 19A member 1 (SLC19A1), and the proton coupled folate transporter (PCFT), also known as Solute Carrier family 46A member 1 (SLC46A1) are two different soluble mechanisms of carrier-mediated transport of folate (44), both found in the apical membrane of the intestine (46). RFC1 has low affinity for reduced folate and even lower affinity for folic acid but the PCFT has a high affinity for folate and folic acid (46). The RFC1 acts in neutral pH and transports folate to all body tissues. The PCFT transports folate in the duodenum and jejunum and works in acidic pH (44). The optimum pH for folate transport is 5 (45), so PCFT predominantly transports folate to enterocytes (46). Folic acid from supplements or fortified foods, is mostly reduced to 5-methylTHF. Some folic acid is also found as free folic acid (unmetabolised) circulating in the blood. Plasma folate, 5-methylTHF in particular, is bound to low-affinity proteins. 50% of folate is

bound to albumin. Little folate is bound to high-affinity folate receptors. When folate is transported to the different body tissues, the RFC1 is responsible for its transport because of its pH (pH= 7.4) (44).

Monoglutamated forms are found in plasma and urine but tissue folate is predominantly found in the polyglutamated form. To be kept inside the cell, folate needs to be transformed again to the polyglutamated form by folylpoly-gamma-glutamate synthetase (FPGS) (47). Red Blood Cell (RBC) folate is commonly found as 5-methylTHF polyglutamates. This form of folate is used in the remethylation of homocysteine to methionine by MTR and its cofactor cobalamin producing tetrahydrofolate (THF). THF can be converted to 5,10-methyleneTHF by methylenetetrahydrofolate dehydrogenase (MTHFD1) needed for purine synthesis. THF can also be produced in the reduction of DHF by DHFR enzyme. 5,10-methyleneTHF is reduced to 5-methylTHF in a reaction catalysed by the MTHFR enzyme. 5-methylTHF acts as the methyl donor needed for the remethylation by MTR. MTHFR is the most important reaction in the folate cycle because it is irreversible and it is the only enzyme that generates 5-methylTHF needed for DNA synthesis and methylation (48).

Folate status and deficiency

Serum or plasma folate status reflects recent folate intake (in a defined moment of time) as well as folic acid supplement use. It is the most used method for evaluating folate status (49). RBC folate indicates long term folate status (approximately 120 days) reflecting folate reserves. During erythropoiesis, erythrocytes concentrate folate but when they reach maturity

they cannot discharge folate, so they store it (18). It is very important in the first stages of life development.

Originally folate deficiency was considered when folate status was <7 nmol/L (50). Now the accepted cut-off for folate deficiency is <10 nmol/L (51). The WHO also adheres to this threshold (52). For RBC folate the cutoff for folate deficiency was <340 nmol/L (52). For the prevention of NTDs the WHO recommends RBC folate status of >906 nmol/L in women of childbearing age (53).

Folate deficiency is prevalent in some countries with no mandatory folic acid fortification. In a Spanish cross-sectional adult population study, the prevalence of folate deficiency was 18.8% overall and 24.2% in women of fertile age (54). A cross-sectional study of a Mediterranean population found that approximately 50% of healthy women of childbearing age had RBC folate concentrations lower than 305 nmol/L and all of them were <906 nmol/L (the limit for the prevention of NTDs). Surprisingly in this study, 51% of these women reported taking supplements (55). Folate deficiency is very low in countries with folic acid fortification. Countries with mandatory folic acid fortification like the USA have experienced an important change in folate status and deficiency before and after folic acid fortification. Prevalence of low serum folate status decreased from 21% to <1% and low RBC folate status from 38% to 5% before and after fortification respectively (56). In the NHANES 2007-2012, 22.8% of women had suboptimal RBC folate status (57). Low folate status may be caused by low dietary intake, poor absorption of folate and impaired folate metabolism due to genetic defects or drug interactions (58). Inadequate folate intake has been associated with impaired DNA synthesis, repair and methylation leading to diseases such as neural tube defects (3,4), megaloblastic anemia (59), preeclampsia and other adverse

pregnancy outcomes (5,6), cancer (7,8), cardiovascular diseases (10,11) among others (60). Some studies suggest a protective effect of folate against the risk of NTDs (4,37). Folate metabolism is affected by B-vitamin deficiencies and genetic polymorphisms such as *MTHFR* 677 C>T.

Folate, homocysteine and MTHFR 677 C>T polymorphism

The MTHFR enzyme is a key enzyme in 1C metabolism because it affects the provision of 1C units for nucleotide synthesis and the products of the reaction synthesise methyl groups for some biological processes. The *MTHFR* 677 C>T polymorphism is a mutation located at base position 677 of the gene encoding the MTHFR enzyme. It results in the substitution of cytosine for thymine leading to a valine instead of an alanine in the codon 222 (61). This produces a thermolabile enzyme with reduced activity *in vitro* (61). The *MTHFR* C allele has higher enzyme activity than the T allele (61). The reduced activity of the enzyme results from the inappropriate loss of its flavin adenine dinucleotide (FAD) cofactor (62) and this leads to elevated tHcy status (61). The MTHFR enzyme catalyses the reduction of 5,10-methyleneTHF to 5-methylTHF helped by the coenzymatic form of riboflavin called FAD (Flavin Adenine Dinucleotide), cofactor of the MTHFR enzyme. This reaction is irreversible and is required for the conversion of homocysteine to methionine in the remethylation pathway (48). The binding affinity of the FAD cofactor and the MTHFR enzyme is weaker in the presence of mutant *MTHFR* (63) but high folate status compensates this situation (62,63). Riboflavin is an independent predictor of tHcy (64,65) but only in people with the TT genotype (64). The effect of the *MTHFR* 677C>T polymorphism on tHcy also depends on riboflavin status (66). Supplementation with riboflavin stabilizes

the variant enzyme and helps to maintain adequate levels of folate, thus preventing increased homocysteine. Carriers of the TT genotype are more sensitive to riboflavin status than those with the CC or CT genotypes (67).

A study of more than 7000 newborns from 16 different areas of the world found that the prevalence of the variant form of this polymorphism (*MTHFR* 677 TT) varies between countries and ethnicities. They found that the USA and Canada had the lowest prevalence (5-10% approximately and 5.8% respectively) compared with Italy (26.4%) and Mexico (32.2%) that had the highest (68). In Spain the prevalence of the homozygote variant was reported to be 18.1% in a representative sample of an adult population from Southern Catalonia (54) and 11.8% in the Wilcken et al. study (68).

Folate is the major determinant of tHcy concentrations (65). Low folate status is associated with elevated fasting tHcy (69,70). Homocysteine metabolism is affected by both environmental and genetic factors. tHcy concentrations increase with age and are higher in men than in women (65,71). Riboflavin status has also been associated with tHcy (65). Independently of the level of folate intake, people with the *MTHFR* 677 TT genotype have lower plasma folate status than those with the CC or CT genotypes. When plasma folate status is high, tHcy status is similar among all of the genotypes (72).

tHcy is a risk factor for CVD (73), adverse pregnancy outcomes (6) and other diseases. Reducing tHcy concentration by 25% lowers the risk of ischemic heart disease and stroke by 11 and 19% respectively (74). Supplementing with folic acid helps to reduce tHcy concentrations (75). Higher tHcy concentrations were associated with male sex and older age (65,71). Smoking, high BP, elevated cholesterol status and lack of exercise are other factors related to elevated plasma tHcy (71). Smoking and drinking were

positively associated with increased plasma tHcy concentrations (76) but another study reported that smoking and coffee were positively associated but in case of alcohol the association was negative (77). McNulty et al. reported that elevated tHcy levels were limited to people with the *MTHFR* 677 TT genotype with poor riboflavin status (67) and that supplementation with riboflavin (1.6 mg/d) for 12 weeks decreased tHcy concentrations (78).

Following mandatory folic acid fortification in the USA, folate status was improved and tHcy reduced. Mean tHcy in adult participants decreased from 10.1 to 9.4 µmol/L before and after fortification respectively in the Framingham Offspring Study cohort and the prevalence of high tHcy considered as tHcy >13 µmol/L was reduced from 18.7% to 9.8% (79).

The *MTHFR* 677 C>T genotype was an independent predictor of plasma folate, RBC folate and plasma homocysteine concentrations in Northern Chinese women of childbearing age (80). The TT genotype was the most common genetic cause of elevated tHcy in humans in a Dutch cohort (aged 20-65 y) (72). Folate status was lower in the *MTHFR* 677TT and *SLC19A1* 80AA genotypes compared with wildtype genotypes in Spanish adults aged 18-75 years (54). Serum folate and RBC folate levels were lower and plasma tHcy was higher in people with the *MTHFR* 677 TT genotype compared to the CC genotype in different populations (61,80–84). A publication in the Framingham Offspring Study cohort showed that supplementing enriched grain products with folic acid improved folate concentrations and decreased homocysteine (79). TT genotypes had higher plasma Hcy levels in CAD patients compared with CC and CT genotypes (85). A trial with women of childbearing age taking different doses of folic acid supplements (100, 400, and 4000 µg/day and 4000 µg/week) found that the *MTHFR* 677 TT genotype was associated with lower folate concentrations compared to the CC and CT

genotypes, at all doses. Doses of 100 µg/day or 4000 µg/week of folic acid did not reduce high tHcy concentrations in people with the *MTHFR* TT genotype (80).

Folate and homocysteine in pregnancy

Folic acid supplementation during pregnancy protects against folate sensitive NTDs (3,37). This led to the recommendation to take 400 µg/day of folic acid during preconception and the first trimester of pregnancy to women planning to get pregnant (40). The USA and Canada implemented the mandatory fortification of flour with folic acid in 1998 with the aim of the reducing of the incidence of NTDs (38,39). In Spain the RDA for pregnant women is 500 µg/day (43). In the USA and Canada it is 600 µg/day (25).

Folate is crucial during pregnancy for fetal growth and cell replication. If women do not take folic acid supplements during pregnancy, serum and RBC folate decrease remarkably as pregnancy progresses (86–88). Folate deficiency, as well as megaloblastic anemia, can be prevented through folic acid supplementation (86). Before 12 GW to 15 GW of pregnancy plasma folate increases (89), it increases only in the case of women with low plasma folate status (87,90). After 15 GW plasma folate decreases (87,89). During gestation maternal folate status decreases due to foetal folate requirements as well as placental development and maternal tissue development. Plasma folate drops due to the physiological changes associated with pregnancy such as haemodilution, increased renal function and maintenance of the materno-placental-foetal unit (91).

Folic acid supplementation during pregnancy is associated with lower tHcy status compared to no supplementation (91,92). In case of stopping folic acid

supplementation in the Reus-Tarragona Birth Cohort (RTBC) plasma folate concentrations decrease as do RBC folate concentrations, but these decrease later (87). Taking supplements of 400 µg/day of folic acid during the 2nd and 3rd trimesters of pregnancy increase maternal RBC folate and cord blood folate status compared to unsupplemented mothers. As a result, maternal folate concentrations remain stable and the natural rise of homocysteine levels in late normal pregnancy does not occur (90). Cord blood folate is higher than that of the mothers at labour (87,93).

Different studies have found an association between high maternal tHcy and pregnancy complications. High tHcy status or hyperhomocysteinemia is associated with lower birth weight than babies born to mothers with normal homocysteinemia (92), small for gestational age (94) and preeclampsia (95,96), among others. Plasma folate was inversely associated with tHcy in pregnancy (87). RBC folate and plasma cobalamin concentrations were negatively associated with maternal homocysteine (97). Maternal plasma tHcy was inversely associated with birth weight independently of maternal weight, height, GW at labour and baby's sex (97). A previous study from our group showed that tHcy decreases from preconception to mid pregnancy approximately. On the contrary in late pregnancy it increases in pregnant women who do not take folic acid supplements (91,92). The decrease in tHcy that takes place in early pregnancy is not due to folic acid supplementation, hemodilution or serum albumin decrease, hence it is hypothesised to be related to a physiological effect of pregnancy itself. However, in a study carried out by our research team, folic acid enhanced the reduction because it was greater in mothers who take folic acid supplements (91). As Spain has no mandatory folic acid fortification, this study helped to investigate tHcy variation during pregnancy. In another study by our group, plasma folate was

inversely associated with tHcy throughout pregnancy in the mothers with folate status below the median (14.1 nmol/L) during the first trimester of pregnancy. However in mothers with plasma folate status above the median in the first trimester, due to regular folic acid supplement use or use of supplements >400 µg/d, there was no association between plasma folate and tHcy while supplementation lasted (87). Pregnant women have 50-60% lower tHcy concentrations compared to non-pregnant women (98). tHcy concentrations at preconception and labour were very similar in the unsupplemented group in the Pre-C study in Spain (92). Fetal cord tHcy was significantly lower than tHcy at labour in two studies in Spain and in Ireland (92,99).

Birth weight (BW) is regularly used in perinatal epidemiology as an indicator of health status of the babies. Folic acid supplementation is positively associated (100) and tHcy negatively associated with birth weight with (92,101). Babies born to mothers in the highest tertile of tHcy had lower birth weight compared to babies with mothers in the lowest tertile of tHcy (92). Low maternal folate status in pregnant women was associated with higher body mass index (BMI) in the offspring (102).

3. Riboflavin

Structure, characteristics and functions

Riboflavin (also known as vitamin B_2) is an essential and water-soluble vitamin from the B-vitamin group.

In 1916, McCollum thought that 2 factors composed the diet: fat-soluble A present in foods like butter fat or fish oil and water-soluble B found in milk, egg yolk and wheat embryo. In 1926, Goldberger and others discovered that pellagra was associated with a lack of vitamins and they thought that this yellow pigment was useful for preventing pellagra so they called it anti-pellagra factor. He thought it was the same water-soluble component that McCollum discovered. One year later, the British Committee on Accessory Food Factors defined vitamin B_2 as the most heat-stable component of the anti-pellagra factor. In 1932 Warburg and Christian extracted a yellow enzyme from yeast, nowadays known as riboflavin. In the next year riboflavin was isolated from different sources and was given its name depending on the source ovoflavin (from eggs), lactoflavin (from milk), hepatoflavin (from liver) and uroflavin (from urine). A few years later, in 1937 Theorell discovered flavin mononucleotide (FMN) and in 1938 Warburg and Christian discovered the structure of flavin adenine dinucleotide (FAD). The essential nature of the vitamin as a food constituent for man was shown in 1939 (103).

The molecule of riboflavin is composed of an isoaloxazine ring attached to a ribitol as a side chain (104). There are 2 coenzymes formed from riboflavin. The first is riboflavin-5'-phosphate, also called FMN. Flavokinase is the enzyme responsible for the phosphorylation reaction from riboflavin to FMN. The second coenzyme is FAD, synthesized from FMN. The enzyme in charge

of this reaction is FAD synthase, that adds adenosine monophosphate (AMP) to FMN (104). FAD is the predominant form of riboflavin found in the body.

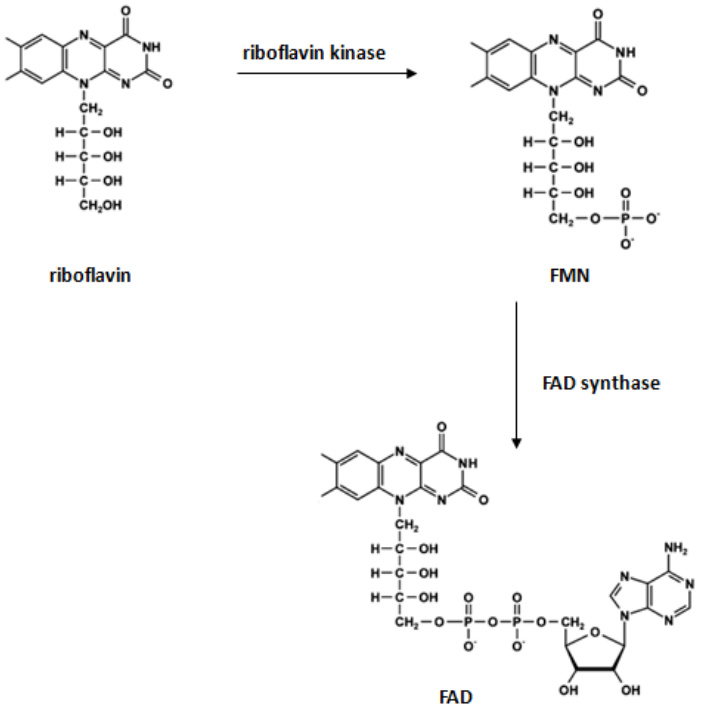

Figure 4. Structure of riboflavin and its cofactors FMN and FAD.
Adapted from (104).

The principal functions of riboflavin cofactors FMN and FAD are in oxidation-reduction (redox) reactions and in energy metabolism reactions such as the respiratory chain, metabolism of fats, ketone bodies, carbohydrates and proteins (104). Riboflavin is an antioxidant nutrient that prevents lipid peroxidation and reperfusion oxidative injury. Riboflavin also plays an important role in 1C metabolism, as the MTHFR enzyme uses FAD as a cofactor and the TT genotype has reduced enzyme activity due to the loss of the FAD cofactor (63). It is also involved in the synthesis and activation of some vitamins like pyridoxine, niacin and folate.

Sources, bioavailability and requirements

Riboflavin is mostly found in meat, poultry, animal viscera, fish, eggs, milk and dairy products like cheese. Broccoli and other green vegetables are other sources of riboflavin (105). In Western diets milk, dairy products and meat are the main source of riboflavin (106). Riboflavin content from different food sources is shown in Table 2.

Table 2. Riboflavin amount in food sources.

Food sources	Riboflavin (mg / 100 g)	Food sources	Riboflavin (mg / 100 g)
Liver	2.6	Yoghurt	0.26
Foie-gras and pâtés	0.85	Beef	0.22
Roquefort cheese	0.7	Spinach	0.19
Almonds	0.67	Whole milk	0.18
Sardine	0.4	Lamb	0.16
Eggs	0.33	Peas	0.15

Adapted from (107).

Natural grain products have low amounts of riboflavin, so some countries decided to fortify bread and cereals to increase riboflavin status (106). In the 1940s, mandatory restoration of riboflavin lost during milling to flour with riboflavin was implemented in the United States.

Even though plants and many microorganisms are able to synthesize riboflavin, animals and humans cannot synthesize water-soluble vitamins and must obtain riboflavin from exogenous sources such as diet. Riboflavin is not stored in the body, so there is a need to consume it every day. If there is an excess of riboflavin in the body it is excreted in the urine (104). Urinary

excretion of riboflavin is in the form of free riboflavin. Another source of riboflavin can be through intestinal microbiota, leading to homeostasis of riboflavin status (108).

The predominant available form of riboflavin in food is FAD. FMN is found in a lower proportion. Both are mostly in a non-covalent bound form because in case of being covalently bound they cannot be absorbed (109). Free riboflavin is scarcely found in food but milk and eggs contain it bound to specific binding proteins (110). Dainty et al. wanted to quantify the bioavailability of riboflavin from milk and spinach using stable-isotope labels with urine and plasma samples and they found that the amount of flavins absorbed from milk or spinach was approximately 60-65% (111). The maximum riboflavin absorbed in a meal or dose is about 27mg, with an estimation of bioavailability of 95% of food flavin (112). The bioavailability of riboflavin also depends on the way of cooking and processing food. Riboflavin is heat-stable but it is light-sensitive, so it can be lost due to UV light exposure. Food containing riboflavin should not be stored in transparent containers. For example when milk was sold in glass bottles, flavins could be degraded, so to avoid this problem it is important that these products are inside an opaque container (104).

The RDA for riboflavin is 1.4 mg/day for adults (113), obtained eating a balanced diet. The requirements of riboflavin dietary intake depending on sex and life stage are shown in the table below. Older people need to consume higher quantities of riboflavin. For babies until 6 months of age and from 7-12 months the RDA is 0.3 and 0.4 mg/day respectively. Children from 1 to 3 years of age the estimated RDA is 0.5 mg/day of riboflavin. From 4 to 8 years of age they need to increase 0.1 mg/day their intake and from 9 to 13 years of age the RDA is 0.9 mg/day. From 14 years of age onwards, the

recommendation is different for women and men. Women from 14 to 18 years of age are recommended to have a riboflavin intake of 1.0 mg/day and from 19 to >70 years of age 1.1 mg/day. Men from 14 to >70 years of age need to consume 1.3 mg/day of riboflavin (114). During pregnancy and lactation there is a higher requirement of vitamins, so the intake is higher than in any other life stage. The RDA for pregnant and lactating women is 1.4 and 1.6 mg/day, respectively (114).

Table 3. Recommended Dietary Allowance of Riboflavin depending on sex and life stage.

Life stage	**RDA (mg/day)**	
	Women	**Men**
Babies until 6 months of age	0.3	0.3
Babies from 7 to 12 months of age	0.4	0.4
From 1 to 3 years old	0.5	0.5
From 4 to 8 years old	0.6	0.6
From 9 to 13 years old	0.9	0.9
From 14 to 18 years old	1.0	1.3
From 19 to 30 years old	1.1	1.3
From 31 to 50 years old	1.1	1.3
From 51 to 70 years old	1.1	1.3
> 70 years old	1.1	1.3
Pregnant women	1.4	-
Lactating women	1.6	-

Abbreviations: RDA; Recommended Dietary Allowance (114).

Ariboflavinosis is the name given to riboflavin deficiency. Although riboflavin deficiency is not very common, it can be found in populations of low socioeconomic status which have a low intake of meat and dairy products (115–118). Surprisingly, some populations in countries like the United Kingdom (119), the USA (120) and France (121) have unexpectedly observed a higher proportion of cases of riboflavin deficiency. It is important to have an

adequate daily intake of riboflavin. The Erythrocyte Glutathione Reductase Activation Coefficient (EGRAC) is a functional indicator of riboflavin status (described below) and increasing EGRAC indicated worsening riboflavin status. EGRAC is affected by riboflavin intake, and decreased when women aged 19-25 years old from the United Kingdom were supplemented with 2 or 4 mg of riboflavin during 4 or 8 weeks. When the supplementation was interrupted, EGRAC returned again to baseline values (122). Riboflavin supplementation increases riboflavin status, so in consequence EGRAC decreases (123)(124).

Some consequences of aribоflavinosis are sore throat, cheilosis, hyperaemia, edema of oral and mucous membranes, angular stomatitis and glossitis (113). Other symptoms of the deficiency are anemia (125), seborrheic dermatitis, swollen tongue and impaired nerve function (113).

Absorption, transport and metabolism

Riboflavin is absorbed in the small intestine. As riboflavin is mainly found as FMN and FAD, they must be hydrolysed to free riboflavin before the absorption by intestinal phosphatases in the enterocytes. Primary absorption of riboflavin consists of a saturable ATPase active transport system. The capacity of absorption increases when riboflavin is increased in the presence of food and bile salts. In human blood some riboflavin is bound to albumin and other proteins as immunoglobulins. A little amount of riboflavin circulates via the enterohepatic system (104).

Riboflavin measurement: functional method

The gold standard method of measuring riboflavin status is the EGRAC assay. This functional method consists of the reduction of the oxidized form glutathione (GSSG) to the reduced form (GSH). The enzyme involved in this reaction is glutathione reductase (GR), an enzyme that is FAD-dependent and is released from the membrane of washed red cells. As this enzyme needs FAD to be activated, samples are analysed in duplicate adding or not exogenous FAD. EGRAC is calculated as the ratio between stimulated (with exogenous FAD added) and non-stimulated (without exogenous FAD added) glutathione reductase activity, as we can see below (126). NADPH is oxidized to $NADP^+$. The change of colour produced from this reaction is measured by using a spectrophotometer set at 340 nm.

$$GSSG + NADPH + FAD \xrightarrow{GR\text{-}FAD} GSH + NADP^+ \quad \textit{Stimulated}$$

$$GSSG + NADPH \xrightarrow{GR\text{-}FAD} GSH + NADP^+ \quad \textit{Non-stimulated}$$

If the EGRAC value equals 1, it means that the enzyme has enough FAD to react and it is saturated. Higher values of EGRAC mean that the enzyme needs more FAD to be saturated. The higher the value of the EGRAC assay is, the lower the riboflavin status. There is not an established criteria for the values of this functional method. Originally, an optimal riboflavin status was considered for EGRAC <1.2, values between 1.2 and 1.4 indicated marginal status and EGRAC >1.4 indicated riboflavin deficiency (104). Nowadays some studies have used another cut-off classifications for measuring riboflavin status. Values lower than 1.3 are considered an optimal riboflavin status,

between 1.3 and 1.4 suboptimal status and values higher than 1.4 mean riboflavin deficiency (127).

The improvement of riboflavin status provoked an increase in the number of circulating red blood cells and haemoglobin concentration, the lower the riboflavin status was at the beginning of the study, the higher was the increase in red blood cell number or hemoglobin concentration in a study with 123 women with riboflavin deficiency (EGRAC> 1.40) randomly assigned to receive 2 or 4 mg riboflavin or a placebo for 8 weeks (122).

4. Blood pressure

Introduction to blood pressure: Definitions and classification

Blood pressure (BP) is the pressure the exerted against the walls of the arteries as blood flows throughout the body. BP regulation is complex as it depends on different factors such as some physiological systems, organs, hormonal signals and vasculature. BP changes depending on the time of day and the heart's needs (128).

BP is estimated as systolic and diastolic blood pressure. Systolic blood pressure (SBP) is the maximum pressure that blood applies to the artery walls during a heartbeat or contraction called systole. Diastolic blood pressure (DBP) is the minimum pressure that blood applies to the artery walls when the heart is relaxed between beats or contractions, also called diastole (128). All BP measurements are given as SBP/DBP mmHg. SBP is always the highest number and DBP the lowest. Both SBP and DBP values are important as they are indicators of human's health and the proper and efficient functioning of vital organs (129). There is a minimum blood pressure needed for optimal functioning of the body and its vital organs. SBP should be be 90 mmHg or higher and DBP 60 mmHg or higher, it can vary depending on the person (130).

Table 4. Classification for BP in adults.

	SEC (131)		ESC/ESH (132)(133)		WHO (129)		AHA (134)		CDC (135)	
BP Category	**SBP**	**DBP**	**SBP**	**DBP**	**SBP**	**DBP**	**SBP**	**DBP**	**SBP**	**DBP**
Optimal	< 120	< 80	< 120	< 80						
Normal	120-129	80-84	120-129	80-84	<120	< 80	<120	< 80[1]	<120	< 80
High normal	130-139	85-89	130-139	85-89						
Prehypertension	-	-	-	-	120-139	80-89	-	-	120-139	80-89
Elevated							120-129	< 80[1]		
Hypertension stage 1	140-159	90-99	140-159	90-99	≥140	≥90	130-139	80-89[2]	-	-
High	-	-	-	-	-	-	-	-	≥140	≥90
Hypertension stage 2	160-179	100-109	160-179	100-109	-	-	≥140	≥90[2]	≥160	≥100
Hypertensive crisis (or grade 3)	≥180	≥110	≥180	≥110	-	-	≥180	≥120[1,2]	-	-

Abbreviations: AHA, American Heart Association; BP, Blood Pressure; CDC, Centers for Disease Control and Prevention; DBP, Diastolic Blood Pressure; ESC, European Society of Cardiology; ESH, European Society of Hypertension; SEC, Sociedad Española de Cardiología; SBP, Systolic Blood Pressure; WHO, World Health Organization. The Spanish Guidelines are based in the European Guidelines of the ESC/ESH (131,136). Adapted from (137). [1]and, [2]or.

BP is classified depending on SBP and DBP measurements. As shown in Table 4 there is not a global consensus in BP classification, there are some differences depending on the organization criteria. In general normal BP is defined when BP is 120/80 mmHg or lower. SBP values between 120-139 mmHg and DBP 80-89 mmHg are considered to indicate prehypertension, occurring in people at high risk of developing hypertension. In case of the European Society of Cardiology (ESC) classification when SBP is 130-139 mmHg and DBP is 85-89 mmHg BP is considered high-normal. SBP between 120-129 and DBP <80 mmHg is considered elevated in case of the American Heart Association (AHA). Values from 140 to 159 mmHg of SBP or 90-99 DBP are considered stage 1 of hypertension (SBP 130-139 and 80-89 in case of the AHA) and measurements higher than these are considered stage 2 of hypertension. Grade 3 hypertension (or hypertensive crisis for the AHA) consist of values of SBP higher than 180 mmHg and DBP higher than 110 mmHg in case of the ESC or 120 mmHg in case of the AHA). High blood pressure or hypertension is when your BP is repeatedly too high. Hypertension is defined when SBP is equal or higher than 140 mmHg and DBP is equal or higher than 90 mmHg (≥140/90 mmHg) (129). The Spanish Guidelines are based on the European Guidelines of the ESC/ European Society of Hypertension (ESH) (131,136).

Depending on the aetiology, there are 2 types of hypertension: essential or primary hypertension and secondary hypertension. Essential hypertension is idiopathic, there is not an identifiable cause for the disease but it is thought that it is due to a combination of genetics and other lifestyle factors. Secondary hypertension is caused by other diseases that can lead to hypertension such as chronic kidney disease and renal artery stenosis (138).

About 90% of the cases of hypertension are essential hypertension and approximately 10% are secondary hypertension (139).

Prevalence of Cardiovascular Diseases and Hypertension

Cardiovascular diseases (CVDs) are the leading cause of death (140). Every year approximately 17 million people die due to CVDs (141) (ischaemic heart disease and stroke provoked 15.2 million deaths in 2016) (140). Unfortunately about 80% of these CVD deaths occur in low and middle income countries (141,142).

Hypertension is one of the most important risk factors for mortality. In 2010 there were 9.4 million deaths caused by hypertension (143). Forty five percent of ischemic heart disease deaths and 51% of stroke deaths are caused by hypertension (141). The prevalence of hypertension is constantly increasing (probably due to ageing, the growth of the world's population and other risk factors) (129). From 1980 to 2008 the cases of hypertension increased from 600 million to 1000 million (144). It is expected that in 2025 the prevalence will increase by 60% compared to the year 2000 when more than a quarter of the population was suffering this disease (142). The prevalence worldwide was 22% in adults over 18 years of age in 2014 (145). The number of cases of hypertension varies between the different countries. Limiting the references to studies with a representative population, from 2009 to 2010 the prevalence of hypertension in the adult population in the USA was 29.5% (146). Data in 2013 showed that there were 78 million adults with hypertension (147). The prevalence was 30% in the UK (148) and 29.6% in 2009 in China (149). Data from 2 Spanish studies found that the prevalence

of hypertension of the adult population in Spain ranged from 33.3% to 42.6% (150)(151).

Since hypertension is usually an asymptomatic disease, not everyone is aware of their condition and this makes it difficult to detect. In the NHANES study from 2009 to 2010 the 74% of adults with hypertension reported being aware of having the disease. Consequently the 26% were unaware, representing a high percentage of people (146). The variability in the awareness by people with the disease depends on the country and type of population. In a Chinese study 41.6% were aware (152), but in the Spanish population the grade of awareness was higher, at 59.4% (151). As mentioned before, most of the CVD and hypertension cases occur in developing countries maybe due to an increased risk of exposure to adverse factors and a lack of control and awareness of these. The main inconvenience in these countries is the scarcity of data available regarding hypertension and not enough resources for a better control of the disease. In low income countries awareness is lower than in other countries and the prevalence is different between urban and rural zones (48.4% and 31.2% respectively)(153).

Established risk factors for hypertension (modifiable and non-modifiable)

Hypertension is a multifactorial disease. Modifiable and non-modifiable factors can affect hypertension. The modifiable factors are lifestyle factors such as diet, smoking, alcohol consumption, obesity, physical activity among others. The non-modifiable risk factors are sex, age, ethnicity, family history of hypertension and genetic factors (129,132,139). Changing the modifiable risk factors and improving lifestyle can help to prevent hypertension.

As different studies have demonstrated, hypertension varies between sexes. Hypertension is more prevalent in men than in women (150,154), although this varies between populations. According to the US NHANES study, it affects 30.2% of men and 27.7% of women (154) and a Spanish study reported 49.9% in men and 37.1% in women (150) However, postmenopause, the prevalence in women is higher than in men (155). As age increases, the prevalence of hypertension also increases (156,157). It was reported to be 7.5% of people aged 18-39 compared with 33.2% of people aged 40-59 and 63.1% of people aged 60 years or more in a study in the United States (154). In a Spanish study the prevalence of hypertension in people between 61-75 years of age was 75.4% (72.5-78.4) and in those older than 75 years was 88.7% (85.6-91.8) (150). Hypertension prevalence was higher in non-Hispanic blacks compared to the other ethnicities in the USA (146)(154). The prevalence in non-Hispanic blacks was 40.3% compared with non-Hispanic whites, non-Hispanic Asians and Hispanics with prevalences of 27.8%, 25.0% and 27.8%, respectively (154).

Heavy smoking is associated with increased blood pressure (158). A Japanese study showed that 24-hour ambulatory BP was significantly lower during the nonsmoking period than in the smoking period and that BP decreased a week after smoking cessation (159). A reduction in alcohol consumption reduces ambulatory systolic BP (160). BMI is positively associated with BP (161). Lack of physical activity is responsible for 5-13% of the risk of developing hypertension. Fitness is associated with lower risk of having hypertension in future life, demonstrating that physical activity is very important for the prevention of hypertension (162).

Measurements

There are two principal ways of measuring BP, office and the out of office measurements. The office or clinic BP measurement is the classical and common way to measure BP in the clinical setting and is carried out by a trained health professional with a sphygmomanometer or an electronic BP monitor (137). This procedure is described in detail in the *Methods* section. Out-of-office measurements are classified as: home BP measurements and Ambulatory Blood Pressure Monitoring (ABPM). In this thesis the focus for out of office BP measurements will be on ABPM. It is a technique that consists of repeated BP measurements during one day at defined intervals of time, normally every 15-30 minutes during the day, and every 30-60 minutes during the night time. It gives information over 24h, day-time BP and night-time BP (163). All the steps needed for the correct measurement of this technique are explained in detail in the Me*thods* section.

BP is variable, it can undergo short-term and long-term variability. Short-term variability is due to fluctuations over 24h including beat-to-beat, minute-to-minute, hour-to-hour, and day-to-night changes which can be detected with ABPM. Long-term variability consists in oscillations in more prolonged periods of time as days, weeks, months, seasons or years detected with office BP, home BP or ABPM (164). Long-term variability can be caused by a poor BP control in treated patients like inadequate treatment, poor adherence and use of an inappropriate methods to measure BP (165).

In both methods (office BP and ABPM), it is extremely important to measure BP carefully and properly because one mistake can lead to poor diagnosis of patients. The principal limitation of office BP compared to ABPM is that office BP consists of one measurement at a precise moment of time and it is not

always representative of real BP because it is obtained under circumstances that can stress or influence the patient like being in the doctor's office, a phenomenon known as the white-coat effect. Other limitations are that it has a higher risk of error because of human characteristics and variability (137). On the contrary, ABPM method obtains multiple measurements during 24h improving the accuracy of the values and making it easier to follow up BP and detect variation between readings (163). The ABPM method is proposed to be the gold standard in the diagnosis of hypertension, reducing the cases of misdiagnosis and saving medical costs (163). Healthcare costs are reduced using ABPM compared to office BP and home BP (166). In fact in 2011 the National Institute for Health and Clinical Excellence (NICE) Guidelines in the United Kingdom recommended ABPM measurements for hypertension diagnosis as a result of the accurate diagnosis (reducing misdiagnosis), the adoption of the most suitable treatment and the cost-effectiveness of the technique (167). Countries like Italy, Germany, the United States or China subsidise the use of ABPM for BP measurement (163). In United States ABPM is the selected method for hypertension diagnosis and treatment in adults in primary care (166). The principal advantages of ABPM are the high number of readings that can be obtained during 24h, the reproducibility of 24h mean values, daytime and night time BP (it is important to take into account that BP measurements during night time can only be obtained with ABPM), diagnosis of white-coat hypertension and masked hypertension (reducing false negatives) in treated and untreated people, interpretation of BP profiles, diagnosis of nocturnal hypertension and dipping patterns, evaluation of 24h BP variability, evaluation of antihypertensive medication efficacy and detection of excessive BP lowering (163). Hypertension classification depends on the technique used. Table 5 describes the criteria for the diagnosis of hypertension depending on the method. We can see that in the case of ABPM

the diagnosis of hypertension is defined when SBP is equal or higher than 130 mmHg and DBP is equal or higher than 80 mmHg (≥130/80 mmHg) (163).

Table 5. Classification of hypertension according to Office BP and ABPM

Office BP		**ABPM**					
		Day time		Night time		24h	
SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
≥140	≥90	≥135	≥85	≥120	≥70	≥130	≥80

Abbreviations: BP, Blood Pressure; DBP, Diastolic Blood Pressure; SBP, Systolic Blood Pressure (132,163).

As mentioned before one of the advantages of ABPM is the diagnosis of white-coat hypertension and masked hypertension. White-coat hypertension is when office BP values are high but out-of-office BP values are normal. White-coat hypertension is diagnosed when office BP is ≥140/90 mmHg, daytime ABPM is <135/85 mmHg and night time ABPM is <120/70 mmHg (163). The prevalence of white-coat hypertension is between 15-30% (168). For a correct diagnosis of white-coat hypertension it would be interesting to record BP over 24h by ABPM and confirm again the diagnosis in 3-6 months (163). The United States Preventive Service and the American Heart Association suggest that it is important to measure out-of-office BP to diagnose hypertension before taking antihypertensive drugs (169)(170). Besides 24h and daytime BP, the European Society of Hypertension (ESH) in 2013 gave importance to mean BP at night considering it useful for the diagnosis of white-coat hypertension (132). Night time BP was also important for the prediction of CVD, non-CVD and total mortality (171). Different studies have evaluated the prevalence of white-coat hypertension considering 3 different criteria: only daytime BP, daytime BP and 24h BP, or daytime, 24h

and night time BP. In the Jackson Heart Study the prevalence of white-coat hypertension was 10.6% considering 24h, daytime and night time BP compared to daytime BP (29.6%) and daytime and 24h BP (21.1%) (172).

Masked hypertension is when office BP values are normal but out-of-office BP values are high. Masked hypertension is diagnosed when office BP is <140/90 mmHg, awake ABPM is >135/85 mmHg and sleep ABPM >120/70 mmHg (163). The prevalence of masked hypertension is between 8-20% depending on the population and whether they are treated or not (173). This disease is difficult to identify because when office BP values are normal clinicians do not suggest checking BP with ABPM thus the disease is often undetected and not treated (174). It is important to detect it and then to have a correct diagnosis of the disease so as to get the correct drug treatment. It is associated with a higher target organ injury a random sample of the normotensive participants including in young people (175).

Due to the multiple and precise readings of this technique ABPM is a better predictor of CVD events and other health outcomes compared to home BP and office BP (176,177). However, these results contrast with the results of the PAMELA Study whose conclusion was that the 3 methods had the same predictability of death (178).

Disadvantages of ABPM are limited availability in general practice, possibility of inaccurate readings, expensive device (although is a cost-effective technique), discomfort at night, objection to use by some patients and difficulty in identifying artefact measurements (163).

The criteria for considering an ABPM recording acceptable is based on the number of valid measurements needed. In adults, it has been estimated that a minimum of 20 valid awake measurements during the day should be

repeated. A 24h recording should contain almost 70% of the expected measurements (163). In children experts consider quality ABPM recordings when there is minimum 1 reading per hour, 40-50 during 24h or 65-75% successful recordings (179).

Health consequences

High blood pressure has become a public health problem due to its relationship with CVDs such as stroke, myocardial infarction and heart failure among others (132,180). The World Health Organization (WHO) aims to prevent and control CVDs and other non-communicable diseases (NCDs) reducing the premature mortality by 25% by 2025 and the modifiable risk factors (181). According to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7), the relationship between BP and risk of CVD events is "continuous, consistent, and independent of other risk factors" (137). Hypertension is the most important modifiable risk factor in CVDs and cardiovascular morbidity and mortality (143) and the strongest risk factor for any type of stroke (182). Hypertension, smoking, waist-to-hip ratio, diet and physical activity are the principal risk factors for stroke and can explain 88.1% of the stroke cases according to the INTERSTROKE study, a case-control study carried out in 22 countries (182). As said before, 51% of stroke deaths are due to high BP (141). High-normal blood pressure is associated with an increased risk of cardiovascular disease (183). The Framingham Heart Study found that SBP, DBP and other BP factors were good predictors of stroke (184). In the Prospective Study Collaboration, Lewington et al. found that BP is strongly associated with vascular mortality in middle and older aged population, with no evidence of a threshold as far down as at least 115/75 mmHg (185). This

observation led to the incorporation of prehypertension to the BP classification in some guidelines as the JNC7, as prehypertension is often underdiagnosed (137). An increment of 20 mmHg in SBP or 10 mmHg in DBP with people's BP from 115/75 to 185/115 mmHg doubles the risk of CVD in people aged 40-69 years (185). The effect of hypertension was higher in people younger than 45 years than in people older than 45 years (182). Small reductions in BP are associated with an important decrease in CV risk especially in hypertensive patients with other risk factors (186).

Treatment

As hypertension is a silent disease it is important to diagnose it and if necessary, to treat it. Uncontrolled hypertension is common in some populations when treated hypertensive people do not reach the target BP. Data from an American study showed that the prevalence of uncontrolled hypertension in the studied population varied from 12.8% to 16.6%. There were no differences between sexes but the prevalence increased with age (187). The EURIKA (European Study on Cardiovascular Risk Prevention and Management in Usual Daily Practice) study revealed that 51.6% of the European population have uncontrolled hypertension (188). In the Chinese population 34.4% of hypertensive people were treated but only 8.2% had their BP controlled. When hypertension remains uncontrolled, the probabilities of having myocardial infarction, heart failure, stroke or kidney disease are increased (189,190). Untreated or not well treated hypertension causes increased morbidity and mortality (161).

There are two types of treatment: lifestyle modifications and drug treatment. Lifestyle modifications are very important for the prevention of hypertension

and the reduction of BP when needed. Its main objective is to reduce BP and prevent CVDs. Some approaches should be introduced to improve people's health such as eating a healthy diet, having a correct weight, practice regular physical activity and stopping smoking and drinking alcohol (137). The Dietary Approaches to Stop Hypertension (DASH) diet, a diet that consists of an increased consumption of fruits, vegetables, low-fat dairy products and reduced consumption of saturated fat and cholesterol, decreases SBP and DBP (191). Also the combination of low sodium intake and the DASH diet was associated with lower SBP compared with the combination of high sodium intake and the control diet (192). Some studies have found that weight loss of approximately 4.5 kg, effectively reduces BP (193,194). As previously stated, regular physical activity reduces BP (195,196). Physical activity is always recommended as additional treatment to drug treatment (132). Lifestyle modifications should be implemented in hypertensive people but also in non-hypertensive people as initial treatment or in addition to drug treatment. In case of not achieving the desired BP reduction with lifestyle modifications, drug treatment should be started. Hypertensive people should reduce their BP to <140/90 and in case of having diabetes or kidney disease the recommendation is <130/80 mmHg (132).

Drug treatment is started if BP rises or is high and depending on CVD risk. The aim of the antihypertensive drug therapy is to reduce morbidity and CVD and renal mortality and avoid target organ damage (137). There are different available options, most treatments start with thiazide-type diuretics. When diuretics are contraindicated for BP reduction other drug treatments should be used as first line of treatment. Monotherapy can control BP of 30% of hypertensive population Then in some cases one antihypertensive drug is not enough for reducing hypertension and two or more might be needed (197–

199). Thiazide-type diuretics can be used alone or in combination with other drugs as angiotensin converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), beta blockers (BBs) or calcium channel blockers (CCBs) (137,200). There are even different treatments to control BP, and in some cases other measures should also be implemented to guarantee some patient groups are properly treated. Multifactorial programmes with strategies to manage BP should be implemented as for example evidence-based guidelines like clinical guidelines (180). There is a need to find some strategies for prehypertension prevention because people with prehypertension have a higher risk of developing hypertension in a short time (201). Following the JNC-7 guidelines people with prehypertension need to reduce and maintain their BP to normal levels following lifestyle advice given by the clinician. Otherwise, drug treatment should be started (137). People with stage 1 hypertension should be treated with drug treatment if lifestyle modifications fail in reducing BP. Stage 2 or 3 hypertension must be treated with medication. The treatment goal for hypertensive people is <140/90 mmHg and <130/80 mmHg for diabetic hypertensive people (137). Antihypertensive therapy is associated with a reduction of stroke by 35-40%, myocardial infarction 20-25% and heart failure by 50% or more (202).

In 2014 the expert panel of the Joint National Committee created a new guideline called the Eighth Joint National Committee (JNC-8) where they mainly updated information about hypertension treatment. Following the JNC-8 guidelines, people from 60 years or older should use antihypertensive therapy with the purpose of decreasing BP when their BP is ≥150/90 mmHg. People younger than 60 years old may need drug treatment for lowering BP when it is ≥140/90 mmHg. Adults with diabetes or chronic kidney disease should start medication if their BP levels are >140/90 mmHg. Comparing with

JNC-7, in JNC-8 the initial antihypertensive treatment can be with thiazide-type diuretics, CCBs, ACEIs or ARBs, not only thiazide-type diuretics. If target BP is not reached in one month, it is recommended to increase the dose or add a second drug (180).

The SPRINT study, in which people older than 75 years participated, compared an intensive treatment to achieve a target SBP of <120 mmHg versus a standard treatment to achieve a target BP of <140 mmHg and found that the intensive treatment reduced CVD by 33% and mortality by 32% (203).

Blood pressure in children

High blood pressure in children is becoming a public health problem. The number of children with elevated BP is increasing, with a prevalence of 2-5% (204,205) being higher because of the increasing prevalence of child overweight and obesity (206,207). BP in children can predict BP in adulthood and may be associated with CVD later in life. This is the reason why it is so important to diagnose elevated BP during the early stages of life (208,209). Children with elevated BP will have a higher risk of developing CVD and high BP in adulthood (210,211). Controlling BP during childhood can help to reduce public health costs, which are high compared to normotensive children (212). If we find the main factors that lead to high BP in children it will be easier to prevent high BP in children and in consequence later in adults.

Boys have higher BP than girls (213,214) and hypertension increases with age (215). The prevalence of elevated BP was 10% at age 5.5 years, 10% at 9.2

years, 7% at 12.5 years, and 9% at 15.6 years, respectively (215). From children with elevated BP at the initial visit, the proportions who had elevated BP at the subsequent visit 3-4 years later was 13% between ages of 5.5 and 9.2 years, 19% between 9.2 and 12.5 years, and 27% between 12.5 and 15.6 years. As in adults, BMI is associated with elevated BP in children (206,209,216). Overweight and obesity were associated with elevated BP in children (217). SBP, DBP and waist circumference were significantly higher in overweight and obese children compared to normal weight children (218). A cross-sectional study with adolescents aged between 11-17 years found that overweight was independently associated with hypertension (219). Regular physical activity reduces BP in pre-pubertal obese children (220).

The BP diagnostic criteria in children depend on sex, age and height. This information is compiled in some tables where there is a precise classification of BP. These tables are attached in the Appendices section. The BP tables include the 50th, 90th, 95th, and 99th percentiles. For office BP, hypertension in children and adolescents is defined when SBP and/or DBP surpasses the 95th percentile on more than 3 occasions. BP above the 90th percentile but lower than the 95th percentile is considered prehypertension (209).

The classification for ABPM in children is different. In Table 6 there is a summary of the classification of BP depending on ABPM (221).

Table 6. Classification of BP according to ABPM.

Classification	Clinic Point BP	Mean Ambulatory SBP (24h)	SBP Load (%)
Normal BP	<P95	<P95	<25
White-coat hypertension	>P95	<P95	<25
Masked hypertension	<P95	>P95	>25
Prehypertension	>P95	<P95	25-50
Ambulatory hypertension	>P95	>P95	25-50
Severe ambulatory hypertension (at risk for end-organ damage)	>P95	>P95	>50

Abbreviations: BP, Blood Pressure; SBP, Systolic Blood Pressure (221).
SBP load refers to the % of valid ambulatory SBP measures above the 95th percentile of BP for age, gender, and height.

The procedure for measuring BP in children is described in the Methods section.

Folate, homocysteine and blood pressure

Some studies have found an association between folate and BP, but the results are controversial. Serum folate is inversely associated with BP in non-pregnant women of childbearing age with high prevalence of folate deficiency (222). Higher folate intake is associated with decreased BP and risk of hypertension. Folate intake was inversely associated with having hypertension as participants in the highest quintile of total folate intake had a lower risk of having hypertension compared with participants in the lowest quintile (HR: 0.48; 95% CI: 0.38-0.62) in the Coronary Artery Risk Development in Young Adults study (223). In a cohort study of women of

childbearing age with high prevalence of folate deficiency, serum folate was inversely associated with BP. Women with folate deficiency had higher BP levels compared to non folate deficient women (222). Other studies say that taking supplements with folic acid and other B-vitamins such as B_6 and B_{12} did not reduce the risk of CVD in patients with vascular disease (224).

Elevated tHcy concentration increases the risk of CVDs (74,225). Some cross-sectional studies reported that elevated tHcy concentrations are associated with increased risk of having hypertension (6,226). Approximately a 5 µmol/L increase in homocysteine was associated with increases in DBP and SBP of 0.5 and 0.7 mmHg respectively in men and of 0.7 and 1.2 mmHg in women (226). However, other studies observed no association (227,228). Plasma tHcy has been reported to be positively associated with SBP in other studies (71,229,230).

Childhood is an important phase for the development of some diseases. Wang et al. found that low maternal folate in late pregnancy mostly in obese mothers can increase child metabolic risk in the forms of overweight or obesity (231). Another study of children found that tHcy, SBP and DBP were significantly lower in the folic acid supplemented compared to the control group of mothers. This may mean that folic acid supplementation may prevent CVD in children in early life (232). A positive association was found in girls between RBC folate and SBP whereas there was no association between vitamin serum biomarkers concentrations and DBP (233).

MTHFR 677 C>T polymorphism and blood pressure

The *MTHFR* 677 C>T polymorphism has been reported to be positively associated with BP, but the evidence is inconsistent. In a cohort study with 3000 Japanese patients Inamoto et al. found that the TT genotype was positively associated with hypertension in women but not in men (234). A case-control study in New Zealand with hypertensive versus normotensive individuals showed that *MTHFR* 677 CT and TT polymorphism increased the risk of essential hypertension (OR=1.57, 95% CI: 1.04-2.37) (235). In addition two studies conducted in the USA and Turkey respectively reported an increased risk of hypertension in homozygous participants for the polymorphism assessed in comparison to wildtype participants (85,236). Being a carrier of the variant T allele was associated with a 60% increase in the risk of hypertension compared to non-T carriers in the New Zealand and USA studies (235,236). Another case-control study in Mexico where there is mandatory folic acid fortification, reported a relationship between the polymorphism and hypertension in adults but not in children (237). Cheng et al. reported that DBP was higher in TT patients in comparison to those with the other genotypes and found no differences in SBP among the three genotypes (238). The *MTHFR* 677 C>T polymorphism is significantly associated with DBP independently of blood lipids (239). Two meta-analyses in China found a positive association between *MTHFR* 677 C>T polymorphism and the risk of developing hypertension (240,241).

On the other hand Fowdar et al. found no association between *MTHFR* 677 C>T and hypertension (242). A study in an Algerian population also found no association between the *MTHFR* 677 C>T polymorphism and the risk of hypertension (243).

Riboflavin and blood pressure

Supplementing with 1.6 mg/day of riboflavin lowered homocysteine in people with the TT genotype, not with the CC or CT genotype (78). Furthermore, riboflavin has been reported to play a role as a modulator between the *MTHFR* 677C>T polymorphism and hypertension.

Riboflavin has been found to reduce blood pressure in different studies (123,124). TT patients with low riboflavin status had higher BP at baseline than CC and CT genotypes. In patients with high riboflavin status this tendency was not observed (123). Supplementing premature CVD patients with riboflavin (1.6 mg/d) for 16 weeks decreased SBP by 13.4 mmHg and DBP by 7.5 mmHg only in the TT genotype, no effect was shown in CC or CT genotypes (123). After 4 years, they continued to corroborate in a follow-up study with a crossover design that blood pressure in people with the *MTHFR* 677 TT genotype remained lower with riboflavin supplementation (124). Previously our group found that TT genotype adults with marginally deficient or deficient riboflavin status, had higher tHcy compared to the CC genotype, independently of folate status (66). TT patients do not react as effectively to antihypertensive drugs as the other genotypes do, as reported in a study in which 63% of TTs were hypertensive and even taking medication failed to achieve target blood pressure with drugs (123). Even though in 2008 different antihypertensive drugs were prescribed to patients, almost 50% of them with the TT genotype could not achieve the expected BP (124). Having hypertension is influenced by the genotype and riboflavin status, independently of the drugs used (124).

Foetal programming of blood pressure

Foetal programming consists of all the permanent adaptations in organ structure and function the foetus makes to adapt to the environment in which it develops, and have a lasting effect later in life (244).

During gestation the embryo grows. In the embryonic phase (0-8 weeks of gestation) when the programming begins, the embryo does not grow a lot physically but all his "information" will be found in the genes. . In the foetal phase (from 9 weeks of gestation until birth) is when growth occurs and cell division is produced. For optimal cell division, nutrients and oxygen are required. If the foetus is in a suboptimal nutritional situation he adapts to it and there is a decrease in the speed of cell division that can have lasting effects in the body (245).

Genetic factors and prenatal environment are very important in the development of chronic diseases later in life (246). Low birth weight is a marker of insufficient nutrient and oxygen status. Low birth weight is associated with a higher risk of developing diseases such as diabetes (247), high blood pressure (248) and others in early life and adults. Also it is associated with more deaths from ischaemic heart disease (249). A meta-analysis demonstrated an inverse association between mortality and birth weight showing a decrease in cardiovascular mortality (HR = 0.88, 95% CI: 0.85-0.91) for every extra kg in birth weight (250). One of the most influencing factors in prenatal environment affecting birth weight and foetal growth is maternal nutrition (251). Suboptimal maternal nutrition during pregnancy affects foetal nutrition, leading to impaired growth during early life and with a higher risk of developing CVD later in life (248,252). Some studies such as the Dutch famine study, in which they found that maternal malnutrition during pregnancy increases the risk of coronary heart disease in

the offspring, have corroborated this (253). Another study in China reported increased risk of hyperglycaemia later in life in children born in the Chinese famine (254). Thus the intrauterine environment appears to have a lasting effect on the development of the offspring. The capacity of one genotype to produce different phenotypes depending on the environment during development is termed developmental plasticity (255). If the *in utero* environment and the postnatal environment are similar then foetal adaptation in utero is adequate and not associated a priori with health problems in later life. On the contrary if the two environments are different, the foetal adaptation in utero will be in vain and may result in disease later (247,256).

As mentioned before not only *in utero* environment influences foetal growth, genetic factors are also important. The mechanism by which the foetus adapts to the different environments is through epigenetics. Epigenetic changes are permanent heritable changes in the genome. These changes are usually due to DNA methylation, microRNAs or histones acetylation. (257) The intake of one carbon metabolism nutrients is important for DNA methylation because they can generate, transfer and release methyl groups (258). The Pune Maternal Nutrition Study based in Pune (India), investigated the effect of macro and micronutrients and foetal birth weight and growth and found that energy intake and protein were not associated with birth size but micronutrients were related to foetal growth and that small babies had mothers that were shorter and weighed more compared with babies that have an adequate growth (259). Yajnik and collaborators have studied the relationship between one carbon metabolism and offspring growth for years. They found no association between plasma cobalamin at 18 GW and height and fat mass in offspring at 6 years of age (260). In 2014 they reported an

association between maternal plasma folate and insulin resistance in children at 9.5 and 13.5 years old (261).

Impaired DNA methylation has been associated with elevated blood pressure (262). High blood pressure or hypertension is one of the diseases that may have its origins in foetal programming. It begins in utero and it lasts until later in life. It is believed that changes in foetal blood flow can alter the blood vessel wall provoking an increase in blood pressure (263). Different studies have investigated the relationship between maternal nutritional status and BP but the results are inconsistent. A randomised control trial in Nepal showed that the offspring of mothers who took multivitamin supplements had lower SBP at 2.5 years (264). Maternal supplementation with folic acid and iron or zinc were not associated with BP in the children at 6-8 years old (265). The Generation R prospective cohort study found no association between maternal first trimester folate and homocysteine with child BP, but found an association between low maternal cobalamin (lowest quintile) and low DBP but not SBP (266). Also Krikke et al. in the Amsterdam Born Children and their Development study (ABCD) concluded that maternal nutritional status during pregnancy could program cardiometabolic health of the offspring but they did not find significant associations between maternal folate and cobalamin with BP in the offspring (102). On the contrary in the Boston Birth Cohort (BBC) children born to mothers with cardiometabolic risk factors and high folate status (those whose mothers had folate levels above versus below the median; range: 30.33-185.51 nmol/L) had 40% less probability of having elevated SBP compared to children born to mothers with cardiometabolic risk factors but with low folate (below the median, range: 6.64–30.31 nmol/L) status [OR= 0.60 (0.40-0.90)] (267).

Mechanisms by which 1C metabolism may influence blood pressure

It is not clear by which mechanism 1C metabolism affects BP but it may involve endothelial function (268). The vascular endothelium is a layer of cells that is in contact with the blood and plays an important role in cardiovascular health and disease. The more BP increases the more endothelial function is impaired, it depends on the grade of hypertension (269).

Folic acid has proven to be protective against CVDs. Nitric oxide (NO) is a vasodilator that has beneficial properties for health such as anti-inflammatory, antithrombotic and others. High doses of folic acid improve NO bioavailability when there is impaired endothelial function (270–272) and reduces plasma tHcy (79). Folic acid supplementation improves endothelial nitric oxide synthase (eNOS) coupling, it increases NO bioavailability and prevents CVDs. On the other hand, homocysteine also has a role in impaired endothelial function. It decreases the bioavailability of NO resulting in impairment of the vasodilator properties of endothelial cells (273), increasing oxidative stress (274), stimulating the proliferation of vascular smooth muscle cells and modifying the elasticity of the vascular wall without affecting eNOS expression (275). NO production is reduced through the formation of peroxynitrite in the reaction between superoxide and NO (276,277). This leads to vasoconstriction of blood vessels increasing BP levels. Figure 5 shows this hypothesis schematically.

Antioxidants such as vitamin C, E or others have been used as a treatment for hypertension (278). Other treatments have also been tested. Supplementation with B-vitamins can decrease homocysteinemia and may subsequently lower BP.

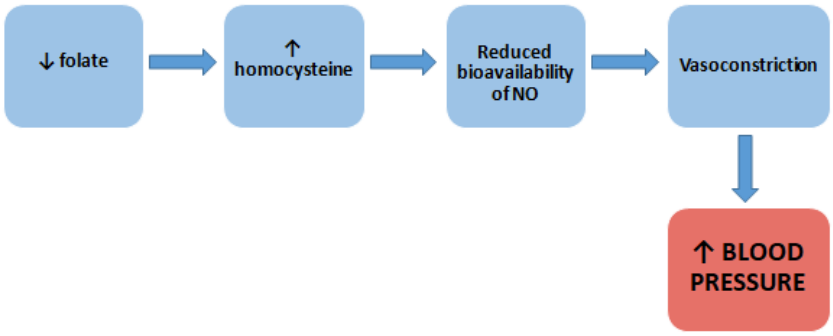

Figure 5. Scheme of the proposed hypothesis for an involvement of 1C metabolism in the development of elevated blood pressure.

Another possible mechanism by which BP is regulated is through DNA methylation and epigenetic modifications. DNA methylation takes place in the carbon 5' position of cytosine in the CpG islands (279). DNA methylation is the link between genes, environment and phenotypes such as BP and it regulates gene expression (279). The variant genotype of the *MTHFR* 677 C>T together with low folate status modifies DNA methylation because people with the TT genotype have lower DNA methylation compared to CC (280). There is some inconsistency about what genes influence BP as the different meta-analyses give different results. Some meta-analysis have reported different genes that regulate BP but it is not clear which ones are more important. Genome-wide association studies (GWAS) have uncovered numerous SNPs associated with BP. One meta-analysis reported that *TSPAN2* is a candidate gene for BP regulated by heritable DNA methylation (281). Another meta-analysis found associations between SBP or DBP and common variants in regions near the genes *CYP17A1, CYP1A2, FGF5, SH2B3, MTHFR, c10orf107, ZNF652 and PLCD3* genes (282). In a meta-analysis with the Global BPgen Consortium, 4 loci were significant for SBP (*ATP2B1, CYP17A1, PLEKHA7, SH2B3*), 6 for DBP (*ATP2B1, CACNB2, CSK-ULK3, SH2B3, TBX3-TBX5,*

ULK4) and 1 for hypertension (*ATP2B1*) (283). Further studies should be done to identify the genes involved in BP mechanism.

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HYPOTHESIS AND OBJECTIVES

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1. Population study

Hypothesis

In the absence of mandatory folic acid fortification and B vitamin supplement use,

Moderately elevated tHcy increases the risk of having diagnosed hypertension.

The *MTHFR* 677 C>T polymorphism increases the risk of having diagnosed hypertension.

Objectives

Main objective

To investigate the relationship between components of one carbon metabolism and diagnosed hypertension in a representative sample of an adult population unexposed to mandatory folic acid fortification and B vitamin supplement use.

Specific objective

- To investigate the lifestyle, nutritional and genetic factors associated with tHcy.

- To investigate the relationship between tHcy and diagnosed hypertension.

- To investigate the relationship between the *MTHFR* 677 C>T polymorphism and diagnosed hypertension.

2. Reus-Tarragona Birth Cohort

Hypothesis

Maternal tHcy and the *MTHFR* 677 C>T polymorphism during pregnancy are associated with blood pressure in the offspring during childhood.

Objectives

Main objective

To investigate the association between components of maternal one carbon metabolism and blood pressure in the children aged 7.5 years.

Specific objectives

- To describe maternal one carbon metabolism nutrient and metabolic parameters, according to *MTHFR* 677C>T genotype throughout pregnancy.

- To describe one carbon metabolism indicators according to child *MTHFR* 677C>T genotype in children aged 7.5 years.

- To compare one carbon metabolism nutrient and metabolic parameters during pregnancy with those in children at 7.5 years.

- To investigate the association between maternal *MTHFR* 677C>T genotype and lifestyle factors and prehypertension and hypertension in children at 7.5 years.

- To investigate the association between child *MTHFR* 677C>T genotype and lifestyle factors and prehypertension and hypertension in children at 7.5 years.

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MATERIAL AND METHODS

UNIVERSITAT ROVIRA I VIRGILI
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1. Population study

1.1. Design and study population

The population study is a cross-sectional study that was carried out between 1998 and 2002 by the Unit of Preventive Medicine and Public Health, Universitat Rovira i Virgili and the primary health centres that collaborated in the study.

Participants aged between 18-75 years were randomly selected from a representative sample, stratified by age and sex, from the town halls' population registers from 3 villages of Tarragona province (Cambrils, El Morell and la Pobla de Mafumet).

1563 people were selected and were sent a letter explaining the aims and characteristics of the study. After a few weeks, the study team called them inviting them to participate in the study. In case of not being interested or not answering after 3 phone calls (made at different parts of the day and on different days), the candidate was replaced for the next on the list with similar characteristics of age and sex.

1325 participants were invited to participate in the study. Of these, 812 agreed. For the purposes of addressing the hypothesis laid out in this thesis, 788 were included because they met all of the inclusion criteria (Figure 6).

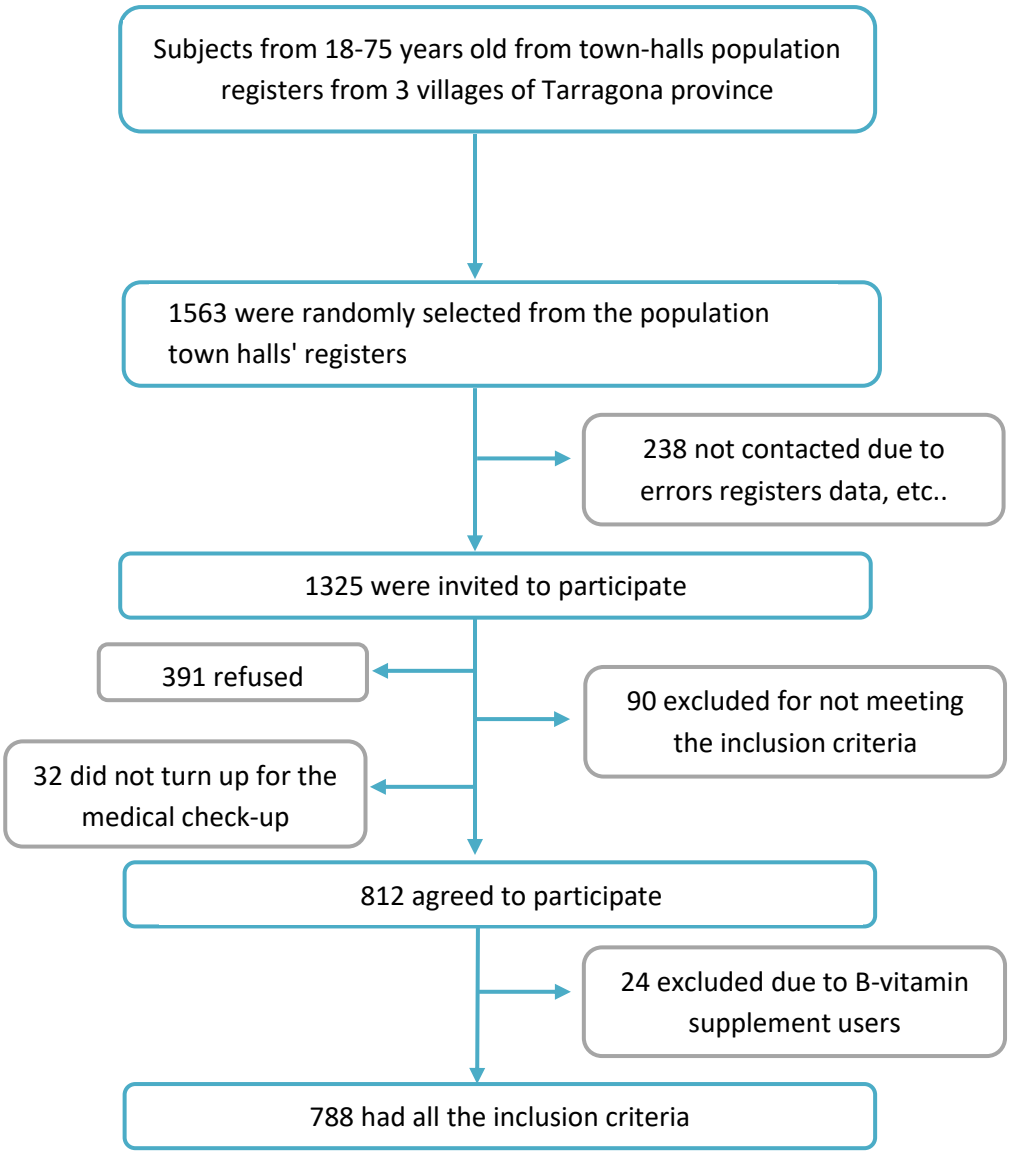

Figure 6. Participant flow chart from the population study.

The inclusion criteria were that participants had to be of Caucasian ethnicity and come from a family established in Spain for a minimum of 3 generations to ensure that there was an established transgenerational relationship between environment and genetics. The exclusion criteria were the use of B-

vitamin supplements, altered renal function, the use of drugs that affect folate metabolism (containing active ingredients such as valproic acid or methotrexate), being pregnant or having given birth in the last 6 months and being in lactation period. There is no mandatory folic acid fortification in Spain, so the Spanish population and the participants were not exposed to this policy. The study was approved by the Hospital Universitari Sant Joan, Reus and by the Jordi Gol Gorina Foundation ethics committees. All participants provided their signed informed consent in accordance with the Declaration of Helsinki.

1.2. Anthropometric data and lifestyle, clinical history and dietary intake

Anthropometric data and lifestyle

A medical check-up was carried out on all participants and the study team collected data ranging from anthropometric measurements to lifestyle habits. Data such as age, weight, height, skinfold thicknesses, waist perimeter and blood pressure (by the study clinicians) was collected. Participants were also asked about their smoking habit, alcohol intake and drug use. As there is no mandatory folic acid fortification of flour in Spain all the participants were unexposed to folic acid in the form of B-vitamin supplements and had a low exposure from fortified foods.

Clinical history

Clinical history, diagnosed diseases and drug treatments were recorded for each participant by the study doctors to describe the health status of all the patients. All the diseases had been previously diagnosed and were classified using a numeric code called “Código Internacional de Enfermedades” (CIE-9)

given by the "Ministerio de Salud del Gobierno de España" (284). In order to choose which diseases to include in the analysis, an extensive search was carried out, considering the frequency of the diseases recorded. The most frequent was hypertension.

Dietary intake

Participants completed a 3-day dietary record on non-consecutive days, including 1 holiday. Subsequently they were interviewed by a dietitian and shown photos of food portions to validate the quantities consumed. As part of this interview, participants were questioned on use of B vitamin supplements to ensure that they were not using them.

1.3. Blood samples collection and processing

A fasting blood sample was collected from all the participants from the antecubital vein in EDTA-K_3 treated vacutainers and a non-anticoagulant vacutainer to obtain plasma and serum. The tubes were kept at 4ºC before being processed, always in less than 2 h from collection. The processing was performed in the laboratories of the Faculty of Medicine and Health Sciences, URV and the Centre of Biochemical Research, IISPV, both in Reus.

Whole blood, plasma, serum, washed red cells and leukocytes were obtained from the blood samples. Plasma and serum were separated from their respective vacutainers. Once the plasma was removed from the EDTA-K_3 vacutainer, erythrocytes were washed with physiologic serum always on ice. All the aliquots were stored at -80ºC in Biobanc IISPV (Reus, Spain) until their posterior analysis.

1.4. Biochemical and genetic determinations

Plasma aliquots were sent to the laboratories of Profs John M Scott and Anne M Molloy in Trinity College Dublin (Ireland) for the determinations of plasma folate, red blood cell folate and plasma cobalamin by microbiological assays. Plasma folate and RBC folate were determined using *Lactobacillus caseii* and plasma cobalamin with *Lactobacillus leichannii.*

Washed red cells were used for the determination of riboflavin status by EGRAC assay and pyridoxine status by the erythrocyte aspartate aminotransferase activation coefficient (EASTAC) assay. These assays were done on the COBAS Mira autoanalyser (Roche, Basel, Switzerland) following the Mount technique (126).

Plasma tHcy was determined by fluorescence polarization immunoassay on an IMx autoanalyser (Abbott Laboratories, Abbott Park, IL, USA) in the central hospital lab of University Hospital Sant Joan, Reus. DNA was extracted from leukocytes using the DNA Puregene extraction kit (Gentra Systems, Minneapolis, MN, USA). The methylenetetrahydrofolate reductase 677C>T (*MTHFR* 677 C>T) polymorphism was determined using the previously described technique (61) in the Centre of Biochemical Research laboratory, University Hospital Sant Joan, Reus.

1.5. Data handling and statistical analysis

The statistical analysis was performed using SPSS version 23.0. The normality of continuous variables was checked with the Kolmogorov-Smirnov test. Descriptive data with a normal distribution were expressed as medians (P25, P75). To compare means between groups, ANOVA test was used. The variables that did not follow a normal distribution were natural log

transformed in order to apply the parametric statistics tests. These ones are reported as geometric mean (CI 95%). Categorical variables were expressed as % (CI 95%) and compared between groups using the chi-square test. Confidence intervals of categorical variables expressed in % were calculated using the Confidence Interval Analysis program (CIA) (Southampton, Southampton, UK). Hardy-Weinberg was used to check allele frequencies distributions as previously described (54).

tHcy was classified in tertiles. tHcy tertiles for women were <7, 7.7-9.6 and >9.6 µmol/L, respectively. For men, tHcy tertiles ranges were <9.3, 9.3-11.1 and >11.1 µmol/L. Plasma folate deficiency was considered when concentrations were <7 nmol/L (50). Plasma cobalamin deficiency was considered when concentrations were <220 pmol/L (285). Multiple linear regression was used to assess the associations between factors possibly associated with tHcy and tHcy in all the population and then separately by sex. Interaction between independent variables were assessed by including their product in the model (eg. the interaction between *MTHFR* 677C>T genotype and smoking was assessed by including the product *MTHFR* genotype*smoking as an independent variable in the model). In the case of interaction between independent variables they were not included in the same multiple regression model. So in the case of interaction between smoking and *MTHFR* 677C>T genotype the models were stratified by *MTHFR* 677 C>T genotype. Multiple logistic regression analysis was used to explore the effect of the age and sex specific high tertile of tHcy (3^{rd} tertile compared to 1^{st} and 2^{nd}) on having diagnosed hypertension. Cut offs for the 3^{rd} tertiles were ≥9.09 µmol/L in women ≤50 years, ≥10.60 µmol/L in women >50, ≥10.88 µmol/L in men ≤50 years, ≥11.59 µmol/L in men >50. Models were adjusted for low versus mid-high socioeconomic status, regular alcohol intake

(moderate [<16 d/day in women and <24 g/day in men] versus none; high versus none [≥16 g/day in women and ≥24 g/day in men], current smoking (cigarettes/day) and total plasma cholesterol (mmol/L). Multiple logistic regression analysis was also used to assess the probability of having hypertension in case of having the variant allele of the *MTHFR* 677 C>T (CT or TT). Age, sex, BMI, low versus mid-high socioeconomic status, regular alcohol intake (moderate [<16 d/day in women and <24 g/day in men] versus none; high versus none [≥16 g/day in women and ≥24 g/day in men], current smoking (cigarettes/day), plasma folate (nmol/L), plasma cobalamin (pmol/L), EGRAC and total plasma cholesterol (mmol/L) were used as potential confounders. Participants without diagnosed hypertension but office BP measurements >140/90 mmHg were excluded from these analyses. In the second MLRA participants with no office BP and BMI>30 kg/m^2 and 5 participants with plasma creatinine concentrations >97 mmol/L in women and >124 mmol/L in men indicating possibly impaired renal function were also excluded. Significance level was set at $p < 0.05$.

2. Reus-Tarragona Birth Cohort

2.1. Design and study population

The Reus-Tarragona Birth Cohort (RTBC) is an ongoing longitudinal cohort study. The study is being carried out by the Area of Preventive Medicine and Public Health of Universitat Rovira i Virgili and the Areas of Obstetrics and Gynaecology of Hospital Universitari Sant Joan, Reus (HUSJR) and Hospital Universitari Joan XXIII, Tarragona (HUJXXIII) in Tarragona province (Spain).

The RTBC study has two phases: the pregnancy phase and the follow-up phase in the children at 7.5 years old. Both phases of the study were conducted in agreement with the Declaration of Helsinki and approved by the Ethics Commitees of both Hospitals. Moreover, all the participants had to sign an informed consent to join the study and in case of the follow-up phase children's legal representative and verbal assent was obtained from the children.

The purpose of the study was and still is to find biochemical and genetic factors during pregnancy that can influence pregnancy outcomes, foetus development and child growth at 7.5 years. The study was identified as NCT01778205 in ClinicalTrials.gov.

2.2. Pregnancy phase

The study began in 2005 and it is ongoing. By May 2018, 619 complete pregnancies were followed. Pregnant women attending a prenatal visit before or at 12 GW in both of the Hospitals mentioned before were eligible to participate in the study and were informed and invited to participate by the obstetricians of both hospitals.

Inclusion criteria were to have a viable singleton pregnancy confirmed. Exclusion criteria were twin pregnancies, pregnancies of more than 12 GW at the first prenatal visit, chronic diseases or surgical interventions affecting nutritional status and use of any medication that affects folate or cobalamin metabolism. Potential participants had to read and sign the informed consent before entering the study.

Following the Spanish Obstetrics and Gynaecology Society recommendations, pregnant women with low obstetrical risk were recommended to take every day 400 µg of folic acid supplement until the end of the first trimester and 2 µg of cyanocobalamin also included in this supplement (286). Women with complications in previous pregnancies such as neural tube defects (NTDs) were recommended to take a 5 mg or higher daily dose of folic acid supplement. 40 mg of iron supplements were also recommended to all women starting at the end of the first trimester of pregnancy to prevent anaemia. In the case of women with anemia they were treated according to its severity and the clinicians' criteria with 80 or 120 mg or iron per day.

The study design is shown in Figure 7. Participation in the study consisted of providing 5 blood samples (4 from the mother and 1 from the umbilical cord), completing 2 habit and lifestyle questionnaires and 2 food frequency questionnaires (FFQ).

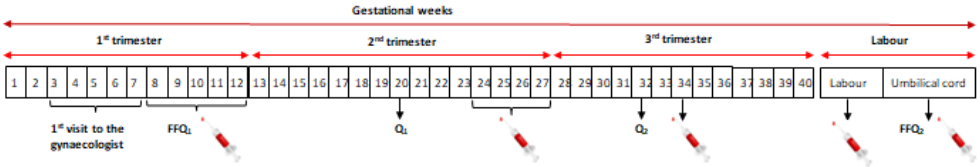

Figure 7. Structure of the Reus-Tarragona Birth Cohort Study pregnancy phase.
The syringes represent the different blood samples that are collected from the participants during the pregnancy. FFQ_1 and FFQ_2 are the food frequency questionnaires and Q_1 and Q_2 are the habits and lifestyle questionnaires.

2.2.1. Clinical history, dietary intake and lifestyle data

Clinical history

Age, 1st trimester weight, height and parity from the pregnant women were obtained from the clinical history of the pregnancy check-ups from obstetricians. BMI was calculated from this data. Blood pressure measurements were controlled and recorded throughout pregnancy. Gestational hypertension was defined as systolic blood pressure >140 mmHg and/or a diastolic blood pressure >90 mmHg on two or more check-ups (6 hours apart) after the 20th GW.

Newborn sex, date of birth, birth weight, gestational age at labour and previous adverse obstetrical outcomes were also recorded.

Blood analysis including routine haematology and biochemical results (eg. glycaemia etc) were obtained from the same routine prenatal blood samples collected as described above.

Dietary intake

The participants completed two validated food frequency questionnaires to estimate their dietary habits before and during pregnancy. The first questionnaire was carried out on the first visit at 12 GW and was targeted at dietary habits in the year before the pregnancy. The second questionnaire was carried out the day after giving birth and referred to dietary habits during the pregnancy.

Both questionnaires consisted of 45 food items and questions regarding appetite during the specified period of time, quantity of salt consumed, the type of milk, yoghurt and bread consumed as well as water intake. In the case of the second questionnaire, they were also asked about nausea and sickness and how they took their iron supplements. The questionnaires are contained in the Appendices section.

Lifestyle

Two questionnaires were completed regarding lifestyle and use of supplements during the pregnancy. The first questionnaire was at 20 GW and encompassed the preconception period until 20 GW. The second questionnaire was at 32 GW and encompassed 20 GW until 32 GW.

The women were asked about the use of folic acid, iron and multivitamin supplements during the pregnancy but also during the preconception period (3 months before the pregnancy). Participants were shown photographs of available supplements on the market to facilitate the identification of the supplements used and to improve reliability of the information. Commercial brand, time of initiation and duration of use were recorded. Based on this information, vitamin content of the supplements was recorded.

Information regarding breakfast habits, tobacco, alcohol consumption, toxic substance use, physical activity, sunbathing, socioeconomic status and planning of pregnancy was also recorded. In relation to tobacco, participants were asked about the number of cigarettes consumed per day at the moment of completion of the questionnaire and in the five previous years. They were specifically asked whether they had stopped smoking before or during the pregnancy. Frequency of alcohol consumption and toxic substances and time since last use in case of cessation, were recorded. Smoking data collected by questionnaires was corroborated with plasma cotinine determination in the mother at <12 GW, 24.27 GW and from the cord (see below). An example of both questionnaires is in the Appendices section.

2.3. Follow-up phase in children at 7.5 years old

Mothers that participated in the pregnancy phase of the study were contacted when their children were 7.5 years old. A letter was sent to the parents or legal representatives explaining the aim of the study, the different parts of it and inviting the children to participate. After two weeks, a phone call was made to contact the parents and ask whether they had any doubts regarding the study and give them extra information in case they needed it. A summary of the study was explained and they were asked their interest in participating. In case of agreement, two visits were established in Hospital Universitari Sant Joan de Reus or Hospital Universitari Joan XXIII. In case of not contacting the mother or the legal representative in the first phone call, other phone calls were made at different times of the day to make sure that the contact information was not wrong. There was a margin of 4 months after the letter and the first phone call to schedule the visits in both Hospitals.

521 pregnancies resulting in live births were recruited between 2005-2010. 104 were not eligible for inclusion in the child follow-up phase because parents were uncontactable or had refused permission to be recontacted. By May 2018, 417 children were eligible to participate. From these, 408 families were successfully contacted and 9 were pending contact. Finally, 212 children participated in the study and were followed-up and 196 refused to participate. So the participation rate in the child phase was 52.0%.

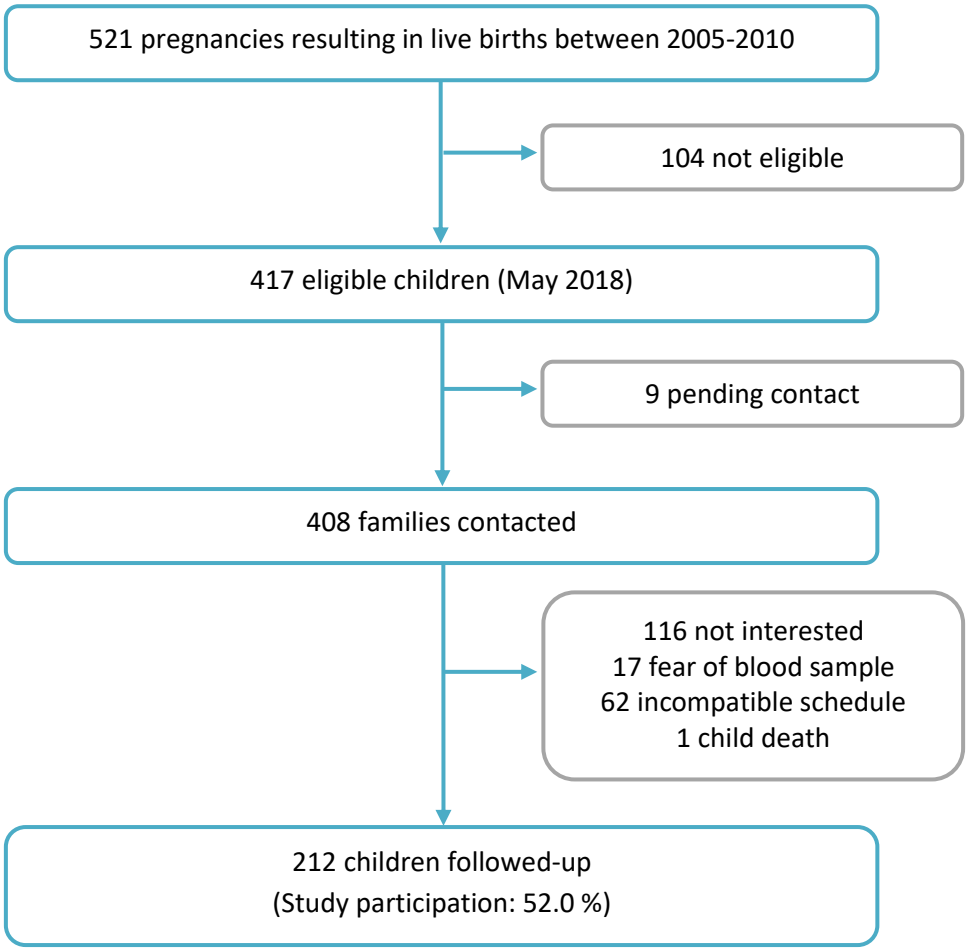

Figure 8. Recruitment participant flow chart from the follow-up phase of the Reus-Tarragona Birth Cohort Study.

The first visit consisted of a point blood pressure measurement and anthropometric measurements (weight, height, mid-upper arm, waist circumference and other circumferences, tricipital, bicipital, subscapular and suprailiac skinfold thickness), body composition (fat mass and fat free mass) and a fasting blood sample. This visit was carried out in the morning to facilitate collection of a fasting blood sample. The second visit consisted of neuorological developmental tests (not described or considered in this thesis) and three questionnaires completed by the parents or legal guardian. These questionnaires were about clinical history, food frequency questionnaire and physical activity respectively. As in this second visit there was no need for fasting, the hour scheduled for the visit was flexible and was arranged by the parents depending on their availability and when it was more suitable for them. It was carried out in the Faculty of Medicine of Universitat Rovira i Virgili in case of Reus or in Hospital Universitari Joan XXIII in Tarragona.

After the second check up or at an agreed time, children were fitted with an Ambulatory Blood Pressure Monitoring (ABPM) for 24h to record their 24 h ambulatory BP.

2.3.1. Anthropometric measurements and body composition

In all of the visits there were always two members of the study, one trained member of the team took the anthropometric measurements from the children following the WHO guidelines (287) and the other noted all the results on a data collection sheet prepared for the check-up. The same team member took the measurements to all the children to reduce the possible bias of the different team members. Children were asked to come in fasting conditions to the visit due to the blood sample.

Always in front of their parents or legal representative, children were asked to remove the necessary clothing and shoes (even hair accessories if necessary), down to their underwear, in order to accurately carry out all the measurements. Everything done was explained first to the parents and the child to make sure they had understood all the procedures before doing them and let them know that none of them was painful.

Weight was measured with an electronic scale (Tanita BC-420MA, Tanita Corporation, Tokyo, Japan). The precision of the apparatus was of 0.100g. Children had to stand still on the scale with their feet on the two metallic platforms.

Height was measured with a stadiometer. The precision of the apparatus was 0.1 cm. The procedure was to ask children to stand still with their head straight, knees extended and feet together joining their heels.

Head, mid-upper arm, chest, waist, hip and thigh circumferences were measured with a measuring tape (Seca GmBH & Co Kg, Hamburg, Germany). The precision of the tape was 0.1 cm. All the circumferences were measured twice.

The procedure for the head circumference measurement was to circle the head with the measuring tape on the forehead. For the mid-upper arm circumference the measurement was done in the mid-point between the tip of the acromion and the olecranon process. Chest circumference was measured at the nipple line. The waist circumference was measured with the child with his feet together in the mid-point between the iliac crest top and the last palpable rib. Hip circumference was measured at the widest area of the buttocks. For the thigh circumference they were asked to stand with one

foot in front of the other and they were measured the mid-point between the waist and the knee.

Tricipital, bicipital, subscapular, suprailiac and thigh skinfold thicknesses were measured with a Harpenden skinfold calliper (Holtain Ltd, Crymych, Wales) with a precision of 0.2 mm. All these measurements were measured 3 times.

Children were explained that the study member had to pinch their skin in some body zones and that this procedure was not painful in any moment. If in any of the measurements the children felt pain they had to warn it to the study member and then he will stop.

All the children behaved well and tolerated the measurements except a few of them that it was difficult to complete the whole procedure and measurements were made just once rather than in triplicate.

The procedure to measure the different skinfold thicknesses was first of all to mark with eyeliner the exact zone for the measurement with the skinfold calliper. For tricipital and bicipital skinfold thicknesses the measurements were done at the mid-point between the tip of the acromion and the olecranon, in the anterior and posterior arm respectively. For subscapular skinfold thickness the measurement was done in the inferior angle of the scapula. The suprailiac skinfold was measured on the iliac crest top. For the thigh skinfold thickness children had to stand with one foot in front of the other and they were measured the mid-point between the waist and the knee.

Tetrapolar bioelectrical impedance analysis (Tanita BC-420MA, Tanita Corporation, Tokyo, Japan) was used for calculating body composition. The

measurement was done in duplicate at 50 kHz. Total fat mass, fat-free mass and total impedance were recorded.

2.3.2. Office blood pressure measurements

BP was measured with BP monitor (Omron M6 AC, Omron Healthcare Co, Kyoto, Japan). The precision of the apparatus was of 1 mmHg.

Children were asked to sit quietly for 5 minutes, sitting with their back supported, feet on the floor and arms resting palm up in the arm rest of the chair so the cubital fossa was at heart level. They could not speak during the test and could not cross their legs. Then an adjustable cuff was placed on the arm and the trained member reminded all the steps previously explained to the participant to guarantee that the measurement was carried out successfully. A total of 3 measurements were needed. The subsequent measurements were performed after 1 minute. Room temperature was kept comfortable (21-23 ºC) (132).

2.3.3. Ambulatory Blood Pressure Monitoring (ABPM)

Children were asked to wear a 24-h blood pressure monitor known as ABPM in order to record BP values during 24h and thus record BP during one day and to differentiate day from night measurements. In case of acceptance, we agreed a day with them to wear the device. They had to wear it 24h non-stop (even when they were sleeping). Parents were given instructions about how the monitor worked, what to expect during the measurement and how to fit the monitor. A telephone number for contacting the study team was

provided to the parents in case they had more questions or something went wrong.

Like in the office BP measurement, children had to wear an appropriate cuff depending on their arm circumference. Children with an arm circumference from 12 to 20 cm wore a child cuff and those with 17-26 cm wore the small adult cuff. Children were asked to continue with their daily life in order to have reliable results and to keep their arm still during the cuff inflation. If the ABPM was not able to record a measurement, the measurement was repeated after 2 minutes automatically. After 24h a member of the study met the participant's parents to take off the monitor and to bring it back for analysis. Then all the data was downloaded from the apparatus to the computer and checked. Day-time and night-time periods were specified according to their answer in the questionnaire about the sleeping hours. We considered successful ABPM values if the recordings were valid in ≥50% of the cases. If a child obtained less than a 50% of valid measurements during the 24h follow-up, he was asked to repeat the recordings and wear it again another day. In case of refusing, all the ABPM measurements from that child were considered non-valid and were excluded from the study.

2.3.4. Clinical history, dietary intake and physical activity

Clinical history

A member of the study team interviewed the parents regarding their child's clinical history from birth until 7.5 years. They were asked about breastfeeding pattern (duration of exclusive breastfeeding and mixed breastfeeding), chronic diseases and other illnesses (all classified using the International Classification of Diseases-10 criteria). They were also asked

whether the child suffered from allergies and about parental smoking habits and socioeconomic status.

Dietary intake

A validated food frequency questionnaire of 105 items, supplement use and nutrition habits was completed by the parents.

Physical activity

A physical activity questionnaire was also completed by the parents to record information regarding leisure time activites, extracurricular physical activity, mode of transport to school and length of the journey.

2.4. Blood samples collection and processing

In the pregnancy phase, fasting blood samples for the study were drawn at the same time as the routine pregnancy samples at <12, 24-27 and 34 GW according to the regional health authorities protocol (286). Non-fasting blood samples were collected from the pregnant women on admission to the hospital with confirmed labour and from the umbilical cord vein after the birth but before expulsion of the placenta.

In the follow-up phase in children at 7.5 years, a specialised nurse collected the blood sample from the children while they were lying on the clinical examination bed. The nurse explained carefully to them all the procedure she was going to carry out, trying to relax them and keep them from moving.

All blood samples were kept at 4 ºC after collection. The processing was started at the hospital with a portable centrifuge to make sure that plasma

was separated in less than 30 minutes and serum in less than 1 hour to minimize the possible variations in the 1C metabolism metabolites. Then the rest of the processing was done in the Universitat Rovira i Virgili laboratory, always on ice. Samples were transported at 4ºC from the hospital to the laboratory.

The same blood sample collection procedure was performed in the pregnancy and the child follow-up phase. In both phases, blood samples were collected from the antecubital fossa vein into two different vacutainers, a 10 mL dipotassium ethylenediaminetetraacetic acid (K_2EDTA) vacutainer for plasma and a 10 mL dry vacutainer for serum. Moreover in the last blood sample of the pregnancy phase (umbilical cord vein) two vacutainers of each type were used. Whole blood, plasma, serum, washed red cells and leukocytes were obtained from the blood samples.

Whole blood was obtained from the EDTA vacutainer, 50 µL was removed before centrifuging and was diluted with 450 µL 10% ascorbic acid solution prepared the same day of the processing. The solution was mixed very well and the mix was kept for 30 minutes at room temperature. After that, the solution was separated into two aliquots of 250 µL each and were frozen at -80 ºC. This procedure was performed for blood samples at 12, 24-27 and 34 GW in the pregnancy phase and the blood sample in the child follow-up phase for the posterior red blood cell folate analysis.

Plasma was obtained from the EDTA vacutainer, centrifuged at 1500 g for 15 minutes at 4 ºC, divided in four aliquots of 1 mL each and stored at -80 ºC. The same procedure was done for the serum tube.

Leukocytes were obtained from the EDTA tube. Once the plasma was removed, PBS was added to the EDTA tube that contained red blood cells and

the buffy coat and it was mixed very well by inversion. This was added to a Falcon tube that contained 30 mL of haemolysis solution and left 20 minutes at room temperature. Then the mix was centrifuged at 2000 g for 5 minutes at room temperature and the supernatant was discarded. The pellet was resuspended in 20 mL of haemolysis solution, centrifuged again at 2000 g for 5 minutes and the leukocytes were set free. 450 µL of PBS was added to the sample and resuspended and to finish all the procedure, 10 mL of Cell Lysis Solution (Qiagen GmbH, Hilden, Germany) were added to the Falcon. The Falcon was kept in the dark at room temperature from between 1 month and 12 months (after 24 months the samples may not be stable so the maximum of 12 month time frame was imposed). Later on, the leukocytes obtained were used for DNA extraction.

DNA extraction was performed from maternal and cord leukocytes with the Puregene Kit (Qiagen GmbH, Hilden, Germany). 3.33 mL of Protein Precipitation Solution were added to the Falcon tube and vortexed at high speed for 20 seconds until the sample was homogeneous. After that, the mix was incubated on ice for 30 minutes and centrifuged at 2000 g for 15 minutes at 4 ºC. The supernatant was removed considering that the precipitated DNA wasn't discarded and 10 mL of 100% cold isopropanol were added. The Falcon was carefully mixed until the DNA got visible, centrifuged at 2000 g for 5 minutes at 4ºC and the supernatant was removed again and dried for 30-40 minutes at room temperature on an absorbent paper. 1200 µL of DNA Hydration Solution were added to the sample to rehydrate the DNA and was left 3-4 days at room temperature in a shaker. Afterwards, the DNA quantification was performed using a NanoDrop 1000 spectrophotometer at a wave length of 260 nm. To perform the technique 2 µL of hydrated DNA were needed.

The time of processing and number of aliquots obtained in the collection were recorded in a notebook in order to make a register of all the samples and control how long it took from the processing of the samples until they were frozen.

2.5. Biochemical and genetic determinations

BeVital A/S (Bergen, Norway) was the company that analysed the samples. The samples analysed were collected 18 months ago maximum and were organized in batches. For every sample, it was sent plasma aliquots of 0.5 mL and 250 µL of whole blood diluted with ascorbic acid solution.

For the genetic determinations 120 ng of lyophilised DNA were sent. The same SNPs were determined in both phases. In case of had genotyped the cord blood sample, that child sample was not sent because we already had the SNP determined.

Plasma folate and cobalamin, red blood cell folate, tHcy, methylmalonic acid and cotinine were determined by chromatography-tandem mass spectrometry (288), as previously explained (88).

2.6. Data handling and statistical analysis

The statistical analysis was performed using SPSS version 23.0. The normality of continuous variables was checked with the Kolmogorov-Smirnov test. Descriptive data with a normal distribution were expressed as means (95% CI). To compare means between groups, ANOVA test was used. Variables that did not follow a normal distribution were natural log transformed in order to apply the parametric statistics tests and were expressed as the geometric

mean (95% CI). Plasma folate, RBC folate, plasma cobalamin, holotranscobalamin (holoTC), methylmalonic acid (MMA), EGRAC and tHcy were not normally distributed. Categorical variables were expressed as % (95% CI) and were compared between groups with chi-square test. Confidence intervals of categorical variables expressed in % were calculated using the CIA program (Southampton, Southampton, UK). Hardy-Weinberg was used to check allele frequencies distributions as previously described (54).

Anthropometric and bioelectrical impedance variables were calculated as mean (95% CI). BMI was calculated as weight (kg) divided by height squared (m^2). According to the WHO references BMI (kg/m^2) was transformed to age- and sex-specific z-scores to take into account age and sex differences in growth (289). The Lambda-Mu-Sigma (LMS) method gives z-scores to consider the asymmetry of the distributions with three parameters: the median (M), the coefficient of variation (S) and the skewness of the distribution (L). Z-score was calculated as: $Z = \frac{(\frac{x}{M})^L - 1}{L * S}$

Overweight and obesity were defined according to the International Obesity Task Force criteria (290). We used these cut-offs because values are based on internationally based percentiles passing through BMI 25 and 30 kg/m^2 at age 18 and are linked to mortality rates. Depending on the method used (office BP or ABPM) different hypertension diagnosis criteria was used. For office BP, hypertension in children and adolescents is defined when SBP and/or DBP >95th percentile on more than 3 occasions. BP higher than 90th percentile but lower than 95th percentile is considered prehypertension (209). ABPM diagnosed criteria is found in Table 6 of the Introduction section. For linear regression analysis and multiple logistic regression analysis we joined the

cases of prehypertension and hypertension into the same variable in order to have sufficient sample size for the analyses.

In the ABPM method, we considered an ABPM registry valid when included at least 50% of the child SBP and DBP successful recordings during 24h. We established this criteria when we saw that it was difficult to measure BP in young children with ABPM because of the number of erroneous readings. However, we can see in the literature that in children experts consider ABPM recordings of adequate quality when there is minimum 1 reading per hour, 40-50 during 24h or 65-75% successful recordings (179). As mentioned before, in adults the official criteria for ABPM readings requires 70% of SBP and DBP successful recordings during 24h (163).

Differences in child characteristics between girls and boys were determined using ANOVA for continuous variables and chi-squared analysis for categorical variables. Multivariate linear regression analysis was used to assess factors associated with fasting plasma total homocysteine in all participants and separately by sex. Maternal and offspring demographic, lifestyle and genetic factors were used as independent variables and SBP and DBP from office BP and ABPM as dependent variables. Dependent variables were natural log transformed before analysis. Independent variables were child characteristics as including sex, BMI z-score, *MTHFR* 677 CT, *MTHFR* 677 TT, plasma folate and maternal variables like *MTHFR* 677 CT, *MTHFR* 677 CT, smoking, BMI, plasma folate and EGRAC. To evaluate the risk of maternal or offspring *MTHFR* 677 C>T polymorphism with prehypertension or hypertension multiple logistic regression analysis was used. Model 1 looked at the association of offspring *MTHFR* 677 C>T polymorphism and hypertension adjusting for the variables sex, child overweight and obesity, child plasma folate, *MTHFR* CT versus CC and *MTHFR* TT versus CC genotypes. Model 2

included the same variables as model 1 and was adjusted for potential confounders associated with the mother including gestational hypertension, smoking, plasma folate, plasma cobalamin and EGRAC. One person was excluded from the analysis due to not matching maternal and offspring genotype. ANOVA repeated measures were used to test the associations between the different 1C metabolism (plasma folate, RBC folate, plasma cobalamin, tHcy and EGRAC) nutrients during pregnancy.

UNIVERSITAT ROVIRA I VIRGILI
A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.
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RESULTS

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A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.
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1. Population study

The results from the population study have been submitted for publication as part of an original article, currently undergoing the peer-review process.

Table 7 shows the baseline characteristics of the studied population, stratified by tHcy tertiles. Women in the 3rd tHcy tertile (>9.6 µmol/L) were older, had higher plasma creatinine concentrations and more of them were hypertensive compared to the other tertiles. Moreover more women in the 3rd tertile had cobalamin deficiency. Men in the 3rd tHcy tertile (>11.1 µmol/L) were older and more of them had low socioeconomic status compared to the other tertiles. Both women and men in the 3rd tertile had lower plasma folate, red cell folate and plasma cobalamin concentrations, and more of them had suboptimal riboflavin status (based on EGRAC category) and folate deficiency compared to the other tertiles. The prevalence of hypertension was 27.2% in the highest tertile of tHcy in women and 14.4% in that of men. The allele frequencies for the *MTHFR* 677 C>T were in Hardy Weinberg equilibrium.

Results

Table 7. Baseline characteristics of the study population according to sex specific fasting plasma total homocysteine (tHcy) tertiles (µmol/L).

	Women			Men		
	1 (<7.7)	2 (7.7-9.6)	3 (>9.6)	1 (<9.3)	2 (9.3-11.1)	3 (>11.1)
Age (years)[1]	39.0 (29.0, 48.5) [125]	42.0 (29.0, 53.0) [125]	45.0 (30.0, 64.0)** [125]	38.0 (26.0, 50.0) [117]	43.5 (31.0, 53.0) [118]	44.0 (31.8, 59.8)** [118]
BMI (kg/m^2)[1]	25.8 (24.9, 26.8) [125]	26.7 (25.7, 27.6) [123]	27.6 (26.4, 28.8) [120]	27.2 (26.4, 28.1) [116]	27.7 (26.9, 28.4) [116]	27.2 (26.5, 28.0) [117]
Overweight[2]	32.8 (25.2, 41.4) [41]	35.0 (27.1, 43.7) [45]	23.3 (16.7, 31.7) [28]	41.4 (32.8, 50.5) [48]	45.7 (36.9, 54.7) [53]	41.0 (32.5, 50.1) [48]
Obesity[2]	18.4 (12.6, 26.1) [23]	21.1 (14.9, 29.2) [26]	31.7 (24.0, 40.4) [38]	24.1 (17.3, 32.7) [28]	28.4 (21.0, 37.2) [33]	25.6 (18.6, 34.2) [30]
Smokers[2]	31.2 (23.7, 39.8) [39]	28.2 (21.1, 36.7) [35]	33.9 (26.1, 42.6) [42]	43.6 (34.9, 52.6) [51]	34.7 (26.8, 43.7) [41]	38.1 (29.9, 47.1) [45]
Alcohol consumption[2,3]	[125]	[125]	[124]	[117]	[118]	[118]
None to low	89.6 (83.0, 93.8) [112]	96.8 (92.1, 98.7) [121]	93.5 (87.8, 96.7) [116]	53.0 (44.0, 61.8) [62]	62.7 (53.7, 70.9) [74]	50.8 (41.9, 59.7) [60]
Low to moderate	8.8 (5.0, 15.1)	2.4 (0.8, 6.8) [3]	4.0 (1.7, 9.1) [5]	27.4 (20.1, 36.1) [32]	28.8 (21.4, 37.6) [34]	29.7 (22.2, 38.4) [35]
High	1.6 (0.4, 5.6) [2]	0.8 (0.1, 4.4) [1]	2.4 (0.8, 6.9) [3]	19.7 (13.5, 27.8) [23]	8.5 (4.7, 14.9) [10]	19.5 (13.4, 27.6) [23]
Diagnosed hypertension[2]	8.8 (5.0, 15.1) [11]	8.0 (4.4, 14.1) [10]	27.2 (20.2, 35.6)*** [34]	8.5 (4.7, 15.0)	12.7 (7.9, 19.9) [15]	14.4 (9.2, 21.9) [17]
Low socioeconomic status[2]	42.4 (34.1, 51.2) [53]	48.8 (40.2, 57.5) [61]	51.2 (42.5, 59.8) [64]	15.4 (10.0, 23.0) [18]	25.4 (18.4, 34.0) [30]	34.7 (26.8, 43.7)*** [41]
Plasma folate (nmol/L)[4]	14.3 (13.1, 15.6) [125]	11.5 (10.4, 12.7) [125]	11.0 (9.9, 12.2)*** [125]	12.6 (11.6, 13.6) [117]	11.3 (10.3, 12.4) [118]	9.1 (8.2, 10.1)*** [118]
Plasma folate < 7 nmol/L[2]	7.2 (3.8, 13.1)	19.2 (13.3, 27.0)	24.8 (18.1, 33.0)***	9.4 (5.3, 16.1)	18.6 (12.6, 26.2)	30.5 (22.9, 39.3)***

Red cell folate (nmol/L)[4]	899 (846, 954) [125]	781 (734, 830) [125]	738 (680, 802)*** [125]	952 (302, 1004) [117]	852 (795, 913) [118]	724 (675, 776)*** [118]
Plasma cobalamin (pmol/L)[4]	377 (355, 401) [124]	352 (331, 375) [125]	316 (292, 343)** [125]	385 (363, 408) [117]	343 (321, 367) [118]	321 (303, 341)*** [118]
Plasma cobalamin <220 mol/L[2]	6.5 (3.3, 12.2)	8.0 (4.4, 14.1)	16.8 (11.3, 24.3)*	3.4 (1.3, 8.5)	8.5 (4.7, 14.9)	11.0 (6.6, 17.9)
Riboflavin deficiency[2,5]	45.5 (37.0, 54.3) [56]	33.6 (25.9, 42.3) [42]	30.6 (23.1, 39.3) [37]	41.4 (32.8, 50.5) [48]	25.6 (18.6, 34.2) [30]	29.8 (22.2, 38.8) [34]
Suboptimal riboflavin status[2,6]	54.5 (45.7, 63.0) [67]	66.4 (57.7, 74.1) [83]	69.4 (60.7, 76.9)*	58.6 (49.5, 67.2) [68]	74.4 (65.8, 81.4) [87]	70.2 (61.2, 77.8)* [80]
EASTAC[4]	1.67 (1.63, 1.71) [123]	1.64 (1.61, 1.68) [124]	1.68 (1.64, 1.71) [120]	1.60 (1.56, 1.64) [116]	1.65 (1.61, 1.69) [116]	1.62 (1.58, 1.66) [113]
tHcy (µmol/L)[4]	6.5 (6.4, 6.7) [125]	8.6 (8.5, 8.7) [125]	11.8 (11.4, 12.2)*** [125]	7.9 (7.7, 8.1) [117]	10.1 (10.0, 10.2) [118]	13.7 (13.2, 14.3)*** [118]
Plasma creatinine (µmol/L)[4]	63.7 (62.3, 65.3) [123]	64.0 (62.5, 65.5) [125]	67.0 (65.3, 68.7)** [124]	80.6 (78.5, 82.7) [116]	81.0 (79.1, 83.0) [118]	82.5 (80.0, 85.0) [118]
Plasma cholesterol total (mmol/L)[4]	5.0 (4.9. 5.2) [125]	5.2 (5.0, 5.4) [125]	5.2 (5.0, 5.4) [123]	5.2 (5.0, 5.4) [117]	5.2 (5.0, 5.4) [117]	5.3 (5.1, 5.5) [118]
***MTHFR* CC[2]**	40.0 (31.8, 48.8) [50]	30.4 (23.0, 38.9) [38]	36.0 (28.1, 44.7) [45]	45.3 (36.6, 54.3) [53]	36.8 (28.6, 45.8) [43]	25.4 (18.4, 34.0) [30]
***MTHFR* CT[2]**	51.2 (42.5, 59.8) [64]	50.4 (41.8, 59.0) [63]	39.2 (31.1, 48.0) [49]	47.0 (38.2, 56.0) [55]	50.4 (41.5, 59.3) [59]	41.5 (33.0, 50.5) [49]
***MTHFR* TT[2]**	8.8 (5.0, 15.1) [11]	19.2 (13.3, 27.0) [24]	24.8 (18.1, 33.0)** [31]	7.7 (4.1, 14.0) [9]	12.8 (7.9, 20.1) [15]	33.1 (25.2, 42.0)*** [39]

Abbreviations: BMI, Body Mass Index; EGRAC, Erythrocyte Glutathione Reductase Activation; *MTHFR*, Methylene Tetrahydrofolate Reductase 677C>T polymorphism.

24 participants were excluded after the medical checkup due to declared B vitamin supplement use. A further 59 participants were excluded from all analyses involving tHcy because their blood samples were not processed within 2 h of collection and 5 participants because they had suspected altered renal function

Results

(plasma creatinine >97 mmol/L for women and >124 mmol/L for men).
[1]median (P25, P75), [2]% (95% CI), [3]category of habitual alcohol intake (none to low [0 to <10 g/d], low to moderate [10-20 g/d in women and 10-40 g/d in men], high [≥30 g/d in women and ≥40 g/d in men); [4]geometric mean (95% CI), [5]EGRAC ≥1.4; [6]EGRAC ≥1.2 - <1.4.
Chi-square test comparing categorical variables and ANOVA comparing continuous variables between tHcy tertiles, $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$.

Table 8 shows the associations between nonmodifiable and lifestyle factors with tHcy by multiple linear regression analysis in the overall population and separated by sex. Ranking by size of standardised β coefficients, *MTHFR* TT vs CC genotype followed by age, sex, plasma cobalamin and plasma folate were the strongest predictors of tHcy in all the participants. *MTHFR* TT vs CC genotype, age and sex were positively associated with tHcy but plasma cobalamin and folate were negatively associated. In women the overall influence of modifiable predictors of tHcy was more important than that of nonmodifiable predictors. The most important predictor in women was age and plasma cobalamin the most inversely associated with tHcy. Smoking, plasma creatinine and plasma folate were also important factors. In men, the R^2 of all the models were similar meaning that they explained similar proportions of variability in tHcy. The most important determinant of tHcy was the *MTHFR* 677TT genotype followed by plasma folate, age and plasma cobalamin. Plasma folate and plasma cobalamin were negatively associated with tHcy.

Age interacted significantly with the *MTHFR* 677 C>T genotype in men but not in women. In this case as there is a significant interaction between them they cannot be included together in the same model so we proceed with subsequent analyses, stratifying by genotype.

Table 8. Multiple linear regression analysis of factors associated with fasting plasma total homocysteine in all participants and separately by sex.

Model	adjusted $R^{2,1}$	Independent variables	Standardized β^2	p value
All participants (*N* = 687)	0.184***	Age group[3]	0.206	0.000
Model 1 (Non modifiable factors)[4]		Sex	0.305	0.000
		MTHFR TT vs CC genotype	0.358	0.000
		MTHFR CT vs CC genotype	0.095	0.025
		*Interaction MTHFR genotype*age group*		0.056
Model 2 (model 1 + modifiable lifestyle factors)[4,5]	0.194***	Age group[3]	0.196	0.001
		Sex	0.237	0.000
		MTHFR TT vs CC genotype	0.348	0.000
		MTHFR CT vs CC genotype	0.084	0.048
		*Interaction MTHFR genotype*age group*		0.071
		Cigarettes per day	0.079	0.030
		Plasma creatinine (µmol/l)	0.097	0.033
Model 3 (Model 2 + 1CM nutrient status)[4,5,6]	0.259***	Age group[3]	0.266	0.000
		Sex	0.198	0.000
		MTHFR TT vs CC genotype	0.325	0.000
		MTHFR CT vs CC genotype	0.069	0.089
		*Interaction MTHFR genotype*age group*		0.030
		Cigarettes per day	0.053	0.138
		Plasma creatinine (µmol/l)	0.111	0.011
		Plasma cobalamin (pmol/L)	-0.198	0.000
		Plasma folate (nmol/L)	-0.173	0.000
		EGRAC	-0.051	0.143

Women (*N*= 349)	0.090***	Age group	0.263	0.001
Model 1 (Non modifiable factors)[4]		*MTHFR* TT vs CC genotype	0.308	0.000
		MTHFR CT vs CC genotype	0.095	0.134
		*Interaction MTHFR genotype*age group*		0.314
Model 2 (model 1 + modifiable lifestyle factors)[4,5]	0.137***	Age group	0.281	0.001
		MTHFR TT vs CC genotype	0.287	0.000
		MTHFR CT vs CC genotype	0.089	0.156
		*Interaction MTHFR genotype*age group*		0.341
		Cigarettes per day	0.165	0.002
		Plasma creatinine (µmol/l)	0.133	0.008
Model 3 (Model 2 + 1CM nutrient status)[4,5,6]	0.211***	Age group	0.371	0.000
		MTHFR TT vs CC genotype	0.271	0.000
		MTHFR CT vs CC genotype	0.068	0.258
		*Interaction MTHFR genotype*age group*		0.162
		Cigarettes per day	0.117	0.025
		Plasma creatinine (µmol/l)	0.161	0.001
		Plasma cobalamin (pmol/L)	-0.251	0.003
		Plasma folate (nmol/L)	-0.161	0.000
		EGRAC	-0.086	0.094
Men (*N*= 337)	0.128***	Age group	0.161	0.040
Model 1 (Non modifiable factors)[4]		*MTHFR* TT vs CC genotype	0.453	0.000
		MTHFR CT vs CC genotype	0.106	0.085
		*Interaction MTHFR genotype*age group*		0.089
Model 2 (model 1 + modifiable lifestyle factors)[4,5]	0.128***	Age group	0.136	0.106
		MTHFR TT vs CC genotype	0.454	0.000

		MTHFR CT vs CC genotype	0.100	0.109
		*Interaction MTHFR genotype*age group*		0.066
		Cigarettes per day	0.015	0.784
		Plasma creatinine (µmol/l)	0.013	0.810
Model 3 (Model 2 + 1CM nutrient status)[4,5,6]	0.129***	Age group	0.217	0.011
		MTHFR TT vs CC genotype	0.422	0.000
		MTHFR CT vs CC genotype	0.091	0.080
		*Interaction MTHFR genotype*age group*		0.025
		Cigarettes per day	-0.009	0.868
		Plasma creatinine (µmol/l)	0.008	0.870
		Plasma cobalamin (pmol/L)	-0.175	0.001
		Plasma folate (nmol/L)	-0.227	0.000
		EGRAC	-0.002	0.974

1CM, 1C metabolism; EGRAC, Erythrocyte Glutathione Reductase Activation assay; *MTHFR*, Methylenetetrahydrofolate Reductase, *SLC19A1*, Solute Carrier family 19A member. [1]Corresponding with each model; [2]From the complete models; [3]≤ 50 y, > 50 y; [4]adjusted for SLC19A1 80GA versus GG and SLC19A1 80AA versus GG genotypes; [5]adjusted for the same variables as Model 1 plus low versus mid-high socioeconomic status, BMI, moderate (<16 g/d in women, <24 g/d in men) versus no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) versus no alcohol consumption, number of cigarettes smoked/ d and plasma creatinine; [6]adjusted for the same variables as Model 3. Missing data is due to some incomplete lifestyle questionnaires or insufficient blood sample for all of the determinations. Only data relating to blood samples processed in <2 h of collection were included in the models. ***P<0.001

Table 9 shows the relationship between factors associated with tHcy attested by multiple linear regression analysis according to *MTHFR* 677 C>T genotype. In the *MTHFR* 677 CC group the most important factor was sex, age group and plasma folate. In the CT group the strongest associations were plasma cobalamin, sex, plasma folate and number of cigarettes/day. Plasma cobalamin and folate were inversely associated with tHcy. In the *MTHFR* 677 TT group only plasma cobalamin and folate were associated with tHcy.

Table 9. Multiple linear regression analysis of factors associated with fasting plasma total homocysteine in all participants and separately according to *MTHFR* 677C>T genotype.

Model	adjusted $R^{2,1}$	Independent variables	Standardized β^{2}	p value
***MTHFR* 677CC genotype (*N*= 241)**	0.117***	Sex	0.298	0.000
Model 1 (Non modifiable factors)[4]		Age group[3]	0.200	0.001
Model 2 (model 1 + modifiable lifestyle factors)[4,5]	0.100***	Sex	0.290	0.002
		Age group[3]	0.162	0.033
		Cigarettes/ day	0.007	0.920
Model 3 (Model 2 + 1CM nutrient status)[4,5,6]	0.140***	Sex	0.268	0.004
		Age group[3]	0.227	0.004
		Cigarettes/ day	-0.002	0.974
		Plasma cobalamin (pmol/L)	-0.064	0.301
		Plasma folate (nmol/L)	-0.217	0.001
		EGRAC	-0.069	0.279
***MTHFR* 677CT genotype (*N*= 322)**	0.126***	Sex	0.327	0.000
Model 1 (Non modifiable factors)[4]		Age group[3]	0.174	0.001
Model 2 (model 1 + modifiable lifestyle factors)[4,5]	0.167***	Sex	0.181	0.017
		Age group[3]	0.125	0.048
		Cigarettes/ day	0.151	0.006
Model 3 (Model 2 + 1CM nutrient status)[4,5,6]	0.239***	Sex	0.157	0.032
		Age group[3]	0.055	0.322
		Cigarettes/ day	0.151	0.006
		Plasma cobalamin (pmol/L)	-0.235	0.000
		Plasma folate (nmol/L)	-0.156	0.005
		EGRAC	-0.073	0.160
***MTHFR* 677TT genotype (*N*= 122)**	0.087**	Sex	0.340	0.000

Results

Model 1 (Non modifiable factors)[4]		Age group[3]	-0.028	0.752
Model 2 (model 1 + modifiable lifestyle factors)[4,5]	0.083*	Sex	0.246	0.043
		Age group[3]	0.067	0.532
		Cigarettes/ day	0.088	0.344
Model 3 (Model 2 + 1CM nutrient status)[4,5,6]	0.266***	Sex	0.146	0.187
		Age group[3]	0.104	0.292
		Cigarettes/ day	0.088	0.344
		Plasma cobalamin (pmol/L)	-0.417	0.000
		Plasma folate (nmol/L)	-0.191	0.032
		EGRAC	-0.008	0.924

Abbreviations: 1CM, 1C metabolism; EGRAC, Erythrocyte Glutathione Reductase Activation assay; *MTHFR*, Methylenetetrahydrofolate Reductase, *SLC19A1*, Solute Carrier family 19A member.

[1]Corresponding with each model; [2]From the complete models; [3]≤ 50 y, > 50 y; [4]adjusted for SLC19A1 80GA versus GG and SLC19A1 80AA versus GG genotypes; [5]adjusted for the same variables as Model 1 plus low versus mid-high socioeconomic status, BMI, moderate (<16 g/d in women, <24 g/d in men) versus no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) versus no alcohol consumption, number of cigarettes smoked/ d and plasma creatinine; [6]adjusted for the same variables as Model 3. Missing data is due to some incomplete lifestyle questionnaires or insufficient blood sample for all of the determinations. Only data relating to blood samples processed in <2 h of collection were included in the models. ***P<0.001, **P<0.01, *P<0.05.

Results

Table 10 shows probability of having diagnosed hypertension when tHcy is in the 3rd tertile compared to the 1st. Age and BMI were significant predictors of hypertension in all models. We can observe that even after adjustment for multiple confounding factors, being in the 3rd tertile of tHcy compared to the 1st, increases the risk of having hypertension by 1.8 times in the overall population studied. Stratifying by age, an association between moderately elevated tHcy and hypertension [OR= 2.5 (1.2, 5.4)] was only observed in the people in the >50 years age group.

Table 10. Association between moderately elevated fasting plasma total homocysteine and diagnosed hypertension.

	All participants		Aged ≤50 years		Aged >50 years	
Model	$R^{2,1}$		R^2		R^2	
1	0.024* [583]	1.9 (1.2, 3.0)[2]	0.006 [418]	1.5 (0.6, 3.5)	0.079** [165]	2.8 (1.5, 5.5)
2	0.202***	1.9 (1.2, 3.0)	0.083**	1.5 (0.6, 3.7)	0.108**	2.5 (1.3, 4.9)
3	0.492***	1.8 (1.0, 3.3)	0.372***	1.2 (0.4, 3.5)	0.351***	2.5 (1.2, 5.4)

Multiple logistic regression analysis was used. [1]Nagelkerke R^2; [2]OR (95% CI) for diagnosed hypertension in participants in the 3rd versus the 1st and 2nd age and sex specific tHcy tertiles are shown. Cut offs for the 3rd tertiles were ≥9.09 µmol/L in women ≤50 years, ≥10.60 µmol/L in women >50, ≥10.88 µmol/L in men ≤50 years, ≥11.59 µmol/L in men >50. Participants without diagnosed hypertension but with point blood pressure measurements >140/90 mm Hg, at the study check-up, were referred for blood pressure monitoring and excluded from the analysis (N= 77). A further 41 participants without diagnosed hypertension but with no point blood pressure measurement and BMI >30 as well as 5 participants with possible impaired renal function (plasma creatinine concentration >124 mmol/L in men and >97 mmol/L in women) were also excluded. Only tHcy determinations performed in samples processed in less than 2 hours of collection were included. Model 1: (basic model) having tHcy in the 3rd tertile compared to thcy in the 1st and 2nd tertiles. Model 2: Included the same variables as model 1 as well as low versus mid-high socioeconomic status. Model 3: Included the same variables as model 2 as well as BMI, category of regular alcohol intake (moderate [<16 g/d in women and <24 g/d in men] versus none; high versus none [≥16 g/d in women and ≥24 g/d in men]), current smoking (cigarettes/ d) and total plasma cholesterol (mmol/L). ***P<0.001, **P<0.01, *P<0.05.

The associations between the *MTHFR* 677 C>T polymorphism and diagnosed hypertension is shown in Table 11. We found no significant association between any variant of the *MTHFR* 677 C>T polymorphism and diagnosed hypertension in the overall population. Having the TT genotype increases the risk of having hypertension compared to the other genotypes in all models of people aged ≤ 50 years [OR= 8.2 (1.3, 53.9)]. No significant association was found between *MTHFR* 677 C>T polymorphism and diagnosed hypertension in people aged >50 years.

Table 11. Association between the *MTHFR* 677 C>T polymorphism and diagnosed hypertension.

	All participants			Aged ≤50 years			Aged >50 years		
Model	$R^{2,1}$	CT vs CC[2]	TT vs CC[2]	R^2	CT vs CC	TT vs CC	R^2	CT vs CC	TT vs CC
1	0.003 [573]	1.2 (0.7, 1.9)[3]	1.4 (0.7, 2.6)	0.037 [410]	3.3 (0.9, 11.7)	4.1 (1.0, 16.9)	0.002 [163]	1.1 (0.6, 2.2)	1.3 (0.5, 3.1)
2	0.433***	1.5 (0.8, 2.8)	1.5 (0.7, 3.4)	0.160***	3.2 (0.9, 11.6)	4.0 (0.9, 17.0)	0.059	1.1 (0.5, 2.1)	1.2 (0.5, 3.0)
3	0.585***	1.2 (0.6, 2.6)	1.7 (0.7, 4.4)	0.472***	3.8 (0.7, 20.3)	8.2 (1.3, 53.9)	0.348***	1.0 (0.4, 2.2)	1.2 (0.4, 3.7)

[1]Nagelkerke R^2 from multiple logistic regression analysis; [2]Methylenetetrahdyrofolate reductase (*MTHFR* 677C>T) genotype.

[3]OR (95% CI) for diagnosed hypertension in participants with the CT vs CC genotype and TT vs CC genotype, globally and according age group. Participants that did not have diagnosed hypertension but point blood pressure measurements greater than 140/90, at the study check-up, were referred for blood pressure monitoring and excluded from the analysis (N= 77). A further 41 participants with no point blood pressure measurement and BMI > 30 and 5 participants with plasma creatinine concentration >124 mmol/L in men and >97 mmol/L in women (indicating possible impaired renal function) were also excluded. Model 1: (basic model) including the predictor variables *MTHFR* 677 CT versus CC and *MTHFR* 677 TT versus CC genotypes. Model 2: Included the same variables as model 1 as well as sex, age and BMI. Model 3: Included the same variables as model 2 as well as plasma folate, plasma cobalamin, erythrocyte glutathionine reductase activation coefficient (functional indicator of riboflavin status) low versus mid-high socioeconomic status, category of regular alcohol intake (moderate [<16 g/d in women and <24 g/d in men] versus none; high [≥16 g/d in women and ≥24 g/d in men]) versus none, current smoking (cigarettes/d) and serum total cholesterol. *** $P<0.001$.

2. Reus-Tarragona Birth Cohort

Descriptive baseline characteristics during pregnancy of the women whose children participated in the child follow-up phase are summarised in Table 12. Their mean age was 32.3 (95% CI: 31.8, 32.8) years, mean weight was 63.3 (95% CI: 61.7, 64.9) kg, mean height was 163.0 (95% CI: 162.2, 163.8) cm and mean first trimester BMI was 23.8 (95% CI: 23.2, 24.3) kg/m^2. Of these women, 19.2% (95% CI: 14.5, 25.1) were overweight and 5.3% (95% CI: (3.0, 9.2)) were obese. Gestational hypertension occurred in 4.3% (CI: (2.3, 8.0) of the pregnancies. In the first trimester of pregnancy 39 of the women smoked. Of these, 2 (0.9% of the women participating in this phase of the study) smoked only during the first trimester, then stopped smoking and 37 (17.5%) continued smoking throughout pregnancy. Regarding alcohol intake, 19.3% of the women reported drinking some kind of alcoholic beverages throughout pregnancy. This included all types and patterns of alcohol intake. Mainly low or sporadic intake of small amounts of alcohol during pregnancy, were reported. Low socioeconomic status was observed in 3.8% of the women.

That the pregnancies were planned was reported by 79.9% of the women. Distributions of the CC, CT and TT genotypes of the *MTHFR* 677C>T polymorphism were 37.6%, 41.9% and 20.5% respectively. Folic acid supplement use was very high during the first trimester of pregnancy (85.0%). Anemia (hemoglobin < 11.0 dg/L) was present in 2.9% of women during the first trimester.

Table 12. Baseline descriptive characteristics of mothers during pregnancy.

Age, years [1]	32.3 (31.8, 32.8) [212]
Weight, kg [1]	63.3 (61.7, 64.9) [209]
Height, cm [1]	163.0 (162.2, 163.8) [210]
BMI, kg/m^2 [1]	23.8 (23.2, 24.3) [209]
Underweight, % [2]	2.9 (1.3, 6.1) [6]
Normal weight, % [2]	72.6 (66.2, 78.2) [151]
Overweight, % [2]	19.2 (14.5, 25.1) [40]
Obese, % [2]	5.3 (3.0, 9.2) [11]
Gestational hypertension, % [2]	4.3 (2.3, 8.0) [9]
Smoking, %	
1st trimester only [2]	0.9 (0.3, 3.4) [2]
All pregnancy [2]	17.5 (12.9, 23.1) [37]
Alcohol consumption, % [2]	19.3 (14.4, 25.4) [38]
Socioeconomic status, % [2]	
Low	3.8 (1.9, 7.3) [8]
Mid	45.8 (39.2, 52.5) [97]
High	50.5 (43.8, 57.1) [107]
Planned pregnancies, % [2]	79.9 (73.8, 84.9) [159]
Previous pregnancies, % [2]	50.5 (43.8, 57.1) [107]
Previous abortion, % [2]	34.6 (28.5, 41.2) [73]
Previous preterm, % [2]	4.7 (2.6, 8.5) [10]
***MTHFR*, %** [2]	
CC	37.6 (31.3, 44.3) [79]
CT	41.9 (35.4, 48.7) [88]
TT	20.5 (15.6, 26.4) [43]
1st trimester folic acid containing supplements, % [2]	85.0 (79.4, 84.3) [170]
1st trimester anemia, % [2]	2.9 (1.1, 7.3) [4]

Abbreviations: BMI, Body Mass Index; *MTHFR*= Methylene Tetrahydrofolate Reductase.
[1]arithmetic mean (95% CI), [2]% (95% CI).

Maternal 1CM nutritional status (plasma folate, RBC folate, plasma cobalamin, tHcy and EGRAC) during pregnancy according to *MTHFR* 677 C>T polymorphism is shown in Figure 9. Plasma folate and plasma cobalamin decreased during pregnancy independently of the *MTHFR* 677 C>T polymorphism. Significant differences were observed in RBC folate between the genotypes but it increased in all genotypes until 24-27 GW, and then decreased. Lower RBC folate was found in pregnant women with the TT compared to CC genotype throughout pregnancy. The pattern of change in MMA and tHcy throughout pregnancy was the opposite to RBC folate, as it decreased until 24-27 GW and then increased. In labour tHcy was at its highest. EGRAC increased during pregnancy in all but the CT genotype. For logistical reasons, samples collected in the labour ward were not prepared for RBC folate or EGRAC determinations.

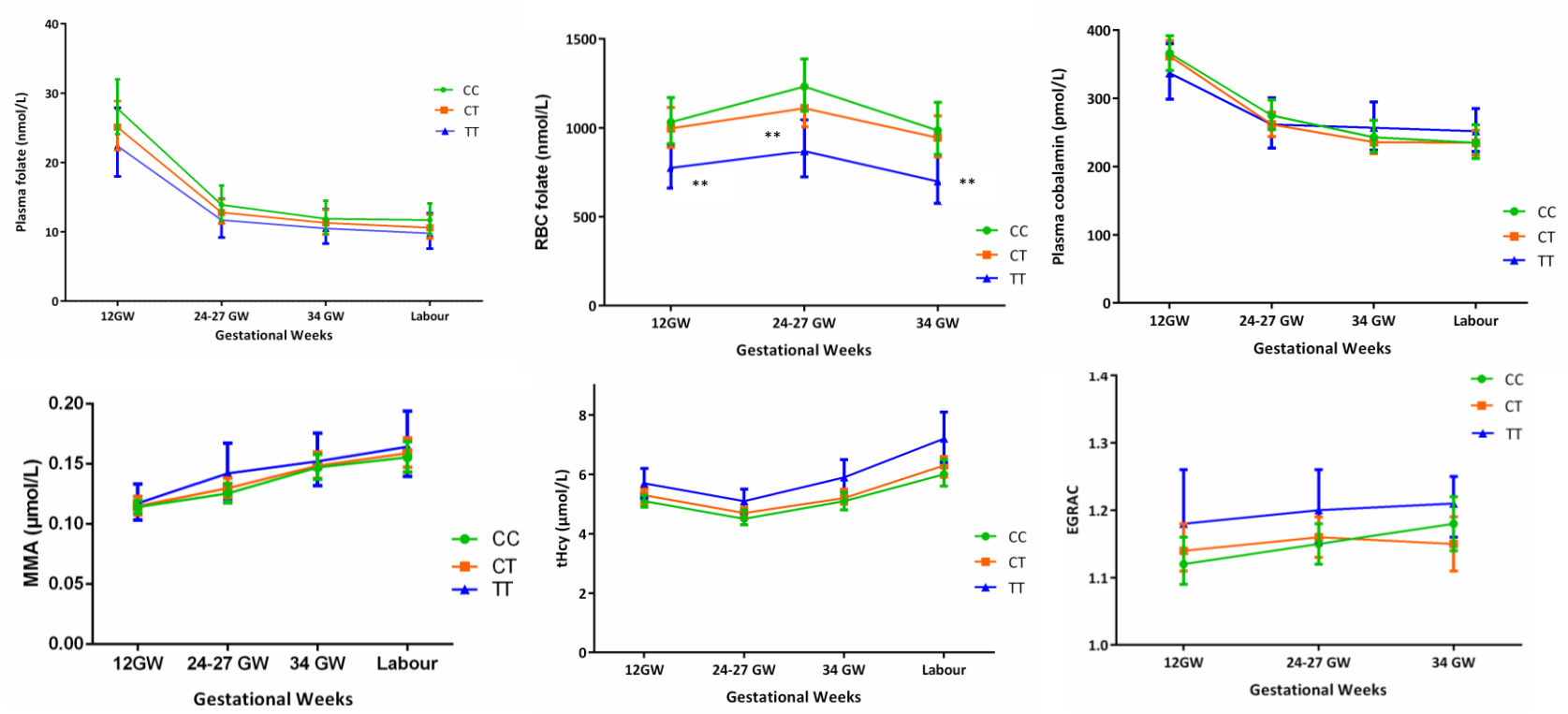

Figure 9. Maternal 1C metabolism (plasma folate, RBC folate, plasma cobalamin, tHcy and EGRAC) nutritional status during pregnancy depending on their *MTHFR* 677 C>T polymorphism.

Abbreviations: RBC folate= Red Blood Cell folate; tHcy= fasting total plasma homocysteine.

ANOVA repeated measures was used to compare changes in plasma concentrations between time points and between genotypes.

** $p<0.01$, TT compared to CC.

For plasma folate, plasma cobalamin and tHcy at 12GW of pregnancy there were 79, 86 and 43 participants with the *MTHFR* 677 CC, CT and TT respectively. At 24-27 GW there were 72, 83 and 39 participants with the *MTHFR* 677 CC, CT and TT respectively, 69, 80 and 39 at 34 GW and 67, 77 and 34 at labour respectively.
For RBC folate at 12GW of pregnancy there were 77, 86 and 43 participants with the *MTHFR* 677 CC, CT and TT respectively. At 24-27 GW there were 72, 83 and 39 participants with the *MTHFR* 677 CC, CT and TT respectively and 67, 80 and 39 at 34 GW respectively.
For EGRAC at 12GW of pregnancy there were 68, 72 and 36 participants with the *MTHFR* 677 CC, CT and TT respectively. At 24-27 GW there were 63, 70 and 34 participants with the *MTHFR* 677 CC, CT and TT and 63, 68 and 36 at 34 GW respectively.

The baseline characteristics of 212 child participants are described in Table 13. 52.8% of the participants were girls and 47.2% were boys and mean age was 90.2 months. Mean weight was 26.6 kg and mean height was 126.9 cm. Mean BMI was 16.5 kg/m^2 and according to the International Obesity Task Force criteria the prevalence of overweight children was 18.6% and obesity was present in 7.1% of the children. No significant differences between sexes were observed for overweight or obesity prevalences.

Girls had lower head circumference compared to boys. Girls also had higher tricipital, bicipital, subscapular, thigh and suprailiac skinfold thicknesses compared to boys. Girls' fat mass was higher than boys'. The prevalence of the different genotypes of the *MTHFR* 677 C>T polymorphism were 37.4%, 43.7% and 18.9% respectively.

Table 13. Baseline descriptive characteristics of children by sex during the follow-up phase at 7.5 years of age.

	Total	Girls	Boys
Sex, % [1]		52.8 (46.1, 59.4) [112]	47.2 (40.6, 53.9) [100]
Age, months [2]	90.2 (89.9, 90.4) [212]	90.1 (89.7, 90.5) [112]	90.3 (89.9, 90.6) [100]
Weight, kg [2]	26.6 (26.0, 27.3) [210]	26.7 (25.7, 27.7) [112]	26.5 (25.7, 27.4) [98]
Height, cm [2]	126.9 (126.2, 127.6) [210]	127.1 (126.1, 128.0) [112]	126.7 (125.7, 127.7) [98]
BMI, kg/m^2 [2] [2]	16.5 (16.1, 16.8) [210]	16.5 (16.0, 17.0) [112]	16.4 (16.1, 16.8) [98]
Overweight, % [1]	18.6 (13.9, 24.4) [39]	20.5 (14.1, 28.9) [23]	16.3 (10.3, 24.9) [16]
Obese, % [1]	7.1 (4.4, 11.4) [15]	8.9 (4.9, 15.7) [10]	5.1 (2.2, 11.4) [5]
Head circumference, cm [2]	51.8 (51.6, 52.0) [210]	51.4 (51.1, 51.6) [112]	52.3 (52.0, 52.7)*** [98]
Arm circumference, cm [2]	19.1 (18.8, 19.5) [210]	19.2 (18.8, 19.7) [112]	19.0 (18.6, 19.5) [98]
Trunk circumference, cm [2]	63.5 (60.5, 66.4) [206]	61.6 (60.6, 62.7) [110]	65.6 (59.4, 71.8) [96]
Waist circumference, cm [2]	56.8 (55.9, 57.7) [210]	56.6 (55.3, 57.9) [112]	57.0 (55.9, 58.1) [98]
Hip circumference, cm [2]	66.3 (65.5, 67.1) [206]	67.1 (65.9, 68.2) [110]	65.5 (64.3, 66.6) [96]
Leg circumference, cm [2]	35.6 (35.0, 36.2) [200]	35.9 (34.9, 36.8) [105]	35.3 (34.6, 36.1) [95]
Tricipital skinfold, mm [2]	10.5 (9.9, 11.0) [205]	11.4 (10.6, 12.2) [108]	9.5 (8.7, 10.3)*** [97]
Bicipital skinfold, mm [2]	6.5 (6.0, 6.9) [204]	7.2 (6.6, 7.8) [107]	5.7 (5.1, 6.2)*** [97]

Subscapular skinfold, mm [2]	6.7 (6.2, 7.3) [206]	7.5 (6.7, 8.4) [109]	5.8 (5.3, 6.4)*** [97]
Thigh skinfold, mm [2]	17.0 (16.0, 18.0) [193]	18.7 (17.3, 20.1) [97]	15.3 (14.0, 16.7)*** [96]
Suprailiac skinfold, mm [2]	10.1 (9.2, 11.0) [201]	11.6 (10.3, 12.9) [105]	8.4 (7.2, 9.6)*** [96]
Fat mass, kg [2]	5.4 (5.1, 5.8) [206]	5.8 (5.2, 6.4) [110]	5.0 (4.6, 5.4)* [96]
Fat free mass, kg [2]	21.2 (20.8, 21.5) [206]	20.9 (20.4, 21.3) [110]	21.5 (21.0, 22.1) [96]
***MTHFR*, %** [1]			
CC	37.4 (30.8, 44.4) [71]	38.2 (29.4, 47.9) [39]	36.4 (27.1, 46.8) [32]
CT	43.7 (36.8, 50.8) [83]	44.1 (34.9, 53.8) [45]	43.2 (33.3, 53.6) [38]
TT	18.9 (14.0, 25.1) [36]	17.6 (11.5, 26.2) [18]	20.5 (13.3, 30.0) [18]

Abbreviations: BMI= Body Mass Index; *MTHFR*= Methylene Tetrahydrofolate Reductase.
The N is always shown as [ ]. [1]% (95% CI), [2]arithmetic mean (95% CI).
Differences between sexes were calculated with chi-square test for categorical variables and ANOVA for continuous variables.
***P<0.001, *P<0.05.

Table 14. Baseline office BP and ABPM in children at follow-up at 7.5 years.

	Total	Girls	Boys
BP at check-up (Office BP)			
SBP mean, mmHg [1]	103.8 (102.7, 105.0) [183]	104.0 (102.3, 105.7) [100]	103.7 (102.3, 105.1) [83]
DBP mean, mmHg [1]	64.1 (62.7, 65.6) [183]	65.5 (64.3, 66.9) [100]	62.4 (59.8, 65.2)* [83]
Normal tension, % [2]	84.9 (78.2, 89.8) [124]	78.2 (67.8, 85.9) [61]	92.6 (83.9. 96.8) [63]
Prehypertension,% [2]	8.9 (5.3, 14.6) [13]	11.5 (6.2, 20.5) [9]	5.9 (2.3, 14.2) [4]
Hypertension, % [2]	6.2 (3.3, 11.3) [9]	10.3 (5.3, 19.0) [8]	1.5 (0.3, 7.9) [1]
ABPM			
Systolic ABPM mean, mmHg [1]	103.1 (102.1, 104.0) [170]	101.9 (100.6, 103.2) [90]	104.3 (102.7, 105.9)* [80]
Diastolic ABPM mean, mmHg [1]	61.5 (60.8, 62.1) [170]	61.3 (60.4, 62.1) [90]	61.8 (60.6, 62.7) [80]
Systolic ABPM day mean, mmHg [1]	106.9 (100.8, 108.0) [165]	105.4 (104.0, 106.8) [88]	108.6 (107.0, 110.3)** [77]
Diastolic ABPM day mean, mmHg [1]	65.9 (65.1, 66.7)[165]	65.7 (64.6, 66.9) [88]	66.1 (64.9, 67.3) [777
Systolic ABPM night mean, mmHg [1]	98.9 (97.8, 100.0) [160]	98.1 (96.6, 99.5) [85]	99.8 (98.0, 101.6) [75]
Diastolic ABPM night mean, mmHg [1]	56.6 (55.9, 57.3) [160]	56.4 (55.5, 57.4) [85]	56.8 (55.8, 57.9) [75]
Valid readings [1]	37 (36, 39) [170]	37 (34, 39) [90]	38 (36, 41) [80]
Normal tension, % [2]	92.0 (85.9, 95.6) [115]	85.3 (75.0, 91.8) [58]	100.0 (93.7, 100.0) [57]
White coat hypertension, % [2]	6.4 (3.3, 12.1) [8]	11.8 (6.1, 21.5) [8]	-
Masked hypertension, % [2]	-	-	-
Prehypertension,% [2]	1.6 (0.4, 5.6) [2]	2.9 (0.8, 10.1) [2]	-
% hypertension [2]	-	-	-

Abbreviations: ABPM= Ambulatory Blood Pressure Monitoring; BP= Blood Pressure; DBP= Diastolic Blood Pressure; SBP= Systolic Blood Pressure.

[1]geometic mean (95% CI), [2]% (95% CI).

Chi-square test for categorical variables and ANOVA for continuous variables, **P<0.01, *P<0.05.

Table 14 describes mean offspring BP readings at 7.5 years depending on the method used, BP at the check-up (office BP) or ABPM. BP is classified in categories depending on the technique used, as different criteria for diagnosing hypertension correspond with each method.

Mean SBP and DBP at check-up were 103.8 and 64.1 mmHg, respectively. Differences between girls and boys were found only in DBP. Looking at ABPM, mean SBP and DBP were 103.1 and 61.5 mmHg, respectively. Mean systolic ABPM in girls was lower than in boys. Mean diastolic ABPM was similar between sexes. As ABPM is measured for 24h, data is collected day and night. Mean daytime systolic ABPM for girls was lower than in boys. The mean number of valid readings per child was 37. Classifying BP using office BP criteria in children (previously explained in the Introduction section) we found that 84.9% of children were normotensive, 8.9% had prehypertension and 6.2% of them had hypertension. The prevalence of normal BP, prehypertension and hypertension with ABPM was 92.0%, 1.6% and 0.0%, respectively. With ABPM we added a new category called white coat hypertension in which the prevalence was 6.4%.

Baseline anthropometric, biochemical and genetic characteristics of children at 7.5 years with hypertension and prehypertension versus children with normal tension are shown in Table 15. There were more girls in the hypertensive group compared to the normal blood pressure group. Significant differences between groups were found in weight, BMI, arm, trunk, waist and hip circumference, tricipital, bicipital, subscapular, thigh and suprailiac skinfold and fat mass. Hypertensive children weighed more and had higher BMI, arm, trunk, waist and hip circumference, tricipital, bicipital, subscapular, thigh and suprailiac skinfold thicknesses and fat mass. No differences in any of the 1CM biochemical parameters were observed between the blood

pressure categories. The *MTHFR* 677 TT genotype was found in 23.1% of children with normal BP and 10.0% of the prehypertension or hypertension group.

Table 15. Baseline descriptive characteristics of children with prehypertension + hypertension vs normal tension.

	Prehypertension + hypertension	Normal tension
Sex, % [1]		
Girls, % [1]	77.3 (56.6, 89.9) [17]	49.2 (40.6, 57.9)* [61]
Boys, % [1]	22.7 (10.1, 43.4) [5]	50.8 (42.1, 59.4) [63]
Age, months [2]	89.6 (89.1, 90.0) [22]	90.1 (89.8, 90.4) [124]
Weight, kg [2]	29.4 (26.4, 32.4) [22]	25.8 (25.0, 26.6)** [124]
Height, cm [2]	126.4 (124.1, 128.7) [22]	126.7 (125.9, 127.6) [124]
BMI, kg/m^2 [2]	18.4 (16.7, 20.0) [22]	16.0 (15.7, 16.3)*** [124]
Overweight, % [1]	45.5 (26.9, 65.3) [10]	12.9 (8.1, 19.9)*** [16]
Obese, % [1]	27.3 (13.2, 48.2) [6]	4.0 (1.7, 9.1)*** [5]
Head circumference, cm [2]	52.0 (51.1, 52.8) [22]	51.8 (51.5, 52.1) [124]
Arm circumference, cm [2]	20.2 (19.0, 21.5) [22]	18.7 (18.3, 19.1)** [124]
Trunk circumference, cm [2]	64.2 (60.8, 67.6) [21]	61.6 (60.9, 62.3)* [123]
Waist circumference, cm [2]	59.9 (56.2, 63.7) [22]	55.7 (54.7, 56.8)** [124]
Hip circumference, cm [2]	68.8 (65.5, 72.1) [21]	65.2 (64.3, 66.1)** [124]
Leg circumference, cm [2]	36.3 (33.8, 38.9) [20]	35.4 (34.6, 36.2) [122]
Tricipital skinfold, mm [2]	13.7 (11.4, 15.9) [21]	9.6 (9.0, 10.2)*** [122]
Bicipital skinfold, mm [2]	9.6 (7.7, 11.5) [21]	5.8 (5.4, 6.3)*** [122]
Subscapular skinfold, mm [2]	10.3 (7.2, 13.4) [21]	5.9 (5.4, 6.4)*** [123]
Thigh skinfold, mm [2]	23.1 (18.9, 27.2) [18]	15.3 (14.2, 16.4)*** [119]
Suprailiac skinfold, mm [2]	15.7 (11.7, 19.6) [21]	8.5 (7.6, 9.5)*** [121]
Fat mass, kg [2]	7.4 (5.5, 9.2) [22]	4.9 (4.5, 5.3)*** [123]

Fat free mass, kg [2]	21.6 (20.3, 22.9) [22]	20.9 (20.4, 21.3) [123]
SBP (mmHg) [2]	114.4 (111.3, 117.6)[22]	102.1 (101.0, 103.2) [124]
DBP (mmHg) [2]	82.3 (62.5, 102.2) [22]	62.4 (61.4, 63.4) [124]
Plasma folate (nmol/L) [3]	17.7 (14.6, 21.6) [17]	18.4 (16.4, 20.6) [91]
RBC folate (nmol/L) [2]	936 (718, 1153) [15]	974 (889, 1060) [90]
Plasma cobalamin (pmol/L) [3]	659 (593, 733) [17]	628 (593, 666) [90]
MMA (µmol/L) [3]	0.15 (0.12, 0.14) [17]	0.13 (0.12, 0.14) [91]
tHcy (µmol/L) [2]	5.6 (5.0, 6.2) [17]	5.6 (5.4, 5.9) [91]
***MTHFR*, %** [1]		
CC	35.0 (18.1, 56.7) [7]	31.5 (23.5, 40.7) [34]
CT	55.0 (34.2, 74.2) [11]	45.4 (36.3, 54.8) [49]
TT	10.0 (2.8, 30.1) [2]	23.1 (16.2, 31.9) [25]

Abbreviations: ABPM= Ambulatory Blood Pressure Monitoring; BP= Blood Pressure.
[1]arithmetic mean (95% CI), [2]% (95% CI), [3]geometric mean (95% CI).
Chi-square test for categorical variables and ANOVA for continuous variables, $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$.

Figure 10 shows baseline 1C metabolism status of children depending on different genotypes of the *MTHFR* 677 C>T polymorphism. No significant differences in status of any of the 1C metabolism nutrients were found between genotypes.

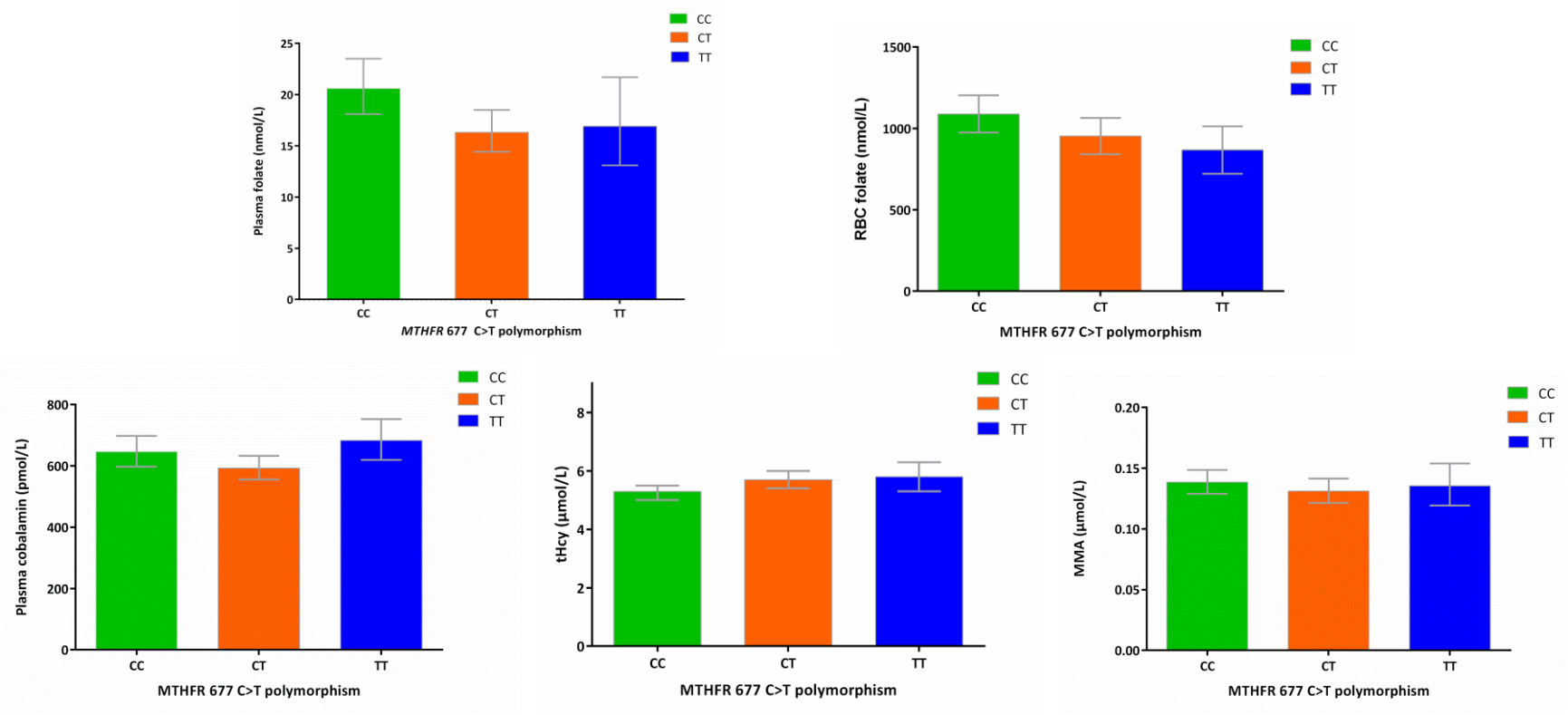

Figure 10. Child 1C metabolism (plasma folate, RBC folate, plasma cobalamin, tHcy and MMA) nutritional status depending on their *MTHFR* 677 C>T polymorphism.
Abbreviations: MMA= Methylmalonic acid; RBC folate= Red Blood Cell folate; tHcy= fasting total plasma homocysteine. Plasma folate, plasma cobalamin and MMA were natural log transformed. ANOVA was used to compare the differences between genotypes.
Geometric means are reported and 95% CI is indicated with error bars. Data for 51 children with the CC genotype, 58 children with the CT genotype and 28 with the TT genotype is reported except for the case of RBC folate where there were 49, 60 and 28 in the CC, CT and TT genotype respectively.

Table 16. Maternal biochemical characteristics during pregnancy, labour and during offspring follow-up at 7.5 years.

	<12 GW	24-27GW	34GW	Labour	Cord	Children 7.5 years
Plasma folate (nmol/L) [1]	25.3 (23.1, 27.7) [209]	13.0 (11.7, 14.3) [195]	11.2 (10.1, 12.5) [190]	10.9 (9.7, 12.1) [178]	24.4 (22.5, 26.4)*** [174]	18.1 (16.6, 19.6)^{1,†††,§§§} [148]
RBC folate (nmol/L) [1]	949 (879, 1024) [207]	1097 (1021, 1179) [195]	888 (814, 970) [188]	-	-	979 (913,1044)^{2,†††,§§§} [148]
Plasma cobalamin (pmol/L) [1]	359 (343, 374) [209]	267 (254, 281) [195]	244 (231, 258) [190]	238 (225, 251) [177]	336 (305, 371)*** [174]	626 (599, 654)^{1,†††,§§§} [147]
holoTC (pmol/L) [1]	71.4 (66.1, 77.2) [177]	57.2 (53.2, 61.4) [170]	64.1 (59.1, 69.4) [163]	59.7 (54.5, 65.3) [151]	167.4 (145.7, 192.4)*** [148]	-
MMA (µmol/L) [1]	0.12 (0.11, 0.12) [208]	0.13 (0.12, 0.14) [195]	0.15 (0.14, 0.16) [190]	0.16 (0.15, 0.17) [178]	0.27 (0.26, 0.28)*** [174]	0.13 (0.13, 0.14)^{1,†††,§§} [148]
EGRAC [1]	1.14 (1.12, 1.17) [177]	1.16 (1.14, 1.18) [168]	1.17 (1.15, 1.20) [169]	-	-	-
tHcy (µmol/L) [1]	5.3 (5.1, 5.5) [209]	4.7 (4.6, 4.9) [195]	5.3 (5.1, 5.5) [190]	6.3 (6.1, 6.6) [178]	5.2 (4.9, 5.4)*** [174]	5.5 (5.3, 5.7)^{2,†††,§§§} [148]

Abbreviations: EGRAC= Erythrocyte Glutathione Reductase Activation assay; GW= gestational weeks; holoTC= holotranscobalamin; MMA= methylmalonic acid; RBC= Red Blood Cell; tHcy= total fasting plasma homocysteine.

All variables during pregnancy were expressed as geometric mean (95% CI). In children: [1] geometric mean (95% CI); [2] arithmetic mean (95% CI). Differences between maternal biochemical characteristics at labour and cord were assessed with a paired T-test. Differences between first trimester of pregnancy and children at 7.5 years were also tested with paired T-test. Labour-cord: ***P<0.001; 1st trimester-children: †††P<0.001; labour-children: §§P<0.01, §§§P<0.001

Table 16 shows the pattern of changes in 1CM nutrients during different time points of pregnancy and offspring follow-up at 7.5 years. Plasma folate decreases as pregnancy progresses but in the cord it increases. RBC folate status increases but decreases at 34GW. Plasma cobalamin follows the same pattern as plasma folate, decreases throughout pregnancy but in the children at 7.5 years it is very high. holoTC increases until the end of the pregnancy. MMA does the opposite to cobalamin, increases until late pregnancy including in the cord but children have similar results as the mothers in early pregnancy. EGRAC stays stable from early until late pregnancy. HoloTC and EGRAC were not determined in the children. tHcy at early pregnancy decreases but then increases between 34GW and labour. Plasma folate, plasma cobalamin, holoTC and MMA were higher in the cord compared to labour. Significant differences were found with a paired T-test in plasma folate, plasma cobalamin, MMA and tHcy in children at 7.5 years compared to first trimester of pregnancy.

We checked the corresponding *MTHFR* 677 C>T genotypes between the mother-child dyads of our study (Table 17) before proceeding with the multivariate analysis. The genotypes in one mother-child dyad were incoherent (a CC child born to a TT mother) and we assume that this was due to a genotyping/ database error. We excluded this dyad from analyses involving genotypes.

Table 17. Checking mother-child genotypes of the *MTHFR* 677C>T

		Offspring *MTHFR* 677C>T polymorphism frequency (%)		
		CC	CT	TT
Maternal *MTHFR* 677C>T polymorphism frequency (%)	CC	66.2 (54.6, 76.1) [47]	28.9 (20.3, 39.4) [24]	0.0 (0.0, 9.6) [0]
	CT	32.4 (22.7, 43.9) [23]	51.8 (41.2, 62.2) [43]	41.7 (27.1, 57.8) [15]
	TT	1.4 (0.2, 7.6) [1]	19.3 (12.2, 29.0) [16]	58.3 (42.2, 72.9) [21]

MTHFR= Methylene Tetrahydrofolate Reductase.
The N is always shown as [ ]. All results are expressed as % (95% CI).
One mother-child dyad was excluded from this table and further genetic analyses due to incongruence in the genotypes.

First of all we checked whether there was a correlation between office and ABPM systolic and diastolic blood pressures. The correlation between office SBP and systolic ABPM is 0.4 (p=0.000) and the correlation between office DBP and diastolic ABPM is 0.2 (p = 0.011).

In Table 18 we investigated whether demographic, biochemical, lifestyle and genetic factors were associated with SBP and DBP using both methods, office BP and ABPM by multiple linear regression analysis. Our aim was to investigate which variables were significantly associated with SBP and DBP. Both models of office BP (SBP and DBP) were significant. However, in the case of the ABPM models, only the basic model (including child sex and BMI Z-score) was significant for SBP.

Child's BMI z-score is the strongest variable in the complete office SBP model and sex the most important in the office DBP model. Child's BMI z-score was positively associated with both office SBP and DBP. In the office SBP model, child's plasma folate and maternal smoking were also associated with SBP. In the office DBP model, male versus female sex was negatively associated with

DBP and was the most important variable in the model followed by child's BMI z-score and child's *MTHFR* 677 TT vs CC+CT genotype. In the only significant ABPM model (basic model, SBP) male sex was positively associated with SBP. All of the remaining ABPM models were not significant. However, for informative purposes only, the associations between predictors and the dependent variables were assessed and contrasted with those of the office BP models. None of the variables assessed in either of the ABPM models were significantly associated with SBP or DBP. As BMI z-score is the most influencing factor in the office SBP model, we would expect to find the same result in the SBP ABPM model. Surprisingly BMI is not as important in the ABPM SBP model as is in the office SBP model. The rest of the variables followed more or less the same pattern, child's plasma folate and maternal smoking during pregnancy were positively associated with child SBP in both the office and ABPM models. Looking at the ABPM DBP model, child's sex was not the principal factor as it was in office DBP model.

Table 18. Associations between maternal and offspring demographic, lifestyle, biochemical and genetic factors and office and ABPM BP measurements in the child.

		Office BP		ABPM	
		SBP	**DBP**	**SBP**	**DBP**
Preliminary model		**adjusted R^2=0.086***** [183]	**adjusted R^2=0.038**** [183]	**adjusted R^2=0.025**** [169]	**adjusted R^2=0.001** [169]
	Child's variables				
	Sex (ref: girl)	-0.03	-0.16	0.18*	0.03
	BMI z-score	0.31***	0.15	0.07	0.10
Model 1		**adjusted R^2=0.078***** [181]	**adjusted R^2=0.041**** [181]	**adjusted R^2=0.015** [168]	**adjusted R^2=-0.010** [168]
	Child's variables				
	Sex (ref: girl)	-0.02	-0.15*	0.18*	0.03
	BMI z-score	0.31***	0.16*	0.06	0.11
	Maternal variables				
	MTHFR 677 CT (ref: CC)	-0.03	0.10	-0.04	0.00
	MTHFR 677 TT (ref: CC)	-0.06	-0.03	-0.03	0.03
Model 2		**adjusted R^2=0.089**** [149]	**adjusted R^2=0.186***** [149]	**adjusted R^2= -0.016** [140]	**adjusted R^2=-0.009** [141]
	Child's variables				
	Sex (ref: girl)	-0.04	-0.34***	0.14	0.00
	BMI z-score	0.32***	0.26***	0.00	0.07
	Maternal variables				
	MTHFR 677 CT (ref: CC)	0.06	0.10	0.02	0.02
	MTHFR 677 TT (ref: CC)	-0.02	-0.02	-0.02	0.02
	Gestational hypertension	0.15	0.10	0.06	0.07

	Smoking	0.09	0.03	0.10	-0.07
	BMI	-0.20	-0.16	0.08	0.07
	Plasma folate	0.01	-0.08	-0.01	-0.10
	EGRAC	-0.05	-0.10	-0.06	-0.15
Model 3		**adjusted R^2=0.145*** [97]	**adjusted R^2=0.295***** [97]	**adjusted R^2=-0.029** [101]	**adjusted R^2=-0.039** [101]
	Child's variables				
	Sex (ref: girl)	-0.11	-0.41***	0.1	-0.05
	BMI z-score	0.28**	0.29**	-0.03	-0.01
	MTHFR 677 CT (ref: CC)	-0.02	0.03	0.00	0.05
	MTHFR 677 TT (ref: CC)	-0.28*	-0.26*	0.16	0.06
	Plasma folate	0.25*	0.11	0.16	0.06
	Maternal variables				
	MTHFR 677 CT (ref: CC)	0.10	0.20	-0.01	0.02
	MTHFR 677 TT (ref: CC)	0.14	0.22	-0.03	-0.03
	Gestational hypertension	0.18	0.12	0.03	0.09
	Smoking	0.21*	0.06	0.15	0.02
	BMI	-0.08	-0.04	0.09	0.06
	Plasma folate	0.03	-0.10	0.02	-0.18
	EGRAC	0.02	-0.04	-0.08	-0.21

Abbreviations: ABPM= Ambulatory Blood Pressure Monitoring; BP= Blood Pressure; BMI, body mass index; EGRAC= Erythrocyte Glutathione Reductase Activation assay; *MTHFR*, methylenetetrahydrofolate reductase; ref=reference. Multiple linear regression analysis with maternal and offspring demographic, lifestyle and genetic factors as independent variables and SBP and DBP from office BP and ABPM as dependent variables. Dependent variables were natural log transform before analysis. Results are expressed as standardized β
***P<0.001, *P<0.05.

Multiple logistic regression analysis was used to investigate the maternal and child factors that influence the probability of the child having prehypertension or hypertension. In an initial model the interaction between maternal and child *MTHFR* 677C>T genotypes on probability of prehypertension or hypertension was assessed by including the product (maternal *MTHFR* 677C>T genotype)*(child *MTHFR* 677C>T genotype) in the model. We observed a significant interaction between the maternal *MTHFR* 677 C>T and offspring *MTHFR* 677 C>T genotypes on hypertension (P=0.029 for the interaction term), so they were analysed in separate models.

Table 19 shows the probability of prehypertension or hypertension in the child associated with the maternal *MTHFR* 677 C>T polymorphism. The maternal *MTHFR* 677 CT genotype increases the risk of having prehypertension or hypertension 7.4 times. The TT genotype also increases the risk of prehypertension or hypertension although the association is not significant. Child obesity also increases the risk. Maternal smoking during pregnancy increases the risk of prehypertension or hypertension in the office BP models. The same variables cannot be tested in the ABPM model due to insufficient cases among the maternal genotype categories to associate with the outcome.

Table 20 shows the risk of prehypertension or hypertension in the child associated with child *MTHFR* 677 C>T. All models were significant. In the office BP model we can see that the *MTHFR* 677 CT genotype in the child is associated with increased probability (by 6.5 times) of having prehypertension or hypertension. Child obesity increases the risk of prehypertension or hypertension in both models but does not reach significance in the ABPM model. Maternal smoking during pregnancy

increases the risk of prehypertension or hypertension in the children in both models.

Table 19. Association between maternal *MTHFR* 677 C>T polymorphism and prehypertension or hypertension (with office BP and ABPM criteria).

	Prehypertension or Hypertension (Office BP)	**Prehypertension or Hypertension (ABPM)**
Model 1	R^2= 0.305*** [91]	R^2= 0.376** [78]
Overweight (ref: normal weight)	3.2 (0.5, 21.1)	18.8 (1.0, 367.2)
Obesity (ref: normal weight)	11.5 (2.2, 60.2) ***	5.2 (0.8, 34.7)
Model 2	R^2= 0.508*** [85]	R^2= 0.817*** [78]
Overweight (ref: normal weight)	5.8 (0.6, 53.7)	-
Obesity (ref: normal weight)	16.9 (2.5, 114.3)	-
MTHFR 677 CT (ref: CC)	7.4 (1.0, 55.0) *	-
MTHFR 677 TT (ref: CC)	4.2 (0.3, 56.6)	-
Smoking	14.2 (2.0, 105.8) **	-

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; ref=reference. Multiple logistic regression analysis with maternal lifestyle, biochemical and genetic factors as independent variables and hypertension diagnosed with office BP criteria and ABPM criteria dependent variables. R^2 is R^2 Nagelkerke. Model 1 looks at the association of maternal *MTHFR* 677 C>T polymorphism and hypertension with the predictor variables child sex, overweight and obesity and child plasma folate.. Model 2: included the same variables as model 1 as well as maternal *MTHFR* CT versus CC and *MTHFR* TT versus CC genotypes, gestational hypertension, maternal smoking, maternal plasma folate, plasma cobalamin and EGRAC.

One person was excluded from the analysis due to not matching maternal and offspring genotype. ***P<0.001, **P<0.01, *P<0.05.

Table 20. Association between child *MTHFR* 677 C>T polymorphism and prehypertension or hypertension (with office BP and ABPM criteria).

	Prehypertension or Hypertension (Office BP)	**Prehypertension or Hypertension (ABPM)**
Model 1	R^2= 0.364** [85]	R^2= 0.383* [73]
Overweight vs normal weight	3.6 (0.5, 2.5)	14.9 (0.8, 285.5)
Obesity vs normal weight	19.3 (3.1, 117.8)***	2.2 (0.1, 45.6)
MTHFR 677 CT (ref: CC)	3.8 (0.7, 21.3)	1.2 (0.1, 10.5)
MTHFR 677 TT (ref: CC)	1.1 (0.1, 11.4)	0.7 (0.0, 11.5)
Model 2	R^2= 0.538*** [85]	R^2= 0.574** [73]
Overweight vs normal weight	5.5 (0.6, 54.0)	53.0 (1.0, 2822.2)
Obesity vs normal weight	33.0 (3.5, 306.4)	2.2 (0.1, 45.6)
MTHFR 677 CT (ref: CC)	6.5 (1.0, 43.5)*	0.5 (0.1, 10.5)
MTHFR 677 TT (ref: CC)	0.8 (0.1, 13.0)	0.0 (0.0, -)
Smoking during pregnancy	15.6 (2.0, 123.3)**	11.6 (0.8, 179.2)

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; ref=reference. Multiple logistic regression analysis with offspring lifestyle, biochemical and genetic factors as independent variables and hypertension diagnosed with office BP criteria and ABPM criteria dependent variables. R^2 is R^2 Nagelkerke. Model 1 looks at the association of offspring *MTHFR* 677 C>T polymorphism and hypertension with the predictor variables child's sex, child overweight and obesity, child plasma folate, *MTHFR* CT versus CC and *MTHFR* TT versus CC genotypes. Model 2: included the same variables as model 1 as well as gestational hypertension, maternal smoking, maternal plasma folate, plasma cobalamin and EGRAC.
One person was excluded from the analysis due to not matching maternal and offspring genotype. ***P<0.001, **P<0.01, *P<0.05.

DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.
Gemma Ornosa Martín

1. Population study

Our results agree with findings that lower status in plasma folate and plasma cobalamin were associated with increasing tHcy (65). It is well established that women have lower tHcy compared to men, that is the reason why we decided to stratify by sex for the data analysis of the population. In the highest tertile of tHcy (tHcy> 9.6 µmol/L in women and tHcy> 11.1 µmol/L in men) plasma folate, plasma cobalamin and RBC folate were lower in women and men and more participants were in the suboptimal status of EGRAC compared to the lower tertiles. In addition, we observed higher risk of hypertension in adults aged over 50, when tHcy was in the highest tertile compared to the lowest tertile. However, in adults aged 50 or under, the *MTHFR* 677 C>T polymorphism did not appear to mediate its effect on hypertension via elevated tHcy. Our results agree with the literature that tHcy and plasma creatinine concentrations were higher in male participants than in women, and that plasma folate and cobalamin are strongly and negatively associated with tHcy (291). The prevalence of the homozygote variant genotype has been reported to be 11.8% in Spanish Caucasians (68) and our group have previously reported the prevalence in this population (a representative sample of the population from 3 towns in Tarragona province) to be 18%. This is higher than in other countries where mandatory fortification with folic acid is absent such as Italy, France or The Netherlands (68). In a study conducted in China the prevalence of the CC, CT and TT genotypes of the *MTHFR* 677C>T polymorphism were 38.6%, 48.1% and 13.3% respectively (238).

The results confirm that non-modifiable factors such as age and the homozygote form of the *MTHFR* 677C>T polymorphism were the principal factors that explain homocysteine. The most important factor in women was

age, but the *MTHFR* 677 TT genotype was the most important factor in men. Our linear regression analysis of factors associated with tHcy in all participants explained 26% of the variability in tHcy. This reflects the fact that unknown factors, not considered in studies to date, explain an important part of variability in tHcy. We have to take into account that factors that affect hyperhomocysteinemia in young people are not the same as old people. Nygård et al. found that sex, age, folate intake, smoking status, and coffee consumption were the strongest determinants of tHcy concentration in a multivariate analysis that did not consider the *MTHFR* 677 C>T polymorphism (292) but in our study we showed that *MTHFR* TT vs CC, age, plasma cobalamin, creatinine, plasma folate and sex were the strongest determinants of homocysteine. The Framingham Heart Study also found that plasma folate, the *MTHFR* 677C>T polymorphism, age, serum creatinine, plasma cobalamin and sex were major determinants of tHcy concentrations (65,293).

Our group previously reported that riboflavin status modifies the relationship between the *MTHFR* 677C>T polymorphism and tHcy (66). The TT genotype was most strongly associated with tHcy when riboflavin status was low. Separately we also reported that the inverse association between the *MTHFR* 677TT genotype and folate status is potentiated by the *SLC19A1* 80 AA genotype. These nutrient and genetic factors, unconsidered in most studies, may explain the underlying differences in *MTHFR* genotype-tHcy associations between studies. Here we also show that smoking, a highly prevalent toxic habit, also affects the genotype-tHcy relationship. Thus, while the genotype-tHcy association has been replicated in different countries across the globe, other genetic and lifestyle factors, modify this association.

The *MTHFR* 677 TT-tHcy association is stronger in male smokers than nonsmokers. Smoking may influence hypertension through different

mechanisms, either shared with or independently of tHcy, such as impairment of endothelial function, inflammation, lipid modification as well as an alteration of antithrombotic and prothrombotic factors (294). Smokers have decreased NO availability compared to nonsmokers (295). Xu et al. surprisingly found that in a random control trial in hypertensive patients without cardiovascular comorbidities elevated tHcy was associated with lower risk of death from all causes and this relationship depended on the *MTHFR* 677 C>T polymorphism. When tHcy was in the highest quartile the *MTHFR* 677 TT genotype decreased the risk of all-cause mortality compared to the other genotypes. This is a surprising because elevated tHcy is known to be a strong predictor of both cardiovascular and noncardiovascular mortality (296–298). Moreover the authors do not consider that patients with the TT genotype are likely to have responded differently to the intervention with folic acid than other participants. It is well established that folic acid modifies the effect of the TT genotype on tHcy and arguably on hypertension also. Hence it is likely erroneous to conclude from that study that the TT genotype has a protective role against all cause mortality in hypertensive patients (299).

THcy was associated with diagnosed hypertension in people older than 50 years old. The *MTHFR* 677 TT genotype was associated with hypertension in people younger than 50. A positive association between homocysteine and diastolic BP mostly in young adults was found by Nygård et al. (71), while we only found an association between homocysteine and diagnosed hypertension in people aged over 50 years. This may be because age and higher BMI are more present in this group compared to young people and they are strong risk factors. Nygård et al. do not take into account the *MTHFR*

677 C>T polymorphism but we did, as there is evidence that the *MTHFR* 677 C>T polymorphism is related to hypertension (234,235,238).

The *MTHFR* 677 TT genotype was associated with hypertension in people younger than 50. More studies found an association with the *MTHFR* 677 TT genotype and hypertension. Rodríguez-Esparragón et al. found a positive association between *MTHFR* TT and hypertension in people older than 57 years old (300), which is opposite to our finding. A case-control study in Australia reported that the TT genotype increased the risk of having hypertension [OR= 1.42 (1.01-1.99)] only in women. The association was weaker than that observed in our study but there is mandatory fortification of flour with folic acid in Australia and this might modify the relationship between the *MTHFR* genotype and hypertension (235). A Turkish study with 178 hypertensive and coronary artery disease (CAD) patients with no mandatory folic acid supplementation also reported that the TT genotype increased the risk of hypertension [OR= 1.57 (1.04-2.37)] (85). Moreover two meta-analyses in China found a positive association between *MTHFR* 677 C>T polymorphism and hypertension (14)(241). However, a study in a Mexican-Mestizo population found a protective effect of the minor allele of the *MTHFR* 677C>T polymorphism and hypertension (237). Although we did not look for associations with SBP or DBP, Cheng et al. found that DBP was higher in the homozygous TT compared to CT and CC and that there were no significant associations between SBP and the *MTHFR* 677C>T polymorphism (238).

2. Reus-Tarragona Birth Cohort

The baseline characteristics of pregnant women of our study were described previously. In this thesis we describe the characteristics of the women whose children went on to participate in the child follow up phase of the study. Their mean age at pregnancy was 32.3, similar to that of Catalan pregnant women with an average age of 32.2 (301). Data from Catalunya's Statistics Department in 2017 revealed that 20.1% of women from 25-34 years smoke, quite similar to the results of our study (302). The prevalence of the variant genotype of the *MTHFR* 677 C>T polymorphism in pregnant women in our study is 20.5%. This result is higher than the prevalence of the overall Spanish population reported by Wilcken et al (68) and slightly higher than in our study of a representative sample of the local adult population (54). Participant recruitment differed between our pregnancy and population studies. In this pregnancy study, participants were recruited from early prenatal clinics in the participating hospitals, including women of low and high obstetrical risk. In the population study, participants were randomly recruited from the population registers based on their age and sex. Comparing with other pregnancy studies, the prevalence in another Spanish study (89) and a Northern-Irish study were lower than ours (90). 85% of pregnant women of our study confirmed taking folic acid supplements during the first trimester. A Dutch study reported folic acid use in the first trimester by 75.9% of the women (102). Although the results are quite similar in our study the percentage is higher, likely reflecting the start of prenatal control in early pregnancy (an inclusion criteria of our study) and adherence to our study obstetricians' recommendation to all participants to take folic acid supplements during the first trimester of pregnancy. Due to the nature of the

study, all participants personally received this recommendation from their obstetrician before completing the first trimester of pregnancy.

1C metabolism nutrient and metabolite status varies during the different stages of pregnancy (91,92). Kim et al. found that serum homocysteine was higher in pregnant women (24-28 GW) with the TT genotype compared to CC or CT genotype as we also observed (303). Although plasma folate and cobalamin in our study were lower compared to Kim et al's. study, they all follow the same trend, being higher in the CC genotype compared to the others (303). Other studies have found that pregnant women with the TT genotype had lower status of serum folate (304) or RBC folate (305) compared to the CC and CT genotypes, and higher tHcy status (304,305). Plasma folate and cobalamin concentrations decreased significantly throughout pregnancy and were lowest in the third trimester Also, tHcy concentrations are lower in the second trimester but increased in the third trimester (91,306), as we observed in the Reus-Tarragona Birth Cohort (87,88) and in this subset of mothers in the child follow-up phase. This pattern of tHcy change is normal during pregnancy and likely strongly influenced by physiological factors of pregnancy itself (91). Folate status also contributes to changes in tHcy during pregnancy (91) and the late pregnancy increase in tHcy is curtailed when supplementation with folic acid is continued until the end of pregnancy (90). Changes in folic acid use between the first trimester of pregnancy and the second and third trimesters, in the Reus Tarragona Birth Cohort, also affected tHcy (87). RBC folate concentrations increased in all genotypes between <12 GW and 24-27 GW. We interpret this to be due to extensive use of folic acid supplements during the first trimester, as reported by the mothers and as reflected in first trimester plasma folate concentrations. The reduction in RBC folate

concentrations between 24-27 GW and 34 GW may be due both to cessation of folic acid supplement use after the first trimester and mobilisation of maternal folate reserves to meet foetal folate requirements.

Weight and height of our children was lower compared to another study of Spanish children of similar age (307) but comparing with USA data, RTBC children weighed less and were taller. It is possible that differences in dietary habits contribute to these differences (308). Looking at the baseline characteristics of children separated by sex we observed no significant differences in BMI between sexes in our study, in line with another study of Greek children (218) or the IDEFICS study, an epidemiological multicentre European project in which BMI was similar to ours (309). Overweight and obesity were slightly more prevalent, although not significantly so, in girls than in the boys group in our study, contrasting with other studies in which boys were more overweight and obese (218).

Although BP at check-up of children of the study did not reach worrying levels, it is true that our children had slightly higher SBP and DBP (SBP= 104.1 mmHg; DBP=65.1 mmHg) compared to two Dutch children studies (102,266) but lower compared to a Chinese study (310). Overweight and obesity were more prevalent in our study than in these studies, possibly explaining why our child BP status is higher. In the IDEFICS study in which they recruited children from eight European countries including Spain, the results were very similar to ours (309), so BP values of our study are in line with other European studies. The prevalence of paediatric hypertension differs between countries, likely due to the different criteria used, the distribution of reference BP data, age of participants and the methodology used (311). The prevalence of hypertension in children in our study was 6.2% and it varies depending on the country, in Greece the prevalence is 5.2% (312), 6.2% in Italy (313), 5.8% in

Australia (314), 9.0% had hypertension in China (315) and 3.6% in the USA (204).

Urbina et al. recommended using the ABPM method when it is suspected that a child has hypertension and associated office BP measurements with ABPM (221). This is one of the reasons why we decided to use ABPM. In our study we saw that there is a significant correlation between office BP results and ABPM results, being weaker in DBP. This information gives us strength to stand up for the use of the ABPM in the study, despite the difficulty in execution of this technique in young children. Certainly in children a little older than ours, it may be interesting to implement in studies of blood pressure. In a German study, ABPM results in healthy children between 3-6 years old were 110 mmHg during the daytime and 100 mmHg at night. In this study as in ours BP decreases at night (316). Although children in a Chinese study were older than 7.5 years, we concord in some observations regarding ABPM results, such as higher BP in boys than in girls and that BP values during the day are higher than at night and it is observed in both sexes (317).

Hypertensive child SBP in a Chinese study (125.7 mmHg), was higher than in our Reus-Tarragona Birth Cohort, maybe because children in the Chinese study were older (mean age 12.3 years old) (310). This is why age and sex specific percentiles are used to classify BP and establish reference ranges. BMI was significantly different between the hypertensive and normal tension groups. Our results agree with those from other studies in which BMI was associated with hypertension and more obese children belonged to the hypertensive group (318)(311). Other studies have reported higher arm circumference measurements in hypertensive children compared to those with normal blood pressure (319). Child plasma folate and tHcy was similar to other studies. In a Norwegian study tHcy concentration was almost identical

to ours (320), however it was higher in the Greek study (321). Neither of these two studies measured BP. It was difficult to find studies that looked at the relationship between 1CM and BP in children with children of the same age as ours for comparative purposes.

We did not investigate the associations between maternal tHcy and BP in children because pregnancy tHcy is not truly indicative of maternal 1C metabolism status for all of the reasons explained before and we considered it much more reliable to test the *MTHFR* 677 C>T component of the mother (not influenced by pregnancy and folic acid supplement use and other confounders), and also because in young adults from our population study we observed that it is a predictor of hypertension. A study with 1279 mother-child pairs in the USA found a U-shaped relationship between maternal tHcy during pregnancy and child SBP. Being in the lowest or highest quartile of maternal tHcy increased the risk of elevated SBP in the children aged between 3-9 years compared to the 2nd or 3rd tertile, mostly from children born to obese mothers with tHcy in the highest quartile (322). Van den Hil et al. in a study in The Netherlands found no associations between maternal first trimester folate and tHcy concentrations with childhood SBP and DBP in children of 6 years of age, but they did with maternal first trimester cobalamin [0.31 mmHg increase in DBP per standard deviation increase in cobalamin (95 % CI 0.06, 0.56) (266).

Few studies have investigated the relationship between offspring *MTHFR* 677 C>T polymorphism and BP or hypertension. Xi et al. found in a cross-sectional study in China with hypertensive versus control children with a mean of age 12.3 years an association between *MTHFR* 677 C>T polymorphism and hypertension only in the obese group (310). However, a study with Mexican-Mestizo healthy children found no association between the polymorphism

and hypertension (237). Although a Greek study did not explore the relationship between the *MTHFR* 677 C>T polymorphism and SBP and DBP, they did report that age and BMI were positively associated with SBP and DBP in children aged 6-15 years (218).

We found that the maternal *MTHFR* 677 CT genotype is associated with increased risk of prehypertension+hypertension [OR= 7.4 (1.0, 55.0)] in children in the office BP model. This association also exists when we look at the effect of child *MTHFR* 677 CT genotype in having prehypertension+hypertension [OR= 6.5 (1.0, 43.5)]. Notably, this relationship is independent of gestational hypertension. None of these associations were found in the ABPM models. Although it follows the same tendency, maybe we have not found these associations in the TT genotype due to a lack of statistical power. More children with the TT genotype will have to be recruited in order to explore whether it is associated with hypertension. The mechanism by which maternal *MTHFR* 677 C>T polymorphism is associated with probability of hypertension in the child may be through foetal programming. For example, if maternal folate status is impaired by the presence of the variant genotype this may have repercussions on epigenetic mechanisms involved in programming the regulation of BP.

It would also be interesting to look at the associations between maternal *MTHFR* 677 C>T polymorphism and prehypertension+hypertension in children stratified by child *MTHFR* 677 C>T polymorphism but we did not have sufficient numbers in the genotype subgroups to do this.

Strengths and limitations

One of the strengths of our study is that our population was a representative sample from three town hall population registers so it can be extrapolated to the general population. Participants were not taking any B-vitamin supplements and in Spain, as in other countries in Europe there is no mandatory folic acid fortification. This allowed us to explore the direct associations between 1C metabolism, *MTHFR* 677C>T polymorphism and hypertension in a sample not affected by the potential masking effect of folic acid on underlying metabolic- or genetic-tHcy associations.

Limitations of the population study are that as participants had previously diagnosed hypertension, it is possible that they had improved their lifestyle habits to treat the disease and in consequence tHcy and other 1C metabolism nutrients may have been affected. Moreover hypertension can be caused by other predictors independently of 1C metabolism but common predictors of hypertension such as age and BMI were confirmed in the hypertension models. As it was a cross-sectional study reverse-causation cannot be ruled out in the different studied tHcy-BP associations. However, this limitation does not apply to the *MTHFR* 677 C>T genotype-tHcy association.

The principal strength of our mother and child study is the prospective design from early life onwards, that all data from pregnant women (anthropometric, clinical, lifestyle, biochemical and genetic data) was collected from the first trimester of pregnancy until the end of pregnancy. This data is very valuable and important because of the difficulty to collect biochemical, clinical and lifestyle data of the study participants due to the variation of attendance to the first prenatal care visit. Pregnant women decide when to attend to the doctor (also depending on when they found out they are pregnant).

Recognising the limitations of an observational study it also has an element of strength to it because all pregnant women of the study, while receiving the protocolised prenatal healthcare advice from health professionals, did not receive any other type of intervention so the observations may be as expected in pregnant women in general with a range of lifestyle and clinical characteristics. It would be interesting to design a clinical trial to investigate the effect of low folate status on BP, growth and other offspring health outcomes but it would be unethical, so we cannot further explore the causal nature of some of the associations that may be relevant to our hypothesis. However, we adjusted for most of the known potential contributing factors to residual confounding. Mandatory folic acid supplementation of flour was not present in our study because unlike other countries like the USA and Canada, Spain has not implemented this policy, this helped us to investigate transgenerational associations that may otherwise be masked in the presence of high maternal folate status.

The most important limitation of our study is the small sample size in the child follow-up phase at 7.5 years, to date. Although the sample size is increasing because we are still recruiting children, at the time of analysis for this thesis, 212 children were followed-up meaning a participation of 51.6% in the follow-up phase. We are conscious that participation could be improved but it was difficult to recruit more children. Some parents did not want their children to miss school a couple of days because of the study and sometimes it was complicated to arrange the visit with them due to schedule incompatibility (usually visits were done in working hours but we always adapted visits to the parents schedule needs). Another reason for not participating was some parents did not see the need to bring their children to the hospital for participating in the study and have a blood test if they were

healthy, they did not want their children to suffer because of that. In case of rejecting to participate in the study because of the blood sample, we also offered participation without the blood test. Then some people agreed to that. Besides all these inconveniences we had enough statistical power to investigate the transgenerational relationship between maternal 1C metabolism and different outcomes in the offspring. To guarantee enough statistical power to test the hypothesis that maternal *MTHFR* 677 C>T polymorphism is associated with probability of hypertension in the offspring, we had to join prehypertension and hypertension cases into the same group and increase the number of the sample.

Another limitation of the study is the use of ABPM measurement for BP readings. Although this technique has a great potential and gives the opportunity to diagnose some pathologies not possible with office BP, we found that it is difficult to use in children. As mentioned previously, ABPM makes different measurements during 24h (day and night) and although it is not painful, when the cuff inflates it can be uncomfortable for the child and we found out that some of them tried to take the monitor off. We have to take into account that a child moves a lot, but to obtain correct BP readings the child must be still. This sometimes led to errors in the reading and invalid results. In that case we asked the parents to do it again but some refused, losing sample size. Nevertheless we did collect valid ABPM data from the children and will add to this in the ongoing study.

Future perspectives

The results of this thesis are meaningful in order to give advice to the population in a public health context. As this study is a longitudinal study, it

would be interesting to continue the follow-up of the offspring at different ages to evaluate if the results obtained in this thesis are sustained over time. Also replicating this study in other populations would give veracity to our findings and reforce the evidence for an important role of 1C metabolism and *MTHFR* 677 C>T polymorphism in its relationship with BP. In the future, more longitudinal studies should investigate the association with BP or hypertension, because at the moment there are few studies in the literature to do so. Other offspring outcomes should be investigated including lipid profile, glucose and endothelial function biomarkers to corroborate BP results and give a complete view of hypertension disease. Other polymorphisms possibly related to BP and affecting this metabolic network should also be studied.

As previously said one of the strengths of the mother-child study is the early recruitment in pregnancy (< 12 GW) and the complete follow-up during all gestation process including clinical and lifestyle data questionnaires and six blood samples collected during pregnancy and labour. Recruitment is almost at the target number of 800 pregnancies that will lead to new child participants as they reach the age for this phase of the study, thus enabling us to increase statistical power for further analysis.

CONCLUSIONS

UNIVERSITAT ROVIRA I VIRGILI
A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.
Gemma Ornosa Martín

1. Population study

Objectives

Main objective

To investigate the relationship between components of one carbon metabolism and diagnosed hypertension in a representative sample of an adult population unexposed to mandatory folic acid fortification and B vitamin supplement use.

Specific objectives

- To investigate the lifestyle, nutritional and genetic factors associated with tHcy.

The *MTHFR* 677 TT vs CC genotype, sex, age group, plasma cobalamin and plasma folate were the strongest determinants of tHcy.

- To investigate the relationship between tHcy and diagnosed hypertension.

Being in the 3rd tertile of tHcy compared to the 1st and the 2nd tertiles increased the probability of having diagnosed hypertension [OR=1.8 (1.0, 3.3)]. A stratified analysis by age group showed that this was driven by the tHcy-hypertension association in people older than 50 years [OR=2.5 (1.2, 5.4)].

- To investigate the relationship between the *MTHFR* 677 C>T polymorphism and diagnosed hypertension.

Having the *MTHFR* 677 TT compared to the CC genotype increased the probability of having diagnosed hypertension, [OR=8.2 (1.3, 53.9)], in people younger than 50 years.

2. Reus-Tarragona Birth Cohort

Objectives

Main objective

To investigate the association between components of maternal one carbon metabolism and blood pressure in the children aged 7.5 years.

Specific objectives

- To describe maternal one carbon metabolism nutrient and metabolic parameters, according to *MTHFR* 677C>T genotype throughout pregnancy.

The patterns of change during pregnancy of plasma folate, cobalamin, MMA, tHcy and EGRAC did not differ between genotypes.

RBC folate was lower in the TT genotype compared to the CC genotype throughout pregnancy.

- To describe one carbon metabolism indicators according to child *MTHFR* 677C>T genotype in children aged 7.5 years.

No differences between plasma folate, RBC folate, plasma cobalamin, plasma MMA or tHcy were found between the genotypes in children.

- To compare one carbon metabolism nutrient and metabolic parameters during pregnancy with those in children at 7.5 years.

Plasma folate, plasma cobalamin, holoTC and MMA were higher and tHcy lower, in the cord compared to the mother at labour.

Child plasma folate was lower than the mother's in early pregnancy but higher than the mother's in late pregnancy. Child plasma cobalamin, MMA and tHcy were all higher than in the mother's in early pregnancy and tHcy was lower than in the mother's in late pregnancy.

- To investigate the association between maternal *MTHFR* 677C>T genotype and lifestyle factors and prehypertension and hypertension in children at 7.5 years.

Maternal smoking during pregnancy was associated with increased probability of prehypertension / hypertension in the children aged 7.5 years [OR= 14.2 (2.0, 105.8)].

Maternal *MTHFR* 677CT genotype was associated with increased probability of prehypertension / hypertension in the children aged 7.5 years [OR= 7.4 (1.0, 55.0)].

- To investigate the association between child *MTHFR* 677C>T genotype and lifestyle factors and prehypertension and hypertension in children at 7.5 years.

Child *MTHFR* 677CT genotype was associated with increased risk of prehypertension or hypertension, [OR=6.5 (1.0, 43.5)].

Child obesity was associated with increased risk of prehypertension or hypertension, [OR=33.0 (3.5, 306.4)].

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pressure: a prospective birth cohort study. Int J Obes. 2017;41(9):1447–53.

SCIENTIFIC AND ACADEMIC CONTRIBUTIONS AND OTHER MERITS

UNIVERSITAT ROVIRA I VIRGILI
A STUDY OF THE ASSOCIATION BETWEEN ONE CARBON METABOLISM AND BLOOD PRESSURE IN ADULTS AND TRANSGENERATIONALLY BETWEEN PREGNANT WOMEN AND THEIR CHILDREN.
Gemma Ornosa Martín

Articles

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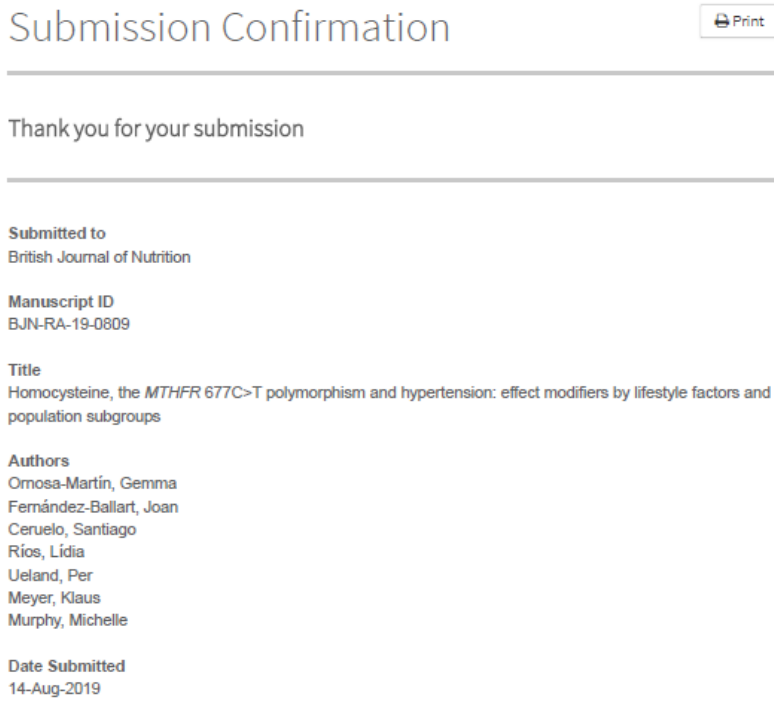

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International conference contributions

Conference: 10th International conference One Carbon Metabolism and Homocysteine. Nancy (France), 2015.
Authors: Ornosa G; Fernandez-Ballart JD; Ceruelo S; Ríos L; Murphy MM.
Title: Associations between homocysteine, the *MTHFR* 677C>T polymorphism and hypertension in adults.
Format: Oral communication.

Conference: 10th International conference One Carbon Metabolism and Homocysteine. Nancy (France), 2015.
Authors: Colomina JM; Cavallé-Busquets P; Fernandez-Roig S; Solé-Navais P; Fernandez-Ballart JD; Ballesteros M; Ornosa G; Ueland PM; Meyer K; Murphy MM.
Title: Effects of the *BHMT* c.716G>A polymorphism on the betaine dimethylglycine pathway during pregnancy.
Format: Oral communication.

Conference: 10th International conference One Carbon Metabolism and Homocysteine. Nancy (France), 2015.
Authors: Solé-Navais P; Ornosa G; Fernandez-Ballart JD; Cavallé-Busquets P; Ballesteros M; Colomina JM; Ueland PM; Murphy MM.
Title: Early pregnancy cobalamin status on anthropometry and adiposity in the offspring at 7.5 years (Reus-Tarragona Birth Cohort).
Format: Poster.

Conference: 11th International Conference on Homocysteine & One-Carbon Metabolism. Aarhus (Denmark), 2017.

Authors: Ornosa-Martin G; Solé-Navais P; Cavallé-Busquets P; Fernandez-Ballart JD; Haro-Barceló J; Ballesteros M; Ueland PM; Meyer K; Murphy MM.
Title: Early pregnancy folate and cobalamin status and overweight and obesity in children at 7.5 years.
Format: Poster.

Teaching and academic activities

Medicine Grade in the Faculty of Medicine and Health Sciences of Universitat Rovira i Virgili (approximately 60 hours/ year; 2014-2017)
- Research and Documentation Bases (70%)

- General Epidemiology (30%)

Other contributions

- Active role in the fieldwork of the pregnancy and follow-up phases of the Reus-Tarragona Birth Cohort.

Involvement in scientific projects

Title: Links between *in utero* one carbon metabolism and development and health in the offspring at 7.5-8 years. **Principal Investigator:** Michelle Murphy. **Duration**: 3 years. **REF**: PI13/02500. **Funding source**: Instituto de Salud Carlos III/ Fondo FEDER.

UNIVERSITAT ROVIRA I VIRGILI
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APPENDICES

UNIVERSITAT ROVIRA I VIRGILI
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12 setmanes

Estudi NUTCIR 1

Nom..... *Data* ....

QÜESTIONARI DE FREQÜÈNCIA DE CONSUM ALIMENTARI 1

INSTRUCCIONS PER OMPLIR-LO

Procuri contestar tranquil·lament aquest qüestionari. Prengui el temps que consideri necessari.

Aquest qüestionari li pregunta la freqüència amb la que vostè consumia de forma **habitual** determinats aliments abans d'estat embarassada.

La freqüència de consum s'ha d'especificar als requadres de la dreta del llistat d'aliments d'aquest qüestionari. Per a cada aliment del llistat ha d'apuntar el **número de vegades** que el consumeix.

- Si el consumeix **tots els dies de la setmana**, posi un 7 en la columna **A LA SETMANA.**
- Si el consumeix **alguna vegada a la setmana,** posi les vegades: 1-2-3-4-5 o 6 en la columna **A LA SETMANA.**

  Pensi sempre en sumar el consum de totes les menjades del dia (esmorzar, dinar, berenar, sopar, altres..). Per exemple, si pren tots els dies llet per esmorzar i alguna vegada a la setmana per sopar: 7 + 4 = 11 vegades a la setmana.
- Si consumeix l'aliment **alguna vegada al mes**, posi les vegades: 1-2-3 etc... en la columna: **AL MES**
- Si no el consumeix **mai** o gairebé mai, deixi la casella en blanc, sense posar res.

Exemple: Una dona esmorza habitualment un got de llet (7 vegades) amb magdalenes(7 vegades), i per sopar a vegades pren llet (4vegades) i a vegades pren iogurt (3 vegades) de postres. A més, pren peix algunes vegades a la setmana per dinar (2 vegades) i altres vegades per sopar (4 vegades). El llegums en consumeix alguna vegada al mes (aproximadament 4 vegades) . Si no menja mai un aliment deixeu en blanc, sense contestar res.

Aquest consum l'apuntaria de la següent manera:

LLISTAT D'ALIMENTS	QUANTES VEGADES MENJA...?	
	A LA SETMANA	AL MES
Llet	*11*	
Iogurt	*3*	
Coc ràpid, magdalenes..	*7*	
...		
Peix	*6*	
...		
Llegum		*4*
Formatge de règim		

QÜESTIONARI DE FREQÜÈNCIA DE CONSUM ALIMENTARI

LLISTAT D'ALIMENTS	QUANTES VEGADES MENJA...?	
	A LA SETMANA	AL MES
Llet		
Iogurt		
Xocolata: tauleta, bombons, "Kit-Kat" "Mars"...		
Cereals inflats d'esmorzar ("Corn-Flakes" "Kellog's")		
Galetes tipus "maria"		
Galetes amb xocolata, crema...		
Magdalenes, coc ràpid,...		
Ensaïmada, Donut, croissant...		

	A LA SETMANA	AL MES
Amanida: Enciam, tomàquet, escarola...		
Mongetes verdes, bledes, o espinacs		
Verdures de guarnició: albergínia, carbassó, xampinyons...		
Patates al forn, fregides o bullides		
Llegums: llenties, cigrons, fesols ...		
Arròs blanc, paella		
Pasta: fideus, macarrons, espaguetis...		
Sopes i cremes		

	A LA SETMANA	AL MES
Ous		
Pollastre o gall d'indi		
Vedella, porc, corder (bistec, empanada...)		
Carn picada: llonganissa, hamburguesa		
Peix blanc: lluç, mero...		
Peix blau: sardines, tonyina, salmó...		
Marisc: musclos, gambes, llagostins, pop, calamars...		
Croquetes, empanadilles, pizza		
Pa (en entrepans, a les menjades...)		

	QUANTES VEGADES MENJA...?	
	A LA SETMANA	AL MES
Pernil salat, dolç, embotits		
Formatge fresc (Burgos,...) o baix en calories		
Formatges: curats o semicurats, cremosos		

	A LA SETMANA	AL MES
Fruites cítriques: Taronja, mandarina		
Altres fruites: Poma, pera, préssec, albercoc, plàtan		
Fruites en conserva (en almívar...)		
Sucs de fruita natural		
Sucs de fruita comercial		
Fruits secs: cacauets, avellanes, ametlles		
Postres làctics: natilles, flam, mató		
Pastels de crema o xocolata		
Bosses d'apetitius ("chips", "cheetos", "fritos"...)		
Llaminadures: gominoles, caramels...		
Gelats		

	A LA SETMANA	AL MES
Begudes ensucrades ("coca-cola", "Fanta"...)		
Begudes baixes en calories (coca-cola light...)		
Vi, sangria		
Cervesa		
Cervesa sense alcohol		
Begudes destil·lades: (Whisky, ginebra, conyac,...)		

Indiqui amb una X la resposta que vostè vulgui assenyalar:

1.- A taula, s'afegeix sal a les menjades?

Mai__ / Alguna vegada__ / Freqüentment __/ Gairebé sempre __

2.- Com definiríeu la vostra gana? Molta __ Força __ Normal __ Poca __ Gens__

3.- Quin tipus de llet prens habitualment?: Sencera __ Semidescremada __ Descremada __

4.- Quin tipus de iogurt prens habitualment?

a) Natural ___ b) Natural descremat ___
c) De sabors ___ d) De sabors descremat ___
e) Amb trossets de fruita ___ f) Amb trossets de fruites descremat ___

5.- Quin tipus de pa prens habitualment?: Blanc __ Integral __

6.- Et poses tomàquet i oli en els entrepans?: Sempre__ Habitualment__ Alguna vegada__ Gairebé mai_

20 setmanas

Nom: Data:

ENQUESTA 1 SOBRE HÀBITS I ESTIL DE VIDA
(referida a la primera meitat de l'embaràs)

ANOTI LES RESPOSTES EN ELS ESPAIS CORRESPONENTS A CADA PREGUNTA.
Aquestes dades serviran a la Universitat Rovira i Virgili per fer un estudi comparatiu entre diferents poblacions. En els resultats mai apareixerà el seu nom.

ÚS DE SUPLEMENTS DE VITAMINES / MINERALS

Per diferents motius, els suplements de vitamines i minerals recomanats no es prenen sempre: per oblit, per sentiment de que no són necessaris, per no trobar-se bé, perquè donen molèsties, etc. Si us plau, contesti sincerament aquestes preguntes per ajudar-nos a valorar la realitat de l'ús dels suplements.

- Ha pres per iniciativa pròpia o receptat per un metge algun tipus de suplement vitamínic / mineral?

Mai n'he pres ☐ **Si n'he pres ☐**

En el cas que sí, escrigui el nom del preparat i indiqui les vegades a la setmana que ho ha pres marcant el quadrat. Marqui el quadrat corresponent als mesos que ho ha pres.

Exemple, una dona que ha pres cada dia FOLIDOCE durant els primers 3 mesos, escriuria...

Nom del preparat	¿Quantes vegades a la setmana?	Mesos de l'embaràs 1	2	3	4	5
ÀCID FÒLIC	■ Cada dia	■	■	■	☐	☐
	☐ La majoria dels dies (4-6 vegades)	☐	☐	☐	☐	☐
Quin? : FOLIDOCE	☐ Alguns dies (1-3 vegades)	☐	☐	☐	☐	☐

Nom del preparat	Quantes vegades a la setmana?	Mesos de l'embaràs 1	2	3	4	5
ÀCID FÒLIC	☐ Cada dia	☐	☐	☐	☐	☐
	☐ La majoria dels dies (4-6 vegades)	☐	☐	☐	☐	☐
Quin?:_____	☐ Alguns dies (1-3 vegades)	☐	☐	☐	☐	☐
FERRO	☐ Cada dia	☐	☐	☐	☐	☐
	☐ La majoria dels dies (4-6 vegades)	☐	☐	☐	☐	☐
Quin?:_____	☐ Alguns dies (1-3 vegades)	☐	☐	☐	☐	☐
MULTI-VITAMINES	☐ Cada dia	☐	☐	☐	☐	☐
	☐ La majoria dels dies (4-6 vegades)	☐	☐	☐	☐	☐
Quin?:_____	☐ Alguns dies (1-3 vegades)	☐	☐	☐	☐	☐

- Va prendre àcid fòlic en els 3 mesos abans de quedar-se embarassada? **Sí ☐** **No ☐**
- Va prendre ferro en els 3 mesos abans de quedar-se embarassada? **Sí ☐** **No ☐**

ESMORZAR (durant l'embaràs)

	Sí	No
Té el costum d'esmorzar?	☐	☐
Esmorza cereals inflats habitualment (p.ex. tipus Kelloggs / Nestlé etc) ?	☐	☐
Pren cafè amb cafeïna?	☐	☐
Pren cafè descafeïnat ?	☐	☐

Nom: Data:

TABAC

- És fumadora passiva (exposada al fum de tabac habitualment a casa o a la feina)? Sí ☐ No ☐
- És fumadora activa? Sí ☐ No ☐

Només per fumadores en els últims 5 anys

	0 cigs/dia	1-5 cigs/dia	6-10 cigs/dia	> 10 cigs/dia
Actualment fumo	☐	☐	☐	☐
Fumava durant els 12 mesos abans de l'embaràs	☐	☐	☐	☐

	No	Abans dels 3 mesos	Entre els 3 i els 6 mesos	Després dels 6 mesos
He deixat de fumar durant l'embaràs	☐	☐	☐	☐

ALCOHOL

	Mai / ocasionalment	< 3 copes / setmana	Cada dia com aperitiu i/o amb els àpats	> 7 copes / setmana
Actualment bec alcohol	☐	☐	☐	☐
En els 12 mesos abans de l'embaràs	☐	☐	☐	☐

	No	Abans dels 3 mesos	Entre els 3 i els 6 mesos	Després dels 6 mesos
He deixat de beure alcohol durant l'embaràs	☐	☐	☐	☐

PES, TALLA, EDAT, ORIGEN I PARTICIPACIÓ EN ESTUDIS

	Abans de l'embaràs	Última vegada que es va pesar abans de realitzar l'entrevista (data; SG)
Pes:	. Kg	. Kg (/ / ; SG)

Talla: . m
Data de naixement:
Participació en altres estudis:

Origen pares:
Origen avis:

Nom: Data:

SUBSTÀNCIES TÒXIQUES

- Ha pres algun altre tipus de substància tòxica (p.ex. marihuana, cocaïna, heroïna, etc...) en els últims 5 anys?

Sí ☐ No ☐

En el cas de que sí hagi pres alguna substància tòxica, especifiqui quines: _____

	No	Ocasionalment	Regularment
Actualment prenc substàncies tòxiques	☐	☐	☐
En els 12 mesos abans de l'embaràs prenia subst. tòxiques	☐	☐	☐

	No	Abans dels 3 mesos	Entre els 3 i els 6 mesos	Després dels 6 mesos
Ho he deixat durant l'embaràs durant els mesos	☐	☐	☐	☐

ACTIVITAT FÍSICA (durant l'embaràs)

- Quina activitat física fa en el treball, estudi o feina de casa?

Hores/setmana

- El meu treball és bàsicament d'estar asseguda i caminar poc (estudiant, docent, conductora de vehicles, dependenta, administrativa)..... ☐ _____
- Al meu treball camino força però no faig cap esforç vigorós (mestressa de casa, fàbrica, venedora, cartera...)..... ☐ _____
- El meu treball és bàsicament de molta activitat física (esportista.)..... ☐ _____

- Quina activitat feu en el temps de lleure? (anotar la prioritària si dues activitats coincideixen en hores)

Horas/semana

- Lectura, televisió i activitats que no requereixin activitat física important..... ☐ _____
- Caminar, anar amb bicicleta, jardineria (no s'inclou el transport d'anar i tornar del treball)..... ☐ _____
- Córrer, esquiar, gimnàstica, jocs de pilota o esports vigorosos regularment..... ☐ _____
- Entrenament esportiu regular per competició..... ☐ _____

- Durant els últims 12 mesos

	Mai	Esporàdicament	Habitualment
Ha tingut el costum de prendre el Sol?	☐	☐	☐

Nom: Data:

PLANIFICACIÓ DE L'EMBARÀS

- Ha buscat / planificat aquest embaràs? **Sí** ☐ **No** ☐
- Durant els 6 mesos abans de l'embaràs

	Cap	DIU	Anticonceptius orals	Pegat anticonceptiu	Anell vaginal	Preservatiu
Quin mètode d'anticonceptiu ha fet servir?	☐	☐	☐	☐	☐	☐

- Cicles sense prendre anticonceptius orals abans de l'embaràs? _____
(Número de regles des de que va deixar de prendre anticonceptius fins que va quedar embarassada)

DADES SOCIODEMOGRÀFIQUES

- Quina és la seva feina en l'actualitat i quin nivell d'estudis ha completat

	Mare	Pare
Feina actual	☐	☐
Nivell d'estudis	Primaris sense finalitzar ☐ Primaris (ESO, EGB, ...) ☐ Secundaris (BUP, Batxillerat, FP, ...) ☐ Superiors (Universitaris) ☐	Primaris sense finalitzar ☐ Primaris (ESO, EGB, ...) ☐ Secundaris (BUP, Batxillerat, FP, ...) ☐ Superiors (Universitaris) ☐ No aplicable (Família monoparental) ☐

- Nombre de persones que formen la unitat familiar _____
- Ingressos nets anuals totals a la llar

Exemple, si la dona té un sou de 20000 €, l'home un de 18000€ i hi ha un avi que viu amb la família i rep una pensió de 6000 €

Menys de 9000 €	>9000 € - 19000 €	>19000 € - 25000 €	>25000 € - 35000 €	Més de 35000 €
☐	☐	☐	☐	■

Menys de 9000 €	>9000 € - 19000 €	>19000 € - 25000 €	>25000 € - 35000 €	Més de 35000 €
☐	☐	☐	☐	☐

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TP-90

ID ☐☐☐

Inicials ☐☐☐☐

Gènere ☐

Centre ☐

Data de entrevista ☐☐ ☐☐ ☐☐ (d d m m a a)

Data de naixement ☐☐ ☐☐ ☐☐ (d d m m a a)

Pes (kg)	☐☐,☐	☐☐,☐
Alçada (cm)	☐☐☐,☐	☐☐☐,☐
Circumferència cranial(cm)	☐☐,☐	☐☐,☐
Circumferència mitjana del braç(cm)	☐☐,☐	☐☐,☐
Circumferència del pit (cm)	☐☐,☐	☐☐,☐
Circumferència de la cintura (WHO) (cm)	☐☐,☐	☐☐,☐
Circumferència de la cintura (CDC) (cm)	☐☐,☐	☐☐,☐
Circumferència malucs (cm)	☐☐,☐	☐☐,☐
Circumferència de la cuixa (cm)	☐☐,☐	☐☐,☐

Plec tricipital (mm)	☐☐,☐	☐☐,☐	☐☐,☐
Plec subescapular (mm)	☐☐,☐	☐☐,☐	☐☐,☐
Plec bicipital (mm)	☐☐,☐	☐☐,☐	☐☐,☐
Plec suprailíac (mm)	☐☐,☐	☐☐,☐	☐☐,☐
Plec de la cuixa (mm)	☐☐,☐	☐☐,☐	☐☐,☐

Pressió arterial Sistòlica (mmHg)	☐☐☐	☐☐☐	☐☐☐
Pressió arterial Diastòlica (mmHg)	☐☐☐	☐☐☐	☐☐☐

Informació impedància bioelèctrica

Ha ingerit líquids avui? ○ Si ○ No

Quantes hores fa de l'últim cop? _____

Ha orinat aquest matí? ○ Si ○ No

Ha realitzat activitat física avui? ○ Si ○ No

Quantes hores fa? _____

A quina resistència? _____kHz

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Reus & Tarragona Birth Cohort

Unitat de Medicina Preventiva i Salut Pública

ID ☐☐☐

Inicials ☐☐☐

Gènere ☐

Centre ☐

TP-90

Data entrevista ☐☐ ☐☐ ☐☐ (d d m m a a)

Data naixement ☐☐ ☐☐ ☐☐ (d d m m a a)

Història clínica

1. Qui respon a la Història Clínica?

- ☐ Mare
- ☐ Pare
- ☐ Altre familiar. Especificar:
- ☐ Tutor/a Responsable no familiar
- ☐ Altres. Especificar

2.

	Si	No	Motiu
Extracció sanguínia			
Carnet de Salut			
BIA			
Quadern d'activitat física			
MAPA			
Diari d'activitat física			
Neurodesenvolupament 1			
Neurodesenvolupament 2			

3. Ha estat diagnosticat d'alguna malaltia o malformació congènita?_____

_____

4. Ha estat diagnosticat d'alguna malaltia crònica? _____

_____

5. Ha estat hospitalitzat per algun motiu?

☐ No ☐ Si Especificar:_____

Edat durant l'hospitalització (anys)?_____

6. Se li ha realitzat algun tipus cirurgia?

☐ No ☐ Si

Quina i Quan?_____

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Inicials:

Reus & Tarragona Birth Cohort

Unitat de Medicina Preventiva i Salut Pública

7. Es va alimentar el nen amb llet materna?

☐ No ☐ Si

Durant quants mesos de manera exclusiva (mesos)?_____
Durant quants mesos en total?_____

8. Quina edat tenia quan es van introduir aliments sòlids a la seva dieta (mesos)?____

9. El seu fill té problemes visuals?

☐ No ☐ Si Necessita portar ulleres?_____

10. El seu fill té problemes auditius?

☐ No ☐ Si

11. Ha estat diagnosticat o ha tingut en algun moment:

	No	Si	Abans dels 2 anys	Entre 2 i 5 anys	Més tard dels 5 anys	Nº de vegades els últims 12 mesos
Refredat amb febre	☐	☐	☐	☐	☐	
Infecció d'oïda	☐	☐	☐	☐	☐	
Infecció a la gola	☐	☐	☐	☐	☐	
Pneumònia	☐	☐	☐	☐	☐	
Bronquitis	☐	☐	☐	☐	☐	
Bronquiolitis	☐	☐	☐	☐	☐	
Gastroenteritis	☐	☐	☐	☐	☐	
Anèmia	☐	☐	☐	☐	☐	
Càries	☐	☐	☐	☐	☐	
Xarampió	☐	☐	☐	☐	☐	
Varicel·la	☐	☐	☐	☐	☐	
Rosada	☐	☐	☐	☐	☐	
Altres. Especificar:	☐	☐	☐	☐	☐	

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Unitat de Medicina Preventiva i Salut Pública

12. Té alguna al·lèrgia alimentària?

	Si	No
- Llet de vaca	☐	☐
- Ous de gallina	☐	☐
- Soja	☐	☐
- Cacahuets	☐	☐
- Fruits secs	☐	☐
- Blat	☐	☐
- Peix	☐	☐
- Marisc	☐	☐
- Altres:	☐	☐

13. Ha disminuït o suprimit el consum d'algun aliment de la dieta del nen degut a l'al·lèrgia?

☐ No ☐ Si Quin/s?_____

Ha substituït aquet/s aliment/s per algun altre?_____

14. Durant el darrer any ha estat prenent algun medicament?

☐ No ☐ Si Quin?_____

15. En la actualitat, està prenent algun medicament?

☐ No ☐ Si

Quin?_____

16. Algun familiar directe ha estat diagnosticat d'algun trastorn psicopatològic (exemples trastorn psicòtic, depressió, trastorn d'ansietat, retard mental, trastorns per dèficit d'atenció amb hiperactivitat, consum de tòxics, trastorn de la personalitat)?

☐ No ☐ Si Qui?_____Tipus de trastorn?_____

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Unitat de Medicina Preventiva i Salut Pública

Qüestionari bàsic de sibilàncies

a. Ha tingut alguna vegada sibilàncies o xiulets al pit durant qualsevol moment del passat? ☐ Si ☐ No

SI HA RESPOST "NO" PASSEU A LA PREGUNTA F

b. Ha tingut alguna vegada sibilàncies o xiulets al pit en els darrers 12 mesos? ☐ Si ☐ No

SI HA RESPOST "NO" PASSEU A LA PREGUNTA F

c. Quants atacs de sibilàncies el vostre fill ha tingut en els darrers 12 mesos? ☐ Cap ☐ 1 a 3 ☐ 4 a 12 ☐ Més de 12

d. En els darrers 12 mesos, quantes vegades, de promig, el somni del vostre fill ha estat pertorbat a causa de les sibilàncies? ☐ Mai s'ha despertat amb sibilàncies ☐ Menys de una nit per setmana ☐ Una nit o més nits per setmana

e. En els darrers 12 mesos, les sibilàncies han estat prou severes per limitar la parla del nen a únicament una o dues paraules entre respiracions? ☐ Si ☐ No

f. El vostre fill ha tingut algun cop asma? ☐ Si ☐ No

g. En els darrers 12 mesos, el tòrax del vostre fill ha sonat sibilant durant o després la realització d'exercici físic? ☐ Si ☐ No

h. En els darrers 12 mesos, el vostre fill ha tingut tos seca per les nits, sense haver estat refredat o amb una infecció de tòrax? ☐ Si ☐ No

Qüestionari bàsic de rinitis

a. Ha tingut algun cop esternuts, el nas "moquejava" o estava tapat quan no tenia la grip ni estava refredat? ☐ Si ☐ No

SI HA RESPOST "NO" PASSEU A LA PREGUNTA F

b. En els darrers 12 mesos, ha tingut algun cop esternuts, el nas "moquejava" o estava tapat quan no tenia la grip ni estava refredat? ☐ Si ☐ No

ID:
Inicials:

Reus & Tarragona Birth Cohort

Unitat de Medicina Preventiva i Salut Pública

SI HA RESPOST "NO" PASSEU A LA PREGUNTA F

c. En els darrers 12 mesos, aquests problemes al nas han estat acompanyats de picors als ulls o estaven plorosos? ☐ Si ☐ No

d. En quin dels darrers 12 mesos es van donar aquests problemes al nas? ☐ Gener ☐ Febre ☐ Març ☐ Abril ☐ Maig ☐ Juny ☐ Juliol ☐ Agost ☐ Setembre ☐ Octubre ☐ Novembre ☐ Desembre

e. En els darrers 12 mesos, quantes vegades aquests problemes al nas han interferit amb les activitats diàries del vostre fill? ☐ Cap vegada ☐ Una mica ☐ Unes quantes vegades ☐ Moltes vegades

f. El vostre fill ha tingut mai al·lèrgia al pol·len? ☐ Si ☐ No

Qüestionari bàsic de èczema

a. Ha tingut alguna erupció cutània amb picors que va estar començant i marxant al menys durant 6 mesos? ☐ Si ☐ No

SI HA RESPOST "NO" PASSEU A LA PREGUNTA G

b. Ha tingut alguna erupció cutània amb picors en algun moment dels darrers 12 mesos? ☐ Si ☐ No

SI HA RESPOST "NO" PASSEU A LA PREGUNTA G

c. Aquesta erupció cutània amb picors ha afectat alguna de les següents zones del cos: els plecs dels colzes, al darrera dels genolls, davant dels turmells, sota les natges o al voltant del coll, orelles o ulls? ☐ Si ☐ No

d. A quina edat va sorgir la primera erupció cutània amb picors? ☐ Abans dels 2 anys ☐ Entre 2-4 anys ☐ 5 anys o més

e. Aquesta erupció es va aclarir completament en qualsevol moment durant els darrers 12 mesos? ☐ Si ☐ No

f. En els darrers 12 mesos, quantes vegades de promig, el vostre fill s'ha quedat despert durant la nit a causa d'aquesta erupció? ☐ Mai en els darrers 12 mesos ☐ Menys de una nit per setmana ☐ Una o més nits per setmana

g. Ha tingut mai èczema? ☐ Si ☐ No

ID:
Inicials:

Reus & Tarragona Birth Cohort

Unitat de Medicina Preventiva i Salut Pública

Carnet de Salut

Creixement

Data	Pes (g)	Talla (cm)

Calendari de vacunacions

	DTPa	Poliomelitis	Haemophilus influenza tipus B	Meningitis C conjugada	XRP	Hepatitis B
2m	✓	✓	✓	✓		✓
4m	✓	✓	✓			✓
6m	✓	✓	✓	✓		✓
12m					✓	
15m				✓		
18m	✓	✓	✓			
4a					✓	
4-6a	✓					

ID:
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Reus & Tarragona Birth Cohort

Unitat de Medicina Preventiva i Salut Pública

Verificar informació embaràs

17. Hàbit tabàquic durant l'embaràs

Era fumadora activa 3 mesos abans de quedar-se embarassada?

☐ No ☐ Si Va mantenir l'hàbit durant tot l'embaràs?_____

☐ No ☐ Si Si no, quan ho va deixar?_____

Va estar en contacte amb alguna persona que fumés davant seu durant l'embaràs?

☐ No ☐ Si Durant quantes hores diàries aproximadament?_____

18. Hàbit tabàquic en l'entorn del nen

La mare fuma actualment o ha fumat al llarg de la vida del nen?

☐ No ☐ Si

Dins de casa, en quin lloc acostuma a fumar?

Si ha deixat de fumar, edat del nen?____

☐ Fora de casa
☐ A casa però sense estar davant del nen
☐ Davant del nen
☐ Altres

	0 cigs/dia	1-5 cigs/dia	6-10 cigs/dia	> 10 cigs/dia
Quants cigarrets al dia fuma?	☐	☐	☐	☐

19. El pare fuma actualment o ha fumat al llarg de la vida del nen?

☐ No ☐ Si

Dins de casa, en quin lloc acostuma a fumar?

Si ha deixat de fumar, edat del nen?____

☐ Fora de casa
☐ A casa però sense estar davant del nen
☐ Davant del nen
☐ Altres

	0 cigs/dia	1-5 cigs/dia	6-10 cigs/dia	> 10 cigs/dia
Quants cigarrets al dia fuma?	☐	☐	☐	☐

20. Altres persones de l'entorn del nen actualment fumen o han fumat al llarg de la vida del nen?

☐ No ☐ Si

Dins de casa, en quin lloc acostuma a fumar?

Si ha deixat de fumar, edat del nen?____

☐ Fora de casa
☐ A casa però sense estar davant del nen
☐ Davant del nen
☐ Altres

	0 cigs/dia	1-5 cigs/dia	6-10 cigs/dia	> 10 cigs/dia
Quants cigarrets al dia fuma?	☐	☐	☐	☐

ID:
Inicials:

Reus & Tarragona Birth Cohort

Unitat de Medicina Preventiva i Salut Pública

Dades sociodemogràfiques

21. Quina és la seva feina actual i quin nivell d'estudis màxim ha completat

	Mare		Pare	
Feina actual/ darrers 12 mesos				
Nivell d'estudis	☐	Sense estudis	☐	Sense estudis
	☐	Primària incompleta	☐	Primària incompleta
	☐	Primària (ESO, EGB)	☐	Primària (ESO, EGB)
	☐	EGB, batxiller	☐	EGB, batxiller
	☐	Formació professional	☐	Formació professional
	☐	BUP, batxillerat	☐	BUP, batxillerat
	☐	COU, PREU	☐	COU, PREU
	☐	Estudis universitaris de grau mig (Diplomatura)	☐	Estudis universitaris de grau mig (Diplomatura)
	☐	Estudis universitaris de grau superior (llicenciatura, màster, doctorat)	☐	Estudis universitaris de grau superior (llicenciatura, màster, doctorat)

22. Quantes persones formen la unitat familiar? ____

23. Ingressos nets anuals totals a la llar

Exemple: si la dona tenia un sou de 20000 €, l'home un de 18000€ i hi havia un avi que vivia amb la família i rebia una pensió de 6000 €

< 9000 €	>9000 € - 19000 €	>19000 € - 25000 €	>25000 € - 35000 €	> 35000 €
☐	☐	☐	☐	☐

≤ 9000 €	>9000 € - 19000 €	>19000 € - 25000 €	>25000 € - 35000 €	> 35000 €
☐	☐	☐	☐	☐

ID:
Inicials:

ID ☐☐☐

Inicials ☐☐☐☐

Centre ☐

TP-90

Data de compleció ☐☐ ☐☐ ☐☐
d d m m a a

(A omplir per l'investigador)

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Qüestionari d'activitat física i desenvolupament

Aquest quadern l'han d'omplir les mares i/o els pares, o bé el tutor legal amb l'ajuda del nen. Les preguntes fan referència a la activitat física, i al desenvolupament del vostre fill.

Les respostes que figurin dins d'aquest quadern, seran confidencials i mai estaran lligades al nom del nen.

Moltes gràcies per la vostra col·laboració.

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Qüestionari d'activitat física

Volem conèixer el nivell d'activitat física del vostre fill durant els **darrers 7 dies** (la setmana anterior). Això inclou esports o ball que el fan suar o que provoca que senti les cames cansades, o bé jocs que el fan respirar ràpidament com ara tocar i parar, saltar a la corda, córrer, escalar o d'altres.

Recordeu:

1. No hi ha respostes correctes o incorrectes – No es tracta de cap examen.
2. Si us plau, responguеu totes les preguntes tan honesta i precisament com pugueu.

1. Activitat física durant el temps lliure: El vostre fill ha realitzat alguna de les activitats següents en els **últims 7 dies** (setmana passada)? Si la resposta és SI, quantes vegades? (Marqueu un sol cercle per filera).

	No	1-2	3-4	5-6	7 cops o més
Saltar a la corda	○	○	○	○	○
Patinar	○	○	○	○	○
Jocs: Tocar i parar, ...	○	○	○	○	○
Caminar (exercici)	○	○	○	○	○
Bicicleta	○	○	○	○	○
Córrer/ Footing	○	○	○	○	○
Natació	○	○	○	○	○
Ballar	○	○	○	○	○
Muntar en monopatí	○	○	○	○	○
Futbol	○	○	○	○	○
Hoquei sobre gespa	○	○	○	○	○
Hoquei sobre patins	○	○	○	○	○
Bàsquet	○	○	○	○	○
Patinatge artístic	○	○	○	○	○
Esquí	○	○	○	○	○
Hoquei sobre gel	○	○	○	○	○

- 2 -

Altres:

_____ ○ ○ ○ ○ ○
_____ ○ ○ ○ ○ ○

2. En els **últims 7 dies**, durant les classes d'Educació Física, quantes vegades va estar molt actiu (jugant intensament, corrent, saltant, fent llançaments)? (Marqueu una sola resposta.)

No fa educació física	○
Gairebé mai	○
Alguna vegada	○
Sovint	○
Sempre	○

3. En els **últims 7 dies**, que feia la majoria de vegades a l'hora del pati? (Marqueu una sola resposta.)

Estar assegut (parlar, llegir, fer els deures)	○
Passejar	○
Córrer o jugar una mica	○
Córrer o jugar bastant	○
Córrer o jugar gairebé sempre	○

4. En els **últims 7 dies**, que feia la majoria de vegades a l'hora de dinar (a part de menjar)? (Marqueu una sola resposta.)

Estar assegut (Parlar, llegir, fer els deures)	○
Passejar	○
Córrer o jugar una mica	○
Córrer o jugar bastant	○
Córrer o jugar gairebé sempre	○

5. En els **últims 7 dies**, quants dies just després de l'escola va practicar esport, ball (dansa) o va jugar a jocs en els quals estava molt actiu? (Marqueu una sola resposta.)

Cap	○
Una vegada en la darrera setmana	○
2 o 3 vegades en la darrera setmana	○
4 vegades en la darrera setmana	○
5 vegades en la darrera setmana	○

6. En els **últims 7 dies**, quants dies per la tarda va practicar esport, ball (dansa) o va jugar a jocs en els quals estava molt actiu? (Marqueu una sola resposta.)

Cap	○
Una vegada en la darrera setmana	○
2 o 3 vegades en la darrera setmana	○
4 vegades en la darrera setmana	○
5 vegades en la darrera setmana	○

7. En **l'últim cap de setmana**, quants cops va practicar esports, ball (dansa) o va jugar a jocs en els quals estava molt actiu? (Marqueu una sola resposta.)

Cap	○
Una vegada en la darrera setmana	○
2 o 3 vegades en la darrera setmana	○
4 vegades en la darrera setmana	○
5 vegades en la darrera setmana	○

8. Quina de les següents afirmacions el descriu millor en els **últims 7 dies**? Llegiu les 5 afirmacions abans de decidir quina resposta el descriu.

a. Tot, o gairebé tot el seu temps lliure el va dedicar a activitats que requereixen poc esforç físic ○

b. Alguna vegada (1-2 vegades) ha practicat activitat física en el seu temps lliure (ex.: fer esport, córrer, nedar, bicicleta o aeròbic) ○

c. Sovint (3-4 vegades) ha practicat activitat física en el seu temps lliure ○

d. Bastant sovint (5-6 vegades) ha practicat activitat física en el seu temps lliure ○

e. Molt sovint (7 o més vegades) ha practicat activitat física en el seu temps lliure ○

9. Marqueu quantes vegades ha realitzat alguna activitat física (tals com fer esport, jocs, ballar o qualsevol altre activitat física) per cada dia durant la **darrera setmana**.

	Cap (0)	Poca (1)	Normal (2)	Bastant (3)	Molta (4)
Dilluns	○	○	○	○	○
Dimarts	○	○	○	○	○
Dimecres	○	○	○	○	○
Dijous	○	○	○	○	○
Divendres	○	○	○	○	○
Dissabte	○	○	○	○	○
Diumenge	○	○	○	○	○

10. Ha estat malalt durant aquest **última setmana**, o alguna cosa ha impedit que practiqués alguna activitat física? (Marqueu una casella.)

Si ○
No ○

Si la resposta és si, què ho ha impedit?

11. Com acostuma a anar el nen a l'escola?

- Bicicleta ○
- Cotxe/ moto ○
- Caminant ○
- Autobús ○

12. Quanta estona tarda en arribar a l'escola?

- 1 a 5 minuts ○
- 6 a 10 minuts ○
- 11 a 15 minuts ○
- Més de 15 minuts ○

13. Penseu en una **setmana habitual**. Indiqueu quantes hores al dia el nen realitza les accions següents:

	Entre setmana (promig dels cinc dies)	Caps de setmana (promig dels dos dies)
Mirar la televisió (DVD, vídeos i pel·lícules al ordinador)		
Jugar a l'ordinador (consoles,)		
Fer deures (sense ordinador)		
Dormir		
Llegir		

14. Té televisió a la seva habitació?

- Si ○
- No ○

Desenvolupament físic

Ens agradaria avaluar el desenvolupament físic del vostre fill utilitzant les figures que es representen en aquesta pàgina.
Aquestes, indiquen diferents estadis de pubertat comunament utilitzats per metges per avaluar el desenvolupament i creixement dels nens.

Els nens passen per els diversos estadis de desenvolupament físic en diferents edats. Alguns d'ells comencen tan aviat com als 6 anys, i d'altres no ho fan fins als 16.

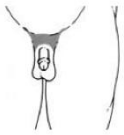
Les figures següents mostren diferents quantitats de pèl púbic masculí. Els nens poden passar per cada un dels diferents estadis representats.

Si us plau, mireu atentament cada una de les diferents figures. És important també que llegiu les descripcions.

Assenyaleu la casella que més concorda amb l'estadi del vostre fill.

○ Lleugera vellositat infantil.

○ Pel escàs, llis i lleugerament pigmentat, usualment arrelat al penis.



○ Pel arrissat, escassament desenvolupat, però obscur, clarament pigmentat i arrelat al penis.

○ No n'estic segur.

Monitoreig Ambulatori de la Pressió Arterial (MAPA)

Es tracta d'una tècnica no invasiva que obté mesures de la pressió arterial durant 24h del dia.

L'ús del MAPA no ha de provocar cap alteració en l'estil de vida del nen, i l'ha de permetre realitzar totes les activitats quotidianes de manera habitual.

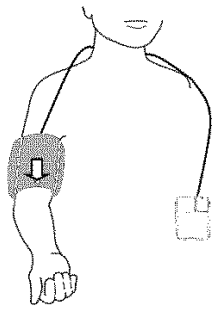
Cal tenir especial cura en el manteniment del MAPA i procurar que no quedi fora de la bandolera.

Per apagar, col·locar la pestanya de la part superior al "0", i per tornar a encendre, moure-la cap a l'altre costat. Sempre que el MAPA estigui posicionat en el braç, ha de romandre encès.

Què faig si...

... el braçalet es mou de lloc?

La fletxa de color blanc ha de quedar uns 2 cm per damunt del plec del braç, i assenyalant al punt mig tal i com es mostra en el dibuix.



...el MAPA pressiona molt fort a l'hora de prendre la mesura?

Serà necessari afluixar el braçalet, treien el "velcro" i col·locant-lo de manera que no pressioni massa, però que no quedi mòbil.

Per a qualsevol dubte, o problema que sorgeixi, 654 660 005.

Moltes gràcies per participar!!!

Unitat de Medicina Preventiva i Salut Pública

- 8 -

TABLE 3. Blood pressure for girls by age and height percentiles

		SBP (mmHg) percentile of height							DBP (mmHg) percentile of height						
Age (years)	BP percentile	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88
11	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92

TABLE 2. Blood pressure for boys by age and height percentiles

		SBP (mmHg) percentile of height							DBP (mmHg) percentile of height						
Age (years)	BP percentile	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92

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