University of São Paulo

School of Public Health

Association of low birth weight and prematurity with blood pressure and biomarkers of renal and endocrine-pancreatic functions in adults without nephropathy or diabetes

Julia Ines Fleitas Branda

Doctoral thesis submitted to the Graduate Program in Epidemiology

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Counselor: Professor Sandra Roberta G. Ferreira Vivolo, MD, PhD, Full Professor

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DEDICATION

Dedico este trabalho aos meus pais que em tudo sempre me apoiaram.

Ao meu querido pai Roberto, que é exemplo de força e tenacidade. Me orienta com sábias palavras e mostra o caminho do trabalho e da incansável leitura.

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SPECIAL DEDICATION

À Profª. Dra. Sandra Vivolo, que mais que orientadora foi minha Mestre, pois me acolheu quando ainda nada sabia e com excepcional sabedoria e competência me mostrou o caminho da ciência. Com sua generosidade me fez levantar voo a objetivos que sozinha não teria alcançado, respeitando sempre minha opinião e compreendendo minhas limitações. Me fez acreditar que sim, eu poderia fazer. Admiro pelo seu talento e inteligência, pelo respeito e o valor que dá à pessoa humana.

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RESUMO

Branda JIF. Identificação precoce de risco para doenças como diabetes mellitus (DM) e doença renal é essencial para propor medidas de prevenção [tese]. São Paulo: Faculdade de Saúde Pública da USP; 2022. **Introdução:** O baixo peso ao nascer (BPN), *proxy* de ambiente intrauterino hostil, tem sido associado a estas doenças no adulto. Em nosso meio, é escassa a literatura sobre associações de BPN com pressão arterial (PA) e função renal e endócrino-pancreática em adultos. O extenso banco de dados ELSA-Brasil permitiu explorar se: 1) indivíduos pré-diabéticos já poderiam apresentar acometimento renal, detectado de forma precoce por biomarcadores; 2) o BPN associar-se-ia no adulto à PA e marcadores de função renal e endócrino-pancreática mais desfavoráveis que nascidos de peso adequado. **Objetivos:** Analisar a associação do BPN com PA e marcadores de função renal e endócrino-pancreática em adultos sem DM e sem nefropatia. Os objetivos específicos foram: no Artigo 1, rever a literatura sobre a prevalência de doença renal diabética em indivíduos pré-diabéticos; Artigo 2, analisar a cistatina C sérica (sCys C) como marcador precoce de disfunção renal em indivíduos sem DM do ELSA-São Paulo; Artigo 3, comparar os valores de PA e de marcadores de função renal (taxa de filtração glomerular estimada - TFGe, razão albumina-creatinina - ACR e sCys C) segundo a presença de BPN e analisar sua associação com PA e marcadores de função renal, em indivíduios sem DM e nefropatia; Artigo 4, comparar os valores de marcadores de função de células β e sensibilidade à insulina (HOMA-β, HOMA-IR, HOMA-AD, QUICKI, TyG e TG/HDL), segundo a presença de BPN e analisar sua associação com marcadores de função de células β e sensibilidade à insulina. **Métodos:** Análises transversais de dados do ELSA-Brasil contemplaram 2 frentes, associação do BPN com PA e função renal e com função pancreática. Foram excluídos indivíduos com >60 anos, IMC <18,5 kg/m², DM, disfunção renal, tireoidiana e hepática. Dados sociodemográficos e de saúde foram coletados por questionários e os clínico-laboratoriais no HU/USP. As variáveis dependentes foram PA, marcadores de função renal e pancreática e a independente o BPN. Variáveis categóricas foram comparadas pelo qui-quadrado e contínuas por teste t de Student ou Wilcoxon. Usou-se regressão linear múltipla para testar associações do peso ao nascer com desfechos e DAG para obter os mínimos ajustes necessários nos modelos. Aplicou-se o escore de propensão para homogeneizar diferenças nos tamanhos amostrais. **Resultados:** Artigo 1:

Verificou-se prevalência de 4,5 a 26,0% de nefropatia diabética no pré-DM. Com base na TFGe, esta taxa variou de 4,5 a 21,3%, na albuminúria de 7,0 a 26,0% e quando combinadas de 12,3 a 17,7%. Artigo 2: Indivíduos com pré-DM tiveram maiores valores de sCys C que os normoglicêmicos [0,67 (0,41–0,95) *vs* 0,48 (0,31–0,81) mg/L, p<0,001] e menores de TFGe (96,3±17,4 *vs* 100,6±17,1 mL/min/1,73m², p<0,001). Normoglicêmicos hiperfiltrantes apresentaram valores menores de sCys C que os normofiltrantes (p=0,035). Comparando a TFGe entre os grupos, observou-se queda gradual à medida que pioravam a sCys C e ACR (p-trend=0,06). Artigo 3: O grupo com BPN apresentou níveis mais altos de PA sistólica (p=0,015), diastólica (p=0,014) e de ACR (p=0,031), e menores de TFGe (p=0,015) que o grupo nascido com peso normal. Os prematuros apresentaram níveis médios de PA mais altos que os nascidos com peso normal, mas não houve diferença em marcadores de função renal. À análise de regressão, níveis de PA sistólica e diastólica associaram-se com BPN, mas tal associação não se manteve após incluir prematuridade no modelo, a qual permaneceu associada com PA (p=0,017). Após aplicar escore de propensão, o BPN associou-se com ACR (p = 0.003) mas não com TFGe. Artigo 4: Indivíduos com BPN ou peso normal relataram valores similares de IMC aos 20 anos e o atual foi ligeiramente menor no grupo BPN. Seus dados cardiometabólicos e função endócrino-pancreática foram normais. Em análise de regressão, o HOMA-β mas não outros índices associou-se ao BPN (p=0,014) independente do sexo, cor, prematuridade e história familiar de DM. Após aplicar o escore de propensão, BPN manteve associação com HOMA-AD e TG/HDL. **Conclusão:** Nossos achados sugerem que indivíduos com alterações iniciais do metabolismo da glicose já podem apresentar biomarcadores de função renal comprometidos. Atentar-se a eventos precoces da vida como o BPN e prematuridade é relevante, uma vez que associações com PA e biomarcadores de função renal e endócrino-pancreática já podem ser identificadas mesmo em indivíduos saudáveis – sem DM e nefropatia. Estudos prospectivos são necessários para avaliar o valor preditivo vislumbrando propor medidas de prevenção.

Palavras-chave: Baixo peso ao nascer, taxa de filtração glomerular, razão albumina-creatinina, HOMA-β, resistência à insulina, DOHaD.

ABSTRACT

Branda JIF. Identifying early determinants of chronic diseases, such as diabetes mellitus (DM) and kidney disease, is essential to propose prevention measures. [thesis]. São Paulo: Faculdade de Saúde Pública da USP; 2022. **Introduction:** Low birth weight (LBW), a *proxy* for hostile intrauterine environment, has been associated with these diseases in adulthood. In Brazil, there is scarce literature on the association of LBW with blood pressure (BP) or kidney and pancreatic functions in adults. The big ELSA-Brasil database allowed to explore whether: 1) pre-diabetic individuals could have kidney function impairment, detectable by renal biomarkers; 2) LBW is associated with less favorable BP levels and kidney and pancreatic function in adulthood compared to normal birth weight. **Objectives:** To analyze the association of LBW with BP and biomarkers of kidney and endocrine-pancreatic function in adults without DM or nephropathy. The specific objectives were: Paper 1: to review the literature on the prevalence of diabetic kidney disease (DKD) in pre-diabetic individuals. Paper 2: to assess serum Cystatin C (sCys C) as an early marker of kidney dysfunction in individuals without DM. Paper 3: to compare BP levels and kidney function biomarkers (estimated glomerular filtration rate - eGFR, albumin-creatinine ratio - ACR and sCys C) according to the presence of LBW and to analyze their associations with BP and kidney function biomarkers in individuals without DM or nephropathy. Paper 4: to compare markers of β–cell function and insulin sensitivity (HOMA-β, HOMA-IR, HOMA-AD, QUICKI, TyG and TG/HDL) according to the presence of LBW and to analyze LBW associations with markers of β-cell function and insulin sensitivity. **Methods:** Cross-sectional analysis of ELSA-Brasil data includes 2 fronts: assessment of the LBW associations with BP and kidney function and with endocrine-pancreatic function. Individuals aged > 60 years, BMI < 18.5 kg/m², DM, kidney, thyroid and liver dysfunction were excluded. Sociodemographic data, lifestyle, birth weight and previous diseases were collected by questionnaires, and clinical and laboratory data in the HU/USP. Dependent variables were BP, biomarkers of kidney and pancreatic functions, and independent variable was LBW. Categorical variables were compared using the chi-squared test and continuous variables by Student *t* test or the Wilcoxon test. Multiple linear regression models were employed to analyze associations between LBW and the outcome variables. Directed acyclic graph (DAG) was used to make the minimum necessary adjustment to the models. The propensity score method was applied to homogenize

differences in sample size. **Results:** Paper 1: Prevalence of DKD ranged from 4.5 to 26.0% in pre-diabetic individuals. Considering eGFR in isolation, the prevalence rates varied from 4.5 to 21.3%, based only on ACR from 7.0 to 26.0% and based on combined criteria the prevalence was between 12.3 and 17.7%. Paper 2: Pre-diabetic individuals had higher sCys C levels than normoglycemic ones [0.67 (0.41–0.95) *vs* 0.48 (0.31–0.81) mg/L, p<0.001] and lower eGFR (96.3±17.4 *vs* 100.6±17.1 mL/min/1.73m², p<0.001). Normoglycemic hyperfiltrating individuals had lower sCys C than normofiltrating ones (p=0.035). Comparing eGFR levels between groups, this gradually decreased as the sCys C and ACR parameters worsened (p-trend=0.06). Paper 3: The LBW group had higher systolic (p=0.015) and diastolic BP (p=0.014) and ACR values (p=0.031), and lower eGFR (p=0.015) than normal birth weight. The preterm group had higher mean BP levels, but no difference in kidney function was detected. In a regression model, BP levels were associated with LBW, but this association disappeared after adding prematurity, which remained associated with BP (p=0.017). Having applied propensity score matching, LBW was associated with ACR (p=0.003), but not with eGFR or BP levels. Paper 4: Individuals with LBW or normal birth weight reported similar BMI at the age 20 years and current BMI was slightly lower in the LBW group. Cardiometabolic and endocrine-pancreatic function parameters were within normal ranges. In regression analysis, log-transformed HOMA-β, but not the other indexes, was associated with LBW (p=0.014) independent of sex, skin color, prematurity, and family history of DM. After applying propensity score matching LBW was associated with HOMA-AD and TG/HDL indexes. **Discussion:** Our findings suggest that individuals with near-normal glucose metabolism disturbance could have some impaired kidney function. Looking at early-life risk factors is relevant since their associations with BP and renal and pancreatic function biomarkers could already be identified even in healthy individuals, without DM and nephropathy. Prospective studies are needed to assess the predictive value aiming at proposing prevention measures.

Keywords: Low birth weight, glomerular filtration rate, albumin-creatinine ratio, HOMA-β, insulin resistance, DOHaD.

GENERAL PRESENTATION

This research was developed as part of a multicenter study entitled the Estudo Longitudinal de Saúde do Adulto (Longitudinal Study of Adult Health) – ELSA-Brasil, conducted by a multi-institutional executive committee under the auspices of research funding bodies[1]. ELSA-Brasil involves 6 public universities or research institutions. The ELSA-Brasil cohort, launched in 2008, investigates aspects of risk, development, and progression of non-communicable chronic diseases (NCCDs), mainly cardiovascular diseases and diabetes mellitus[2].

The present analysis drew on the 2008-2010 baseline data, obtained at the University of São Paulo research center (ELSA-São Paulo). This database has strict quality control and includes sociodemographic, lifestyle and health aspects, together with physical, imaging and laboratory data. Most relevant information to the present sub-study is related to early life events, such as birth weight.

ELSA-Brasil conforms to the requirements for scientific research in humans, having been approved by Research Ethics Committees and informed consent was obtained from all participants.

The two research branches of the current study were approved by the ELSA publications committee and registered under the titles: 1) Assessment of the association of low birth weight with blood pressure and biomarkers of kidney function and 2) Assessment of the association of low birth weight with biomarkers of endocrine-pancreatic function.

This doctoral thesis is being presented in chapters in which the background, *rationale* and hypotheses for the entire work were provided. The Objective chapter includes the general purpose of this investigation, as well as specific objectives which were addressed in each of the 4 manuscripts which compose the present thesis. The Methods chapter contains more detailed information than in the manuscripts; the traditional Results and Discussion chapters were replaced by published or submitted manuscripts. Finally, this thesis includes the main Conclusions of the entire work.

CONTENT

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ABBREVIATIONS

ACR, Albumin-to-creatinine ratio

AH, Arterial hypertension

BMI, Body mass index

BP, Blood pressure

CKD, Chronic kidney disease

CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration

CVD, Cardiovascular disease

DAG, Direct Acyclic Graphs

DKD, Diabetes Kidney Disease

DM, Diabetes mellitus

DOHaD – Developmental Origins of Health and Disease

ELSA-Brasil – Estudo Longitudinal de Saúde do Adulto

GHO – Global Health Observatory

GFR, Glomerular filtration rate

HDL-c, High-density lipoprotein cholesterol

HOMA-AD, HOMA-IR; Homeostasis Model Assessment – Adiponectin, Insulin Resistance

IL, Interleukin

IUGR, Intrauterine growth restriction

LDL-c, Low-density lipoprotein cholesterol

LBW, Low birth weight

NCCD, Non-communicable chronic diseases

QUICKI, Quantitative Insulin Sensitivity Check Index

sCys C, Serum cystatin C

SINASC, Sistema de Informação sobre Nascidos Vivos (system of live birth)

TSH, Thyroid stimulating hormone

TyG, Triglycerides-glucose index

TG/HDL, Triglycerides/HDL-c index

VIGITEL, Surveillance of risk and protective factors for chronic disease by telephone

WHO, World Health Organization

1. INTRODUCTION

Non-communicable chronic diseases (NCCDs) represent major challenges in public health. Cardiovascular disease (CVD), arterial hypertension (AH), type 2 diabetes mellitus (DM) and chronic kidney disease (CKD) are relevant due to their high prevalence rates and deleterious impact on life expectancy of affected people. World Health Organization (WHO) estimates indicate that NCCDs accounted for 70% of all deaths globally in 2015, with CVD representing the leading cause (45% of all deaths due to NCDs), while DM directly responsible for 4% of these deaths[3]. From 1990 to 2015, the proportion of deaths due to NCCDs in Brazil increased from 59.6 to 75.8%[4].

In 2008, the WHO Global Health Observatory estimated that 40% of adults aged over 25 years had AH[3]. As far as DM is concerned, in 2014 the global prevalence was 8.5%, while CKD ranged from 8-16%. AH and DM represent major risk factors for CVD and are the leading causes of CKD worldwide[6].

The 2013 Brazilian National Health Survey (PNS) found that 21.4% of individuals aged over 18 years reported a clinically confirmed diagnosis of AH, 6.2% type 2 DM and 1.4% of CKD[7]. Brazilian registries revealed that rates of new patients on chronic dialysis programs are growing by roughly 8% a year[8] with a total number of 112,000 patients on dialysis in 2014[9]. The VIGITEL (Surveillance of Risk and Protective Factors for Chronic Disease by Telephone Survey) showed a prevalence of self-reported AH of 24.3% in adults with higher rates in women (26.4%) than in men (21.7%). DM was reported by 7.6% of adults, increasing with aging[10].

High prevalence rates of these debilitating morbidities in Brazil and worldwide are a concern because they adversely impact quality of life and survival of individuals and impose substantial costs to health systems.

This scenario justifies efforts to identify and combat the NCCD risk factors. Investigations of early life events may represent a possibility of more timely identification of risk for these morbidities, allowing early implementation of preventive interventions.

1.1. EARLY LIFE EVENTS AND ASSOCIATIONS WITH NCCDS IN ADULTS

There is accumulating evidence on the role of early life events in predicting the risk of cardiometabolic and kidney diseases in adulthood[11,12]. Among those events, a low birth weight (LBW) has been seen as an important *proxy* of a hostile intrauterine environment, associated with several NCCD[13-15].

According to WHO, LBW is defined by a weight of under 2,500 g, being a public health concern in both developed and developing countries. Globally, an estimated 15-20% of newborns have LBW, representing 20 million births annually; rates vary widely from 9% in Latin America to 28% in South Asia[16]. In Brazil, according to the Information System on Live Births (SINASC), LBW rate is around 8%, ranging from 7.4% in the Midwest to 8.4% in the Southeast of the country[17]. Of LBW newborns, 11.5% are pre-term, a condition which has been associated with increased neonatal mortality[18-20].

LBW occurs due to prematurity and/or intrauterine growth restriction (IUGR). In developed countries, LBW occurs predominantly due to prematurity, while in developing countries LBW tends to involve babies who are small for gestational age due to IUGR[21].

The association of LBW with short stature, lower lean mass and high blood pressure, even at school age, has been extensively described[22], as well as with long-term consequences such as obesity, insulin resistance, AH, type 2 DM, and CKD in adults[23-27]. These findings have established a new bridge of causality in which metabolic adaptations occurring early in life culminate in NCCDs in adulthood.

Initial observations suggesting causality of early life events date back to the 1930s in studies examining the relationship between environmental conditions during intrauterine life and infancy associated with survival[28]. Later, in the 1970s, researchers investigated adult offspring of women who had endured food shortages at the end of second world war. The results showed these individuals had different body compositions depending on the period their mothers were exposed to famine. Offspring whose mothers experienced undernutrition in the first 6 months of pregnancy had higher incidence of obesity compared to those whose mothers faced restricted dietary intake in the final trimester of pregnancy[29]. Toward the 1990s, the theory on early life events as a determinant of risk for CVD was reaffirmed by Baker *et al*[30]. Increased mortality rates due to cardiovascular and cerebrovascular diseases in adults suggested that previous conditions of poverty, characterized by poor maternal nutrition during pregnancy and

nutritional deficit in childhood, particularly if followed by subsequent high calorie intake, elevate the vulnerability to NCCD[31].

This phenomenon raised the concept of t fetal origins of adult diseases, later termed the Developmental Origins of Health and Disease (DOHaD). This theory defends that exposures to environmental factors during critical stages of development and growth can have health effects for the individual in the short- and long-terms. This is based on the plasticity of development: a single genotype influenced by specific intrauterine events are able to produce different phenotypes[23]. As an adaptive response to the intrauterine environment, the organism can predict the environment in which it will develop, promoting structural and functional changes in several tissues and organs. Thus, the fetus exposed to hostile intrauterine conditions due to poor nutrition, infection or metabolic alterations, responds with adaptations to the development process in order to promote immediate and future survival[32].

According to the DOHaD theory, adverse conditions during intrauterine life and the initial years of childhood impact the cardiometabolic risk in adulthood[30,33]. The cause-effect pathway explaining the mechanism by which LBW would influence disease development in adult life is complex involving genetic but mainly epigenetic modifications[34]. Epigenetics has been an emerging focus of research in literature, dealing with the interaction of the environment with the genome that produces changes which can then be passed on to future generations. Environmental exposures during the life span, such as those related to diet and nutrition, can result different phenotypes without altering the genome, but changing the way that genes are expressed. Early environment-genome interaction during intrauterine life can promote permanent changes in organs and systems, predisposing to obesity, AH, type 2 DM and CVD. Such diseases share underlying pathophysiological abnormalities such as low-grade chronic inflammation and insulin resistance[35]. The named intergenerational epidemiology has sought to study the risks of future NCCDs arising from early-life exposure to environmental insults[36].

1.2. BIRTH WEIGHT AND ARTERIAL HYPERTENSION IN ADULT LIFE

AH has a multifactorial origin, associated with increased mortality, especially for cardiovascular outcomes. Factors implicated in the genesis of AH include age, sex, race, body weight, excessive salt consumption and sedentarism. Evidence suggested that LBW may be an early risk factor for developing AH in adult life[37,38]. This consistent association has supported the hypotheses on the fetal origin of this adult disease. An inverse association between birthweight and subsequent blood pressure levels has been confirmed in developed and developing countries[39]. However, the effect size of birthweight on blood pressure in adulthood varies widely; some studies have reported increases in systolic blood pressure ranging from 0.6 mmHg to 2-3 mmHg for each decrease in kilograms of birthweight[40,41]. In specific populations, such as the Japanese, a sex-mediated relationship LBW – AH was observed considering that high blood pressure in adulthood was detected only in women born with less than 2,500 g[42].

Several underlying mechanisms have been proposed to explain increased blood pressure in adulthood after intrauterine suffering and LBW. Human and animal studies have identified a role of the renin-angiotensin-aldosterone system via changes in RAS gene expression, angiotensin-converting enzyme hyperactivity and elevated circulating angiotensin II and aldosterone levels, resulting in increased blood pressure[43-45]. The sympathetic nervous system also plays an important role in the AH pathogenesis, since sympathetic hyperactivity was demonstrated in individuals with LBW[46,47]. Elevated oxidative stress and endothelial dysfunction markers, changes in endothelin receptor expression in the vascular wall and high pro-inflammatory cytokines levels, such as interleukin-6 (IL-6), have also been associated with the development of AH in adults who experienced intrauterine growth restriction[48,49]. Animal-based evidence has supported that low intrauterine energy supply induces adaptive fetal programming to the unfavorable environment, resulting in fewer number of cells and lower functional reserve of several organs which increase susceptibility to several diseases during the life course[34].

1.3. BIRTH WEIGHT AND KIDNEY DYSFUNCTION IN ADULT LIFE

Adverse intrauterine conditions affect the embryogenesis of the urinary tract and negatively impact renal function in adulthood. Embryonic development starts in the fourth

week of pregnancy from the urogenital ridge which gives rise to the nephrogenic cord. In the first trimester of pregnancy, the primordial phase for development, the cord divides into 3 segments: pronephros, mesonephros, and metanephros, with this last structure forming the final kidney. This develops from the ureteric bud which gives rise to the ureters, renal pelvis, collecting tubules and ducts and from the metanephrogenic blastema forming glomeruli, Bowman's capsule, convoluted tubules, and Henle loops, thus constituting the functional unit of the kidney, the nephron[50]. The number of nephrons increases only during pre-natal life[51] and nephrogenesis is complete at 32-36 weeks of pregnancy[52]. Glomerular filtration commences at the ninth week and the filtration rate increases after birth. Adequate nephrogenesis is essential to ensure good renal function later in life.

Renal complications in offspring were found in animal models and humans exposed to an adverse intrauterine environment. A lower total number of nephrons was observed in offspring from mothers undernourished during pregnancy, or that born with IUGR, or from fetal suffering due to placental anomalies[53,54]. Also after birth, structural abnormalities were evident in the cortical region of kidney, such as shrinking of the glomerular capillary tuft and enlargement of the space of Bowman's capsule[55]. The smaller glomerular capillary volume of some of the nephrons leads to glomerular hypertrophy of the intact nephrons in an attempt to achieve renal function maturation. Consequently, initial size of the kidneys of underweight newborns is smaller than those of normal weight newborns. During the neonatal period, these kidneys exhibited hypertrophy assuming a disproportional morphology in relation to the body size of the neonate[56]. Glomerular hypertrophy and accelerated renal maturation lead to hemodynamic changes due to increased post-natal demand on the kidney[57]. These structural modifications induce hemodynamic changes increasing the risk of CKD and AH in the long-term. Early abnormalities in intrauterine life that elevate risk for the development of CKD and AH also favor obesity and type 2 DM in adulthood[58,59]. Therefore, obese and or diabetic individuals who had LBW are more prone to progression to CKD[27].

Despite consistent evidence regarding the association between LBW and NCCDs in adult life, it is unclear to what extent early BP monitoring, as well as circulating biomarkers of kidney and endocrine-pancreatic functions in healthy adults would be useful

for screening and identifying individuals at higher risk. A deeper understanding of the particularities of the natural history of these diseases in people submitted to adverse intrauterine conditions could help proposing preventive measures earlier in the life course.

1.4. BIRTH WEIGHT AND B-CELL DYSFUNCTION IN ADULT LIFE

Type 2 DM represents a major burden in public health due to its high prevalence, debilitating micro- and macrovascular complications, and treatment costs. Its genesis is multifactorial involving genetic and lifestyle factors[60] accompanied by accumulation of body fat which produces cytokines promoting low-grade inflammation and insulin resistance[61]. Consistent evidence from animal and human studies has associated LBW with further occurrence of type 2 DM[62].

Rodents submitted to IUGR through undernutrition had a 25% lower insulin storage in pancreatic tissue and 40% reduction in beta-cell mass[63,64]. This condition is disadvantageous in situations of greater insulin demand (for instance, increased adiposity) that may favor hyperglycemia.

Findings in humans revealed that predisposition to DM may start early in the neonatal period and childhood. A German study evaluated glycosylated hemoglobin levels in LBW infants and compared to those from normal-weight controls; the low-weight group had higher glycosylated hemoglobin levels, suggesting that alterations in glucose metabolism start even before birth[65]. Further evidence showed that insulin resistance was present in LBW children early in infancy, which could increase risk for type 2 DM[66,67].

Similar findings have been reported in adults. Some authors claim that insulin resistance is the main abnormality favoring the occurrence of DM in adults born with low weight but not the deficiency in insulin production[68,69]. They attribute the increased risk to the sustained insulin resistance in peripheral organs and increased β-cells insulin production, leading to persistent hyperinsulinemia until pancreatic failure when hyperglycemia develops. However, a study conducted in Denmark, reported that insulin secretion of men with LBW was reduced by up to 30%, unaccompanied by increased insulin resistance[70].

IUGR-induced metabolic abnormalities were investigated in rodents; they exhibited increased glucose production, insulin resistance and expression of PEPCK genes and glucose-6-phosphatase[71]. The increased expression of these genes suggested signaling activation in the liver to promote gluconeogenesis. An explanation for triggering of this response was a compensatory mechanism for the fetal suffering caused by undernutrition in an attempt to maintain fetal glucose homeostasis. The persistence of this mechanism after birth lead to deleterious changes in glucose metabolism. In another experiment in animals submitted to a similar uterine environment, authors found a reduction in glucose uptake by skeletal muscle. This abnormality was accompanied by a reduction in expression of the GLUT4 glucose transporter in muscle and adipose cells and of its translocation to the cellular surface, leading to a reduced glucose uptake of peripheral organs[72].

These metabolic changes associated with LBW, seen as a *proxy* of a hostile intrauterine environment, may disrupt glycemic homeostasis and then increase the risk of type 2 DM, particularly if the child and/or the adult develops excessive body adiposity.

1.5. BIOMARKERS OF KIDNEY AND ENDOCRINE-PANCREATIC FUNCTION

Numerous biomarkers have been proposed for assessing kidney and endocrine-pancreatic function and some of most interest are highlighted in this study.

1.5.1 Renal Function

Serum determinations of urea and creatinine are the most widely employed parameters for assessing renal function, whose alterations are indicative of an advanced stage of functional loss. A more accurate assessment is provided by measures of volume filtered by the glomeruli in unit time, i.e., the glomerular filtration rate (GFR) adjusted for body surface area. The GFR can be measured directly using the clearance of certain exogenous substances fully excreted via the kidneys[73]. Due to financial and technical constraints, in clinical practice GFR is not determined using this method. Instead,

mathematical formulas have been proposed that can yield sufficiently accurate estimates of renal function (eGFR).

Among the several markers of renal function already studied, the urinary excretion of albumin was one of the most used to assess early glomerular dysfunction in patients with prevalent diseases in populations. As far as circulating biomarkers are concerned, serum cystatin C (sCys C) measurement shows clinical and analytical advantages which will be detailed later.

Assessment of the decline in renal function is of great importance given that kidney disease is a serious health issue worldwide[5]. CKD can be defined as a structural or functional abnormality of the kidneys, present for over 3 months, with repercussions on health[74]. Scientific societies currently recommend staging of nephropathy, especially based on albuminuria and eGFR. Persistent albuminuria or reduced eGFR below 60 mL/min/1.73 m^2 are bases for establishing the degrees of renal function deficit[75].

Also, studies have shown that DKD may present phenotypes other than the classical one, characterized by ACR increase and gradual GFR decline. Several factors (age, sex, obesity, kind of pathological kidney injury) have been related to the different DKD phenotypes[76]. Among other clinical courses described are the regression of albuminuria, rapid eGFR decline and non-proteinuric non-albuminuric DKD. These observations reinforce the recommendation for monitoring risk factors as well as several lab parameters for adequate assessment of renal function in at-risk individuals.

1.5.1.1. Albuminuria

Albuminuria results from the injury of the glomerular basal membrane leading to increased permeability to macromolecules. Urinary albumin excretion measurement is a reliable marker of renal damage. Conventional methods for quantifying albumin in urine were insufficiently sensitive for detecting small amounts of protein losses; technical improvement conferred by immunoturbidimetry has allowed an accurate screening and identification of microalbuminuria that characterizes an early functional impairment. This assay based on the formation of antigen-antibody complex was key to improving the efficacy of interventions to prevent progression to advanced stages of nephropathy.

Albuminuria can be measured using 24-hour urine, 12-hour nocturnal samples, isolated morning samples or random samples. Due to difficulties regarding 24-hour collection, together with the influence of physical activity on albumin excretion, it has been recommended to collect overnight or morning isolated samples. Microalbuminuria diagnosis is established based on urinary excretion of albumin within the range 20–200 µg/min or ≥ 30 mg/24 hrs or on albumin/creatinine ratio (ACR) ≥ 30 mg/g of creatinine. Corrections for creatinine is recommended for reducing bias related to concentration or volume of urine collected. Consistent evidence supports that isolated samples represent a reliable alternative to the laborious 24-hour collection[77].

Increased ACR has been widely used for detecting glomerular damage due to systemic diseases, such as AH and DM[75]. In addition to serving as a marker of progression to advanced stages of nephropathy, albuminuria can also predict macrovascular outcomes[78], interpreted as a manifestation of general endothelial dysfunction. Pharmacological and non-pharmacological interventions have proven effective for delaying end-stage renal failure[79].

1.5.1.2. Serum Cystatin C

Despite the easy determination of serum levels of creatinine for assessing kidney function, it is known that this measure is influenced by muscle mass, age and sex[73]. An ideal substance for determining kidney function should have stable production, should be readily filtered by glomeruli and unaffected by concomitant diseases or by tubular activity, as secretion or reabsorption. Some exogenous substances offering these features are inulin, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), iothalamate and iohexol. The use of these substance is limited because the tests are costly and time-consuming.

A more practical accurate marker for assessing renal function is the serum cystatin C. Discovered in the 1960s, cystatin C was consistently shown as a renal marker in the mid-80s, when its serum concentration was found to be inversely correlated with glomerular filtration[80]. Cystatin C is a cysteine protease inhibitor and has the role of inhibiting proteases secreted from lysosomes of injured cells or which have commenced

apoptosis, protecting connective tissue. An important characteristic is that all nucleated cells express cystatin C and its rate of production is stable, uninfluenced by muscle mass, age, sex, diet, or body weight, and freely filtered by the glomeruli[81]. In view of these properties, cystatin C has emerged as a valuable marker for assessing renal function in clinical situations, such as in the pediatric and elderly populations, patients in use of immunosuppressants and chemotherapy, individuals with high muscle mass and those suspected of having mild renal function deficit[82].

Recently, guidelines of international scientific societies have recommended the use of cystatin C only in specific cases, due to the high cost of its assay. Reference values differ between from 0.64 to 0.84 mg/L for men and from 0.57 to 0.74 mg/L for women[83]. Cystatin C determination in sera is recommended principally for mild renal dysfunction given its superior accuracy over creatinine in this condition[75]. Also, in clinical practice, the use of cystatin C levels alone, and in association with GFR calculation, have proven a useful alternative to creatinine measurement[84].

Further investigations of the usefulness and true benefits of serum cystatin C in large studies are warranted, enabling levels to be determined in individuals across a wide age range with different health conditions. In this respect, the Longitudinal Study of Adult Health - ELSA-Brasil seems appropriate to this type of investigation[1].

1.5.2. Endocrine-Pancreatic Function

Estimates of β-cell function have been based on measurements or index that express insulin secretory capacity or functional reserve in pathological conditions. A classical example is in type 1 DM, which is characterized by marked autoimmune destruction of β-cell mass resulting in low circulating levels of insulin, as well as the C-peptide that connects the chains of the proinsulin molecule. The determination of peptide C is useful for the assessment of functional reserve in that type of DM. For the purpose of the present study, exploring associations of early life events with endocrine-pancreatic function in adults at risk for type 2 DM, both estimates of insulin secretion and of insulin resistance are of interest considering the pathophysiological mechanisms.

1.5.2.1. Insulinemia and HOMA Indexes

Measurements of serum insulin express β-cells capacity to produce and release hormone in circulation. These have been employed to indirectly reflect the degree of sensitivity of peripheral tissues to its action. Major actions of insulin in glucose homeostasis include the inhibition of hepatic glucose production and peripheral glucose uptake by muscle, adipose and other tissues. Insulin effects depend on binding to transmembrane receptors and intracellular signaling culminates with the translocation of glucose transporters (GLUT) to the cellular surface, which allows glucose uptake into the intracellular medium[85]. Under pathological conditions (genetic or environment-dependent), glucose uptake by tissues in response to insulin stimuli can be impaired, named insulin resistance. The interpretation of blood determination of insulin alone has limitations; its concentration varies with body adiposity and with degree of resistance of other peripheral tissues.

The gold standard method for assessing insulin sensitivity or resistance is the hyperinsulinemic euglycemic clamp[86]. This is an invasive high-cost procedure, mostly used in the research setting. Alternatives for assessing insulin resistance include mathematically calculated indices, such as the HOMA-IR (Homeostasis Model Assessment of Insulin Resistance), QUICKI (Quantitative Insulin Sensitivity Check Index) and the Matsuda index, among others[87]. Of these indices, the HOMA-IR is the most widely used, whose calculation is based on fasting plasma glucose and insulin values. HOMA-IR results obtained in different populations have shown strong correlation with estimates of insulin resistance using the insulin clamp[88]. Another mathematical model for estimating secretory capacity of β-cells is the HOMA-β. The equations describing these indexes are:

HOMA-IR = [fasting insulin (μUI/mL) x fasting glucose (mmol/L)] / 22.5

HOMA-β = [20 x fasting insulin (μUI/mL)] / [fasting glucose (mmol/L) – 3.5]

The HOMA-IR cut-offs for characterizing individuals as insulin resistant vary according to several factors, mainly age, adiposity and the population studied. In Brazil, the Brazilian Metabolic Syndrome Study suggested a cut-off of 2.71 on the HOMA-IR for a diagnosis of insulin resistance[89]. As far as reference value for HOMA-β is concerned,

there is scarce data in Brazilian population. Most studies have addressed the HOMA-IR and very few use the HOMA-β for assessing endocrine-pancreatic function. In Brazil, a study of 1,100 individuals investigated the functional capacity of β-cells. The group with healthy characteristics had a mean HOMA-β of 115 ± 89[90]. It is important to highlight that when defining the normal HOMA-β value, a number of factors that directly or indirectly influence its results should be taken into account. These include age since there is a natural physiological decline in β-cell function with aging[91]. Excess body adiposity, accompanied by sustained hyperglycemia, can lead to lipotoxicity and glucotoxicity of β-cells, affecting insulin production[92]. Therefore, when assessing secretory function of β-cells, the individual conditions affecting insulin sensitivity must be considered. False low HOMA-β results can occur in individuals of normal weight with preserved lean mass and normal-high insulin sensitivity, as a compensatory reduction in β-cells secretion. In these cases of normal-to-high insulin sensitivity, it was found that a reduction in β-cell function of up to 50% can occur as a compensatory response[93]. Conversely, obese individuals can falsely increase HOMA-β values due to the presence of insulin resistance and hyperinsulinemia; when adjusting for degree of resistance there is a decrease in HOMA results of β-cell function[94].

Thus, tissue sensitivity to insulin action and β-cell function can be assessed indirectly and in a minimally invasive way. Abnormalities in hormonal (endocrine pancreas) action and production often occur concomitantly; in general, during the natural history of type 2 DM, insulin resistance is the initial pathophysiological disturbance, preceding the gradual decline in β-cell function.

1.5.2.2. HOMA-Adiponectin Index

More recently, a variant of the HOMA-IR, the HOMA-adiponectin (HOMA-AD) index, was proposed for assessing insulin resistance that accounts for the effect of adiposity on insulin resistance. It was demonstrated that this adipocyte-derived hormone has a sensitizing effect for insulin action and that its production is inversely associated with the adipose mass[95]. The incorporation of adiponectin concentration to the

denominator of the HOMA-IR calculation was proposed to adjust the index for the individual body adiposity[96]. The HOMA-AD is calculated using the following formula:

HOMA-AD = Fasting glucose (mmol/L) X Fasting insulin (mU/L) / 22,5 X Fasting adiponectin (µg/mL)

The HOMA-AD has been applied to different populations, e.g., in pediatric patients[97], individuals with CKD[98] and, in Brazil, it has been used for the evaluation of non-diabetic young adults[99]. In the latter, the index exhibited good correlation with the HOMA-IR index and with metabolic parameters. On the other hand, a study of individuals with hepatitis C failed to confirm additional advantage of using the HOMA-AD over the HOMA-IR for assessing insulin resistance[100]. Thus, the utility of this index for assessing insulin resistance in individuals at different categories of risk warrants further investigation.

Few studies have proposed HOMA-AD reference values in populations worldwide. In Brazil, a threshold value of 0.95 has been suggested for screening insulin resistance in women[99].

1.5.2.3. Triglyceride-Glucose (TyG) Index

In contrast to HOMA calculations that requires hormone determination (serum insulin and adiponectin) an alternative low-cost index to estimate insulin resistance is the triglyceride-glucose (TyG). The TyG is obtained as the log product of serum triglyceride concentrations multiplied by fasting plasma glucose.

The TyG index has been used and validated in several populations, including adults[101-103] and children[104-106]. In a study conducted in Brazilian adults, the performance of the TyG was compared to the gold standard, the hyperinsulinemic-euglycemic clamp[106]. Strong correlation between the methods for insulin resistance assessment was observed, and the TyG showed to be also associated with the degree of adiposity, metabolic parameters and markers of subclinical atherosclerosis. Other studies on different populations have reinforced the utility of this index as a predictor of cardiovascular events[107,108].

There is no consensus in the literature regarding a cut-off point of the TyG for defining insulin resistance. Nevertheless, the values reported to date by different studies

have proven similar. Espinel-Bermúdez et al. found an optimal cut-off of 4.68 for identifying insulin resistance in a sample of Mexican adults[109], similar to another study of Mexicans that reported a value of 4.55[110].

Particularly for developing countries, the TyG index may represent a promising low-cost surrogate marker for assessing insulin resistance in routine clinical practice. Follow-up studies are still necessary to evaluate its usefulness to predict cardiovascular complications of clinical conditions of insulin resistance.

1.5.2.4. TG/HDL Index

Another estimate of insulin resistance that could be easily calculated in clinical practice is the TG/HDL index. Insulin resistance is considered the key abnormality of the metabolic syndrome, whose diagnosis is based on the presence of at least 3 disturbances. Among its diagnostic criteria, elevated triglyceride and reduced HDL-cholesterol concentrations were included since they were major representatives of the insulin resistance-induced lipid disturbances. In this context, the ratio TG/HDL was proposed and then its utility has been studied in several populations.

TG/HDL has been shown to be a good instrument for the assessment of insulin resistance in Caucasians, African Americans, Hispanics and non-Hispanic whites[111-113]. It was studied in a Brazilian population sample and proved to be the most suitable biochemical indicator of the lipid profile to identify insulin resistance[114]. This index is not only useful for the detection of insulin resistance but also for predicting diseases, such as type 2 DM[115] and cardiovascular outcomes[116]. It was reported that TG/HDL was an independent predictor of coronary heart disease, atherosclerotic disease and cardiovascular mortality[117-118]. Also, in women at increased cardiometabolic risk, such as the postmenopausal ones, this index showed to be a useful marker of insulin resistance and cardiovascular risk[119].

However, large studies conducted in individuals at low cardiometabolic risk is uncommon. Little information is available about the utility of TG/HDL in younger adults who were born with LBW. Considering that LBW individuals at-high risk for developing cardiometabolic diseases, linked by the glucose metabolism disturbance, it is of great

interest to explore the behavior of proxies of insulin sensitivity and β-cell dysfunction in this subset compared with those born with normal weight.

1.6. THE ELSA- BRASIL

The Longitudinal Study of Adult Health – ELSA-Brasil is the largest study in this country examining aspects of adult health. It is supported by several research funding agencies and coordinates by an inter-institutional board[1]. The ELSA-Brasil is being conducted in 6 public universities or research institutions throughout Brazil involving, as its initial population, 15,000 civil servants. The first wave launched in 2008 aimed to estimate the prevalence of NCCDs, describing the sociodemographic characteristics of the participants, as well as examining risk factors particularly for CVD and DM and possible determinants of their progression[2].

The São Paulo arm of the ELSA-Brasil is being carried out at the University of São Paulo and included 5,061 participants at the baseline. Sub-studies of the ELSA-Brasil conducted by our Group have been performed, exploring other aspects within the same research line[120-124]. The ELSA-Brasil has completed the data collection for the second wave, spanning from 2012 to 2014. Its large-scale and longitudinal design provided conditions to develop the hypotheses and analyses of the present study and have the potential to test others in a prospective manner.

1.7. RATIONALE AND HYPOTHESES

In view of the high prevalence rates of NCCDs and their impact on mortality, it is vital to improve prediction, thereby allowing more effective interventions. Risks of type 2 DM, AH and CKD are defined by genetic and environmental factors and evidence indicates a role for the intrauterine *milieu* in determining such risk. LBW has been used as a *proxy* for a hostile intrauterine environment, associated with cardiometabolic risk in adulthood[58,125].

The relationship of LBW with insulin resistance and AH in the life course has been widely reported[40,126,127]. However, in Brazil, there is scarce information regarding

associations of LBW with biomarkers of renal and endocrine-pancreatic function in adults without DM or nephropathy. The assessment of renal function using sCys C has been understudied and controversy remains regarding its usefulness as a marker of renal dysfunction prior to the development of DM.

Since clinical course of DKD can vary[76], the renal assessment should be ample to detect its multiple phenotypes, even before the state of sustained hyperglycemia.

Considering that adults who have experienced restrictions during intrauterine life may have a lower functional reserve of certain organs (reduced β-cells and glomeruli), it is plausible that birth weight could be associated with early markers of dysfunction of these organs.

Slightly abnormal values, screened in adults without DM or nephropathy, may flag an at-risk condition, suggesting the need for preventive measures against disturbances in glucose metabolism and CKD. BP levels also represent an opportunity for testing these associations, given they are elevated in both conditions, insulin resistance and impaired renal function. Therefore, by examining early-life events, high-risk conditions could be identified as potential targets for prevention of important public health problems.

Two research lines involving early-life events were addressed in the present thesis: 1) exploring associations with BP and renal function and 2) with glucose metabolism.

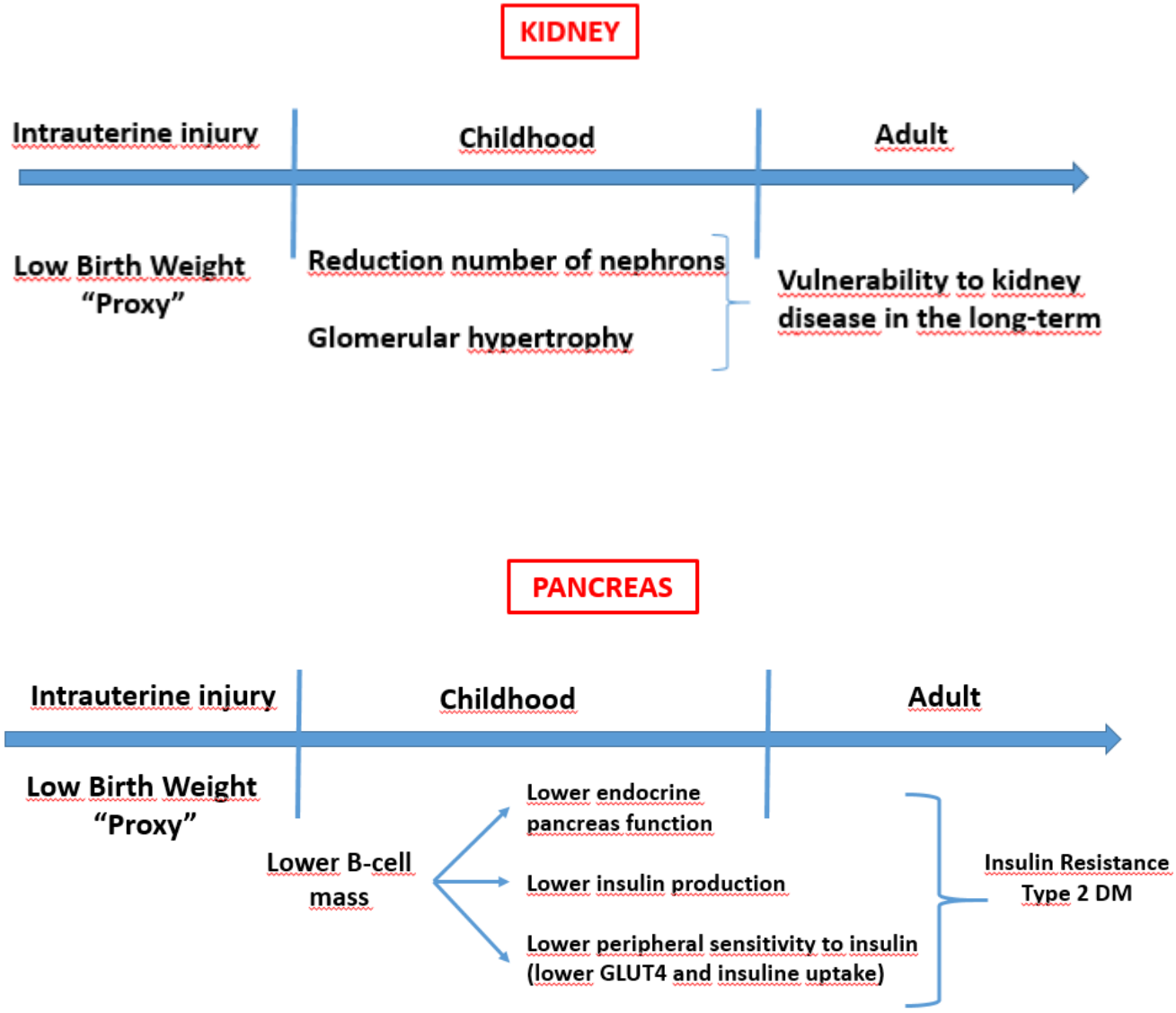
The hypotheses raised were:

1) A proportion of individuals with mild glucose metabolism disturbances (pre-DM) can present laboratory signs of kidney impairment, in whom sCys C determination would help identifying these changes.

2) LBW or prematurity – as *proxies* of adverse intrauterine conditions – would be associated with higher BP and/or renal biomarkers and/or endocrine-pancreatic parameters in adulthood than normal birth weight.

3) There are associations of birth weight with kidney and/or β-cells function markers in individuals without DM or nephropathy.

The rationale of these hypothesis is shown in Figure 1.



2. OBJECTIVES

2.1 GENERAL OBJECTIVE

The general objective was to analyze the association of birth weight and/or prematurity with BP levels and biomarkers of kidney and endocrine-pancreatic function in adults without DM and nephropathy.

2.2. SPECIFIC OBJECTIVES

Since this thesis has been presented as papers for publication, the specific objectives correspond to each one, entitled:

Paper 1: “Prevalence of diabetic kidney disease in prediabetes” aimed to review the literature regarding the prevalence of kidney function abnormality in pre-diabetic individuals.

Paper 2: “Serum cystatin C in early kidney dysfunction in prediabetic participants of the Brazilian Longitudinal Study of Adult Health – ELSA-Brasil” aimed to evaluate sCys C as an early marker of kidney dysfunction in participants without DM from the São Paulo research center, ELSA-Brasil.

Paper 3: “Association of prematurity and low birth weight with blood pressure and kidney function in middle-aged participants of the Brazilian Longitudinal Study of Adult Health – ELSA-Brasil” proposed to: a) compare BP levels and kidney function markers (eGFR, ACR and sCys C) according to the presence of LBW; b) to analyze associations of birth weight with BP levels and kidney function markers (eGFR, ACR and sCys C) in ELSA-Brasil participants without DM and nephropathy.

Paper 4: “Low birth weight, β-cell function and insulin resistance in adults: the Brazilian Longitudinal Study of Adult Health” aimed to: a) compare markers of β-cell function and insulin sensitivity (HOMA-β, HOMAIR, HOMA-AD, QUICKI, fasting glucose, glycated hemoglobin, TyG index and TG/HDL index), according to the presence of LBW; b) to analyze associations of birth weight with the same markers of β-cell function and insulin sensitivity in ELSA-Brasil non-diabetic participants.

3. METHODS

3.1. DESIGN AND ELIGIBILITY CRITERIA FOR THE ELSA-BRASIL

This study was a cross-sectional analysis of the ELSA- Brasil baseline data. The project was approved by the ethics committees of each research center and by the National Research Ethics Commission of the National Health Council[128] and supported by research funding agencies. All participants signed an informed consent and agreed with storage of their biological samples (Attachment 1). The two investigation proposals that compose this thesis (renal and endocrine-pancreatic approaches) were also approved by the ELSA-Brasil publication committee.

The ELSA-Brasil is a nationwide cohort involving six research centers from the Northeast, Southeast and South regions. Its objectives and methodological details were previously described[1]. Briefly, baseline data collections started in 2008 and ended in 2010 including 15,105 public servants who were subjected to interviews, anthropometric measurements, laboratory, and imaging tests. Every employee aged 35–74 years was eligible to participate in the study. Exclusion criteria were intention to leave the institution, pregnancy or having been pregnant in the last four months, cognitive or communication limitations and, if retired, living outside the metropolitan area. The sociodemographic distribution of the ELSA-Brasil population sample was established as follows[129]:

- Sex: 50% female and 50% male;
- Age: 15% between 35-44 years, 30% between 45-54 years, 40% between 55-64 years and 15% between 65-74 years;
- Education: 35% incomplete elementary school, 35% high school and 30% higher education.

The ELSA-Brasil cohort has now completed its second (2012-2014) and third (2016-2019) waves.

3.2. PROTOCOL

Volunteers were interviewed at their workplaces or at the research centers, and sociodemographic data, life habits and medical history were obtained (Attachment 2). Subsequently, they were invited to attend the investigation centers for physical examination and complementary tests, bringing a 12-hour overnight urine sample. Participants were instructed to wear light clothes, without shoes. Body weight and height were measured on a digital scale and fixed rigid stadiometer, respectively, used to calculate BMI. Waist circumference was measured with an inextensible tape using a technique recommended by the WHO[130].

Systolic and diastolic BP were taken in sitting position, after 5-minute rest, three times using an automatic sphygmomanometer (Omron 765CP; Omron, Kyoto, Japan).

Blood samples were obtained after a 12-hour overnight fast. Part of the determinations were immediately performed, and part of the samples was stored at -80°C for later hormones and sCys C determinations. In the urine aliquots, creatinine and albumin concentrations were determined. ACR was calculated using the quotient between albuminuria and creatininuria.

After completion of data collection in the first ELSA-Brasil wave, participants were periodically contacted by telephone for updating data regarding their health status. Three years later, during the second wave, they were invited to perform new examinations and to monitor outcomes.

The present study was based on data from the research center in São Paulo, located at the University Hospital of University of São Paulo. Data of interest, collected by questionnaires, were sociodemographic characteristics, early life events (birth weight), life habits, personal and family history of diseases. Additionally, clinical and laboratory data were considered to address the specific objectives.

3.3. SAMPLE OF THE PRESENT STUDY

ELSA-São Paulo participants of both sexes, without DM and nephropathy, were selected for the current analyses. Individuals aged 35 to 54 years were studied for kidney function (contents of papers 2 and 3 on the Results section of this thesis) and those aged 34 to 59 years for endocrine-pancreatic function (content of paper 4). Sample sizes in the

papers varied due to different exclusion criteria and availability of information in the database.

For papers 2 and 3, 998 individuals without BMI ≥ 35 kg/m^2, self-reported DM or CVD, with sCys C measured, were included. For paper 3, exclusion criteria were:

- newly diagnosed with DM (n = 17) and eGFR < 60 mL/min/1.73 m^2 or macroproteinuria (n = 16);
- BMI < 18.5 kg/m^2 (n = 8) due to possible severe disease and macrosomia (n = 50).
- thyroid dysfunction (TSH < 0.1 or ≥ 10.0 mg/dL), considering that hyperthyroidism can increase sCys C levels, while hypothyroidism can reduce them (n = 18)[75]; severe liver dysfunction (TGO and/or TGP > 3 times the normal value) (n = 2)[131].
- missing data of sCys C (n = 27), eGFR (n = 02), ACR (n = 22) and of LBW (n = 68).

By excluding these participants, the main composition of the sample did not change. A total of 768 participants were included in the present analysis (Figure 2).

To develop paper 4 regarding endocrine-pancreatic function, for 5,061 participants the following exclusion criteria were used:

- Age ≥ 60 years in an attempt to minimize memory bias due to distant birth weight information (n = 623)
- DM (self-reported, under antidiabetic medications or newly diagnosed) (n = 1,036) and eGFR < 60 ml/min/1.73m2 or macroproteinuria (n = 210)
- BMI < 18.5 kg/m2 (n = 39) and macrosomia (n = 186)
- Missing data of plasma glucose (n = 1), insulin (n = 2) or birth weight (n = 330)

A total of 2,634 participants were included in the present study (Figure 3).

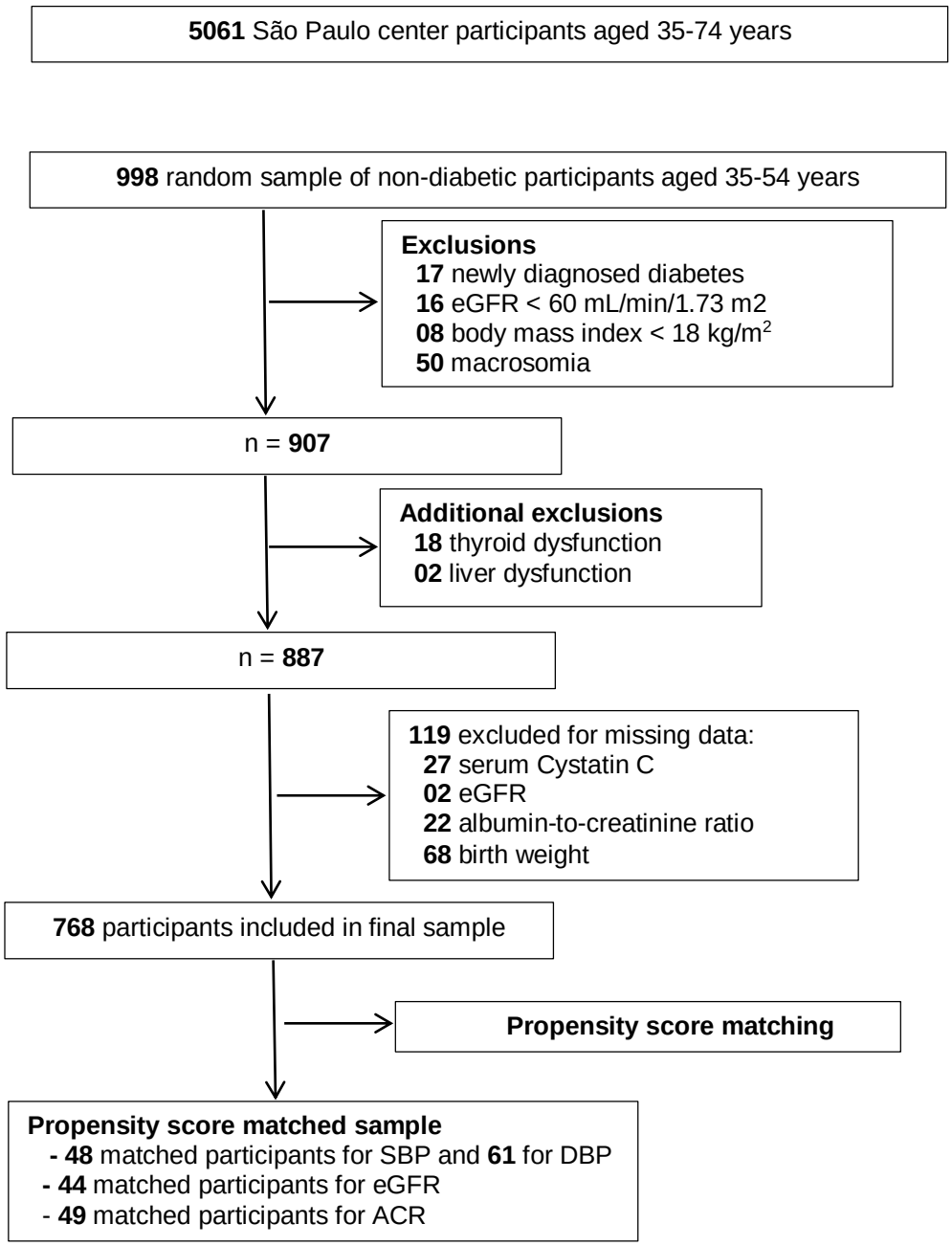


Figure 2. Flowchart of selection process of ELSA-Brasil participants for paper 3 (blood pressure and kidney function), São Paulo center.

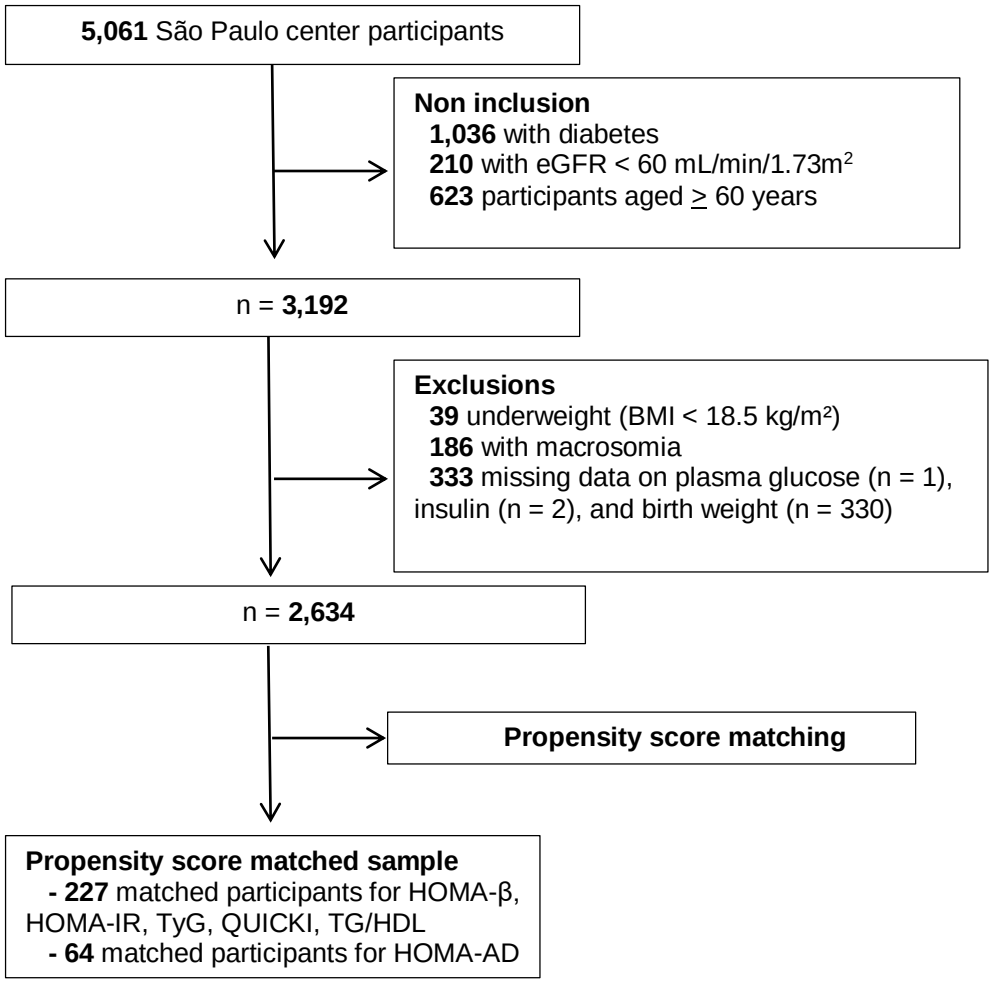


Figure 3. Flowchart of selection process of ELSA-Brasil participants for paper 4 (endocrine-pancreatic function), São Paulo center.

3.4. VARIABLES OF INTEREST

3.4.1. Outcomes

The outcome variables were blood pressure, kidney function biomarkers, endocrine-pancreatic function and insulin sensitivity indexes.

- Blood pressure (mmHg): arithmetic means of the latest two measurements were used. AH was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg and/or use of antihypertensive medication.
- Kidney function biomarkers:

- eGFR (mL/min/1.73m^2): estimated usingthe CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula[75];

- ACR (mg/g of creatinine): calculated from the determinations of albumin and creatinine ratio in a 12-hour nighttime urine sample by immunonephelometric assay (Dade-Behring, Deerfield, Illinois, USA);

- sCys C: determined by ELISA (Elabscience Biotechnology, Houston, Texas, USA). Normal ranges were 0.64- 0.84 mg/L for men and 0.57-0.74 mg/L for women[43].

Altered renal function was defined by eGFR < 60 mL/min/1.73m^2 or ACR > 1,000 mg/g (macroproteinuria).

- Endocrine-pancreatic function and insulin sensitivity indexes:

- Fasting plasma glucose (mg/dL): determined by the hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA);

- Fasting insulin (µUI/mL): determined by immunoenzymatic assay (Centaur; Siemens, Deerfiled, Illinois, USA);

- Glycated hemoglobin (%): determined by high pressure liquid chromatography (Variant; Bio-Rad Laboratories, Hercules, California, USA);

- Adiponectin (µg/mL): determined by ELISA (Enzo Life Sciences, Farmingdale, NY, USA). It was measured in a sub-sample of 1,000 participants; after applying exclusion criteria, 742 were included with adiponectin data available (paper 4).

- Triglycerides (TG) and lipoproteins (mg/dL): total cholesterol were obtained by colorimetric enzymatic method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA). HDL-c was determined by the homogeneous colorimetric method without precipitation, and triglycerides by the glycerophosphate peroxidase method according to the Trinder assay (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA). LDL-cholesterol was calculated using the Friedewald equation.

DM was diagnosed according to the American Diabetes Association criteria[132], as follows: fasting plasma glucose ≥ 126 mg/dL or 2-hour post challenge > 200 mg/dL or glycated hemoglobin ≥ 6.5%. Prediabetes was defined as fasting plasma glucose between 100 – 125 mg/dL or 2-hour post challenge between 140 – 199 mg/dL or glycated hemoglobin 5.7 – 6.4%.

Fasting glucose and insulin were used to calculate the HOMA-β and HOMA-IR indexes according to Matthews et al[88]. HOMA-β was used as a *proxy* of β-cell function, while HOMA-IR, HOMA-AD, TyG index, QUICKI and TG/HDL ratio as indicative of insulin sensitivity.

HOMA-AD was calculated using the formula: fasting glucose (mmol/L) x fasting insulin (mU/L) / 22.5 x fasting adiponectin (µg/mL).

TyG index was obtained by the product of TG and glucose values by the formula: [log (fasting TG (mg/dL) x fasting glucose (mg/dL)] / 2.

QUICKI was calculated as: 1 / (Log insulin (µUI/mL) + (Log glucose (mg/dL).

TG/HDL ratio was calculated by dividing TG (mg/dL) by HDL-cholesterol (mg/dL).

3.4.2. Exposure

The major early-life event was birth weight, specifically LBW, internationally defined[16] by less than 2500 g. This variable was analyzed into two categories, < 2.5 kg and ≥ 2.5 kg. Participants were asked about their weight birth as follow: According to the information you have, what was your birth weight? (< 2,5 kg, between 2,5 and 4,0 kg, > 4,0 kg); Do you know more precisely what your birth weight was? (in kilograms); Were you a premature baby, that is, were you born earlier than expected?.

3.4.3. Adjustment Variables

Several variables were considered as possible confounders in the association of birth weight with BP, kidney and β-cell functions:

- Sociodemographic variables: age (years), sex (male, female), skin color (black, white, brown, yellow, or indigenous, further stratified into white and non-white categories), income, education level and mother's education level.
- Lifestyle: physical activity level, smoking and alcohol abuse.
- Family history of DM and AH.
- Anthropometric data: current BMI (kg/m^2) and at age 20.
- Prematurity (< 37 weeks) was defined by an affirmative answer to the question: "*Were you a premature baby, in other words, were you born earlier than expected*?", twinning and macrosomia (> 4.0 kg).
- AH: when this was not the outcome.

3.5. Statistical Analysis

The sample was characterized using measures of central tendency and dispersion. For continuous variables, the descriptive analysis was presented as mean and standard deviation for variables with normal distribution and as median and interquartile range for variables without normal distribution. To verify the normality distribution, the frequency density graphic step (histogram) was applied, followed by the Shapiro-Wilk statistical test. Categorical variables were presented by absolute (n) and relative (%) frequencies. Some variables with non-normal distribution were log- transformed to achieve normality before analysis.

After stratification by birth weight (< 2.5 kg and ≥ 2.5 kg), continuous variables with normal distribution were compared by Student t test or ANOVA and those non-normal distributed variables by Wilcoxon or Kruskall-Wallis test. Frequencies were compared by the chi-square test. Correlations between continuous variables were tested by Pearson's or Spearman's coefficient.

Sensitivity analyses (presence of obesity, BP, use of antihypertensive agents and prematurity) were performed in paper 2, 3 and 4.

Multiple linear or logistic regression was used to analyze the association between the exposure (birth weight) with outcomes [BP and kidney function biomarkers (paper 3) and endocrine-pancreatic function indexes (paper 4)].

Direct Acyclic Graphs (DAG) were used to build theoretical models to analyze independent associations of exposure with outcomes and to detect minimal sufficient adjustments in the regression models[133,134] (www.dagitty.net).

In order to reduce the sample size difference of the groups with normal and LBW and possible selection bias due to the observational nature of the study, the propensity score matching was employed, creating more comparable groups[135,136].

Statistical analyses were performed using R Project for Statistical Computing (R version 3.5.2.) and a p-value < 0.05 was considered significant.

4. RESULTS

This chapter of the thesis is being replaced by three papers published and one submitted for publication.

Also, our preliminary results were presented in international scientific meetings being published in their official journal as abstract format (Attachment 3) and one oral presentation in the Latin American Congress DOHaD 2020 concluded with a honorable mention award (Attachment 4).

4.1. **PAPER 1**

REVIEW

Prevalence of diabetic kidney disease in prediabetes

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Abstract

Background: Chronic renal failure is a debilitating and expensive complication of diabetes mellitus (DM). It is controversial if kidney disease may occur in prediabetes stages. We systematically reviewed the prevalence of diabetic kidney disease (DKD) as defined by albuminuria or reduced glomerular filtration rate (GFR) in prediabetes.

Methods: We searched for studies reporting the frequency of DKD in prediabetes (impaired plasma glucose, impaired glucose tolerance and glycated hemoglobin). Exclusion criteria were subjects aged < 18 years and type 1 DM. Eleven articles were selected and considered for analysis from a total of 371 abstracts.

Results: DKD prevalence ranged from 4.5% to 26.0% in prediabetics and from 0 to 16.0% in normoglycemics. In two articles, DKD diagnosis was based on eGFR and the prevalence ranged from 4.5 to 21.3%; in six articles, based on albuminuria, between 7.0 and 26.0%; and in three articles, based on combined criteria (eGFR and albuminuria), between 12.3 and 17.7%.

Conclusion: Our findings indicated that DKD occurs in prediabetes stages. Mild glucose metabolism disturbance has a relevant impact on the prevalence of DKD that deserves attention of clinicians and public health authorities. Additional studies with representative samples in different populations are needed to estimate how kidneys are affected by prediabetic states.

Keywords: Diabetic kidney disease; Prediabetes; Glomerular filtration rate; Microalbuminuria

1. Introduction

Kidney disease represents a debilitating complication of DM that develops after years of persistent hyperglycemia, and chronic renal failure is a heavy burden for health care systems. In developed countries, DM is the most common cause of renal replacement therapy and end stage kidney disease (American Diabetes Association, 2018a). The number of diabetic subjects under dialysis has increased in the USA and Europe in parallel to the rates of DM (Collins et al., 2009), (Van Dijk et al., 2005), (Bommer, 2002). Some regional studies regarding renal replacement therapy in Brazil has also shown that DM was among the main causes (Pinto et al., 1997; Cherchiglia et al., 2010). According to the Brazilian Chronic Dialysis Survey, DM as a cause of dialysis has increased reaching 28% in 2010 (Sesso et al., 2011) and 41% in 2016 (Sesso et al., 2017). This has mainly been explained by the increase in prevalence of type 2 DM and the survival of diabetic subjects.

The natural history of type 2 DM is characterized by a long period of slightly elevated plasma glucose which already indicates abnormality in glucose metabolism. It is known that such metabolic instability is sufficient to increase cardiovascular risk and macrovascular complications that may even precede the clinical diagnosis of DM (Vistisen et al., 2018). On the other hand, microangiopathy in retina and kidney typically manifests in subjects with overt DM (fasting plasma glucose ≥ 126 mg/dL or 2-h plasma glucose post 75-g glucose load ≥ 200 mg/dL).

According to the American Diabetes Association (ADA), the term prediabetes has been attributed to the conditions of impaired fasting glucose – IFG (between 100 and 125 mg/dL) and impaired glucose tolerance – IGT (2-h post-load between 140 and 199 mg/dL) that are at increased risk for DM (Unwin et al., 2002). Also, glycated hemoglobin (HbA1c) ranging from 5.7 to 6.4% has been used for this purpose (American Diabetes Associa, 2018b). In an American population-based study, approximately 38% of adults were found to be prediabetics (Menke et al., 2015). In the Rotterdam Study, the prevalence of prediabetes was 14%; most importantly, the lifetime risk of subjects aged 45 years for developing prediabetes was 48.7% and the progression rate of prediabetes to DM was 74% (Lithrat et al., 2016). Lower rates reported in Europe may be in part due to prediabetes diagnosed by

the World Health Organization (WHO) criteria, in which a higher cutoff of fasting plasma glucose (110 mg/dL) is employed (Alberti and Zimmet, 1998).

Despite a clear causal relationship between DM and kidney disease, the presence of DKD in states that precede overt DM is controversial (Echouffo-Tcheugui et al., 2016). This controversy may be at least in part due to underdiagnosis in clinical practice and to heterogeneous criteria for DKD in the early phase of glucose metabolism disturbance. Initial but reversible renal damage in DM has been identified by the presence of glomerular hyperfiltration. Long-term glomerular injury leads to urinary albumin loss that can be assessed by the albumin-creatinine ratio (ACR), commonly used to classify the DKD stages along with reduction in glomerular filtration rate (GFR). Hyperfiltration has been attributed to GFR higher than 125 mL/min/1.73m^2 while reduced GFR is below 60 mL/min/1.73m^2.

As far as we know, a compilation of studies investigating the occurrence of DKD in this phase of the natural history of DM is not available in literature. Few publications have reported evidence of DKD in prediabetic categories (De Nicola et al., 2016). Knowledge about renal damage in prediabetic phases is relevant considering the high prevalence rates of prediabetes in populations and potential benefits of interventions in DKD. In PROSPERO databases, no systematic review addressing this issue was found (PROSPERO).

The present study systematically reviewed the reported frequencies of DKD as defined by albuminuria or reduced GFR in subjects with prediabetic conditions (American Diabetes Association, 2018a; Kidney Disease Improving Global Outcomes - KDIGO, 2012, 2013).

2. Methods

Details of the protocol of this systematic review were registered on PROSPERO and can be assessed on the website (ID Register 103055) PROSPERO. We followed the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA 2009) statement, which includes a 27-items checklist and a four-phase flow diagram to guide this review. We examined medical literature in English language since 2003 – when the ADA criteria for prediabetes were reported (American Diabetes Association, 2015) – using the MEDLINE

database of the National Library of Medicine, LILACS and SciELO. The reference list of scrutinized reports was also scanned to find more relevant articles.

Studies included in this review should report frequencies of kidney disease in adults with prediabetes, irrespective of the criteria used (fasting plasma glucose, 2-h oral glucose tolerance test or/and HbA1c). Diagnostic values for IFG or IGT were provided in mg/dL or in mmol/L; to unify the units in the text and table all the values and ranges are presented in mg/dL. Although several terms were used to define abnormalities of kidney function in prediabetes, we selected DKD to refer to renal damage reflected by the presence of elevated albuminuria (ACR ≥ 30 mg/g or ACR between 22 and 220 mg/g) and reduced eGFR, calculated by using the Modification of Diet in Renal Disease Study – MDRD or the Chronic Kidney Disease Epidemiology Collaboration – CKD-EPI formula (Kidney Disease Improving Global Outcomes - KDIGO, 2012, 2013). Exclusion criteria were age < 18 years and type 1 DM.

To assess the frequency of DKD in prediabetic categories, the medical subject headings (MeSH) checked were “diabetic kidney disease OR nephropathy prevalence OR frequency AND prediabetes”, “diabetic renal disease prevalence AND prediabetes”, “microalbuminuria OR albuminuria AND prediabetes OR impaired fasting glucose OR impaired glucose tolerance”. A total of 371 articles were identified: 360 articles in MEDLINE, five articles in SciELO and six articles in LILACS databases (Figure 1). All 371 abstracts were read, 343 were excluded because 308 were not directly related to the purpose, 11 were not in the English language and 24 were duplicate articles. From the remaining 28 reports, 17 were excluded (14 not related to the frequency of prediabetes and three reviews). Thus, eleven articles were selected for a full-text reading and included in the final descriptive analysis.

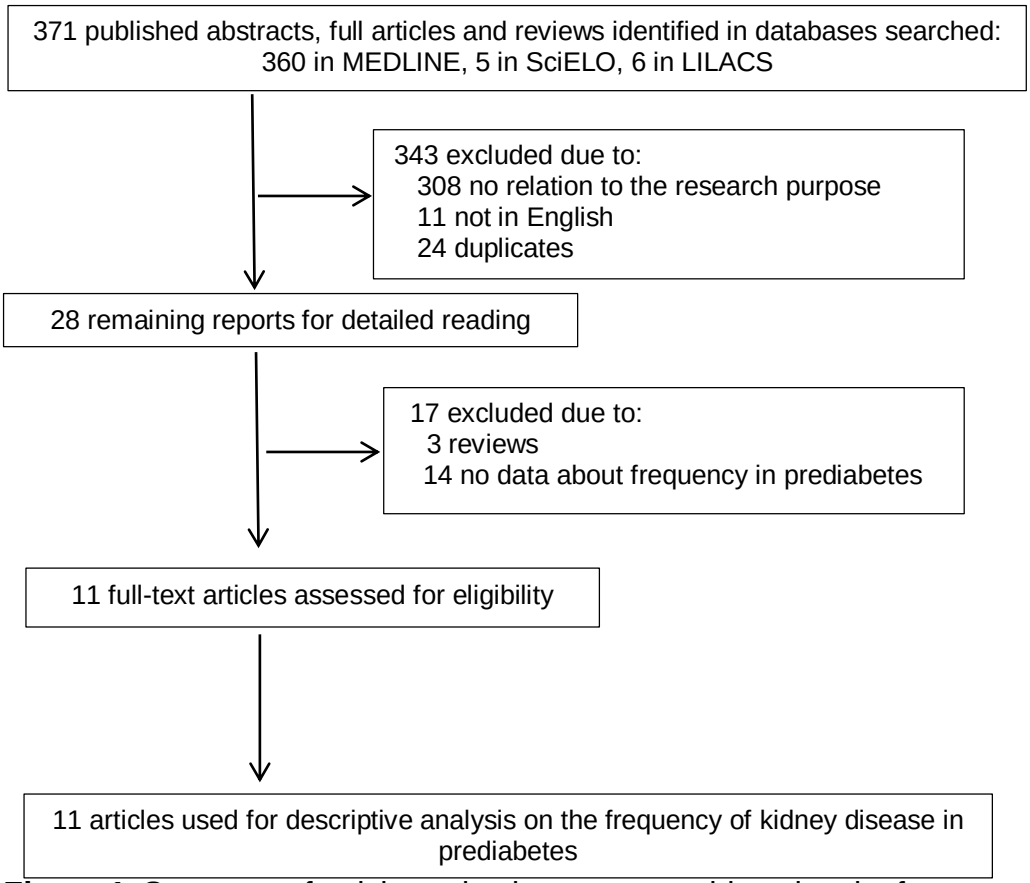

Figure 1. Summary of articles selection process addressing the frequency of kidney disease in prediabetes.

3. Results

Among eleven selected articles, seven studies were originally mentioned as cohorts (Schöttker et al., 2013; Lin et al., 2017; Tapp et al., 2004; Bahar et al., 2013; Won et al., 2015; Kim et al., 2014; Ali et al., 2018) and four had cross-sectional design (Suzuki et al., 2004; Li et al., 2009; Plantinga et al., 2010; Zhou et al., 2013). Cross-sectional analyses of the cohorts were performed at the baseline (Lin et al., 2017) or at the end (Schöttker et al., 2013; Tapp et al., 2004; Bahar et al., 2013; Won et al., 2015; Kim et al., 2014; Ali et al., 2018) of these studies. Therefore, all the studies allowed estimates of prevalence. There was only one cohort conducted in a German population in which the incidence rate was also provided (Schöttker et al., 2013).

The diagnosis of DKD was based on the eGFR (n = 2), on the presence of albuminuria (n = 6; 4 in cohorts and 2 in cross-sectional studies), and three articles that used combined criteria (eGFR and albuminuria).

a. Studies using eGFR criteria

The German population-based cohort ESTHER included subjects aged between 50 and 74 years without abnormalities of renal function, who were recruited between 2000 and 2002 and followed up for eight years (Schöttker et al., 2013). Prediabetes diagnosis was based on FPG (100 – 125 mg/dL) or HbA1c (5.7 – 6.4%); reduced kidney function was defined by eGFR < 60 mL/min/1.73m^2 according to the CKD-EPI (Kidney Disease Improving Global Outcomes - KDIGO, 2012, 2013). At the end of the follow-up, 18.7% of prediabetic subjects characterized by FPG or HbA1c developed reduced GFR. When both parameters of glucose metabolism (FPG and HbA1c) were altered in prediabetic subjects, their prevalence of 21.3% was significantly higher than in normoglycemic subjects. Descriptive analysis showed that age, smoking, body mass index, systolic blood pressure and also lipids and their medications as well as cardiovascular event were associated with DKD. The difference observed in crude prevalence rates [RR 1.33 (95%CI 1.03 – 1.73)] did not persist after adjusting for cardiovascular risk factors [RR 1.06 (95%CI 0.71 – 1.32)].

The REACTION study (Risk Evaluation of cAncers in Chinese diabeTic Individuals) is a nationwide prospective observational study from China that included 250,752 subjects aged ≥ 40 years recruited between 2011 and 2012 (Lin et al., 2017). Prediabetes was defined as FPG (110 – 125 mg/dL) and/or 2-h plasma glucose (140 – 199 mg/dL), and DKD as eGFR < 60 mL/min/1.73m2 using CKD-EPI criteria (Kidney Disease Improving Global Outcomes - KDIGO, 2012, 2013). Comparing DKD crude prevalence between normoglycemic and prediabetic subjects at the baseline, higher rates were found in prediabetic men (2.6% versus 1.7%) and women (1.9% versus 1.2%) than normoglycemic ones, respectively. Prediabetic subjects were older and had a worse lifestyle and higher mean values of blood pressure and lipids ($p < 0.01$). In univariate regression analysis, prediabetes was associated with a higher risk of DKD in for both sexes but after adjustment for confounders, the association remained only in men [OR 1.15 (95%CI 1.02 – 1.32)].

b. Studies using ACR criteria

In the Australian Diabetes, Obesity and Lifestyle study (AusDiab), 11,247 subjects aged ≥ 25 years were examined in 1999 – 2000. IFG was defined by FPG (≥ 110 mg/dL and < 126 mg/dL) and 2-h postload (< 140 mg/dL); IGT by FPG (< 126 mg/dL) and 2-h postload ≥ 140 and < 200 mg/dL (Tapp et al., 2004). Microalbuminuria in men was defined as ACR 22 – 220 mg/g and in women 31 – 220 mg/g and macroalbuminuria as ACR > 220 mg/g of creatinine. At the end of the follow up, a total of 5.1% of subjects with normal glucose tolerance had albuminuria (4.3% micro-, 0.8% macroalbuminuria), while the rate in IFG was 9.3% (8.3% micro-, 1.0% macroalbuminuria) and in IGT 11% (9.9% micro-, 1.1% macroalbuminuria). IFG [OR 1.92 (95%CI 1.44 – 2.56)] and IGT [OR 2.32 (95%CI 1.89 – 2.83)] were associated with an increased risk of albuminuria, but only IFG remained after adjustments for age, sex, smoking, body mass index, blood pressure and lipids [OR 1.38 (95%CI 1.02 – 1.87)]. Except for sex, all the covariates were also independently associated with the outcome.

Data of 154 Japanese men aged 20–70 years was collected in Kyoto University Hospital from 1991 to 2001 (Suzuki et al., 2004). The prevalence of microalbuminuria (ACR 30 – 300 μg/mg of creatinine) was examined in five groups: normal glucose tolerance (NGT), IFG, isolated IGT, combined IGT/IFG and DM. The mean values of ACR were 15.5 ± 2.6 μg/mg in the NGT, 16.2 ± 3.6 μg/mg in the isolated IGT and 42.2 ± 10.7 μg/mg in the IGT/IFG group. Microalbuminuria prevalence was higher in subjects with combined IGT/IFG compared to NGT (26% versus 9%, p = 0.028) but not in IGT (14%). Microalbuminuria was associated only with IGT/IFG, this association persisted after adjustment for age and hypertension [OR 4.41 (95%CI 1.17 – 16.54)], but not when insulin resistance index is added to the model. Partial correlation analysis of ACR with other variables showed that insulin resistance was a strong determinant after adjustment for age.

In a study of 1776 permanent residents aged ≥ 40 years from a single urban community of Shanghai, China, 754 had normal glucose tolerance, 506 IFG (110–125 mg/dL) and/or IGT (140 and < 200 mg/dL) after a 2-h glucose load and 516 newly diagnosed DM ≥ 200 mg/dL (Li et al., 2009). ACR of 30 – 300 mg/g was detected in 4.3% normoglycemic and in 6.6% in prediabetic subjects (7.0% for IGT and 8.6% for IFG). IFG and IGT were positively associated with the presence of microalbuminuria after adjustments

for sex, age, lifestyle factors, body mass index and blood pressure levels [adjusted OR 1.28 and 1.32, respectively, $p < 0.0001$].

A small cohort study was performed from 2009 to 2011 in an Iranian university hospital, including 45 subjects with IFG, 45 with IGT and 45 with normal glucose tolerance, based on the American Diabetes Association criteria (Bahar et al., 2013). Microalbuminuria (ACR 30 – 300 mg/g) was detected in 18.0% of IFG, 14.0% in IGT (15.5% overall prevalence rate in prediabetes, $p=0.005$) and none in the control group. Microalbuminuria rates did not differ between IFG and IGT groups.

Increased albuminuria was investigated in the Korea Health and Nutrition Examination Survey (KNHANES), a national survey conducted at 192 locations since 1998 to 2012 (Won et al., 2015). A total of 5202 subjects were divided into five groups: FPG < 90 mg/dL or normal fasting glucose (NFG1), FPG 90 – 99 mg/dL or NFG2, FPG 100 – 109 mg/dL or IFG1, FPG 110 – 124 mg/dL or IFG2 and FPG ≥ 126 mg/dL as diabetics. Prevalence rates of ACR ≥ 30 mg/g were gradually higher as FPG increased: 4.1% in the NFG1, 6.0% in the NFG2, 7.6% in the IFG1 and 12.3% in the IFG2 and 23.4% in the diabetes group (P for trend < 0.01). After adjustment for age, sex, hypertension and obesity, the prevalence of albuminuria was significantly higher in both prediabetic groups compared to NFG1, and also IFG2 had a greater rate than IFG1. Only IFG2 was significantly associated with albuminuria [OR 1.87 (95%CI 1.19 – 2.94)].

Other results from the Korean population were reported based on the KNHANES 2011 – 2012 (KNHANES V-2,3). A total of 8775 subjects were considered prediabetic when FPG was 100 – 124 mg/dL or HbA1c 5.7 – 6.4%. Mean values of ACR were higher in the prediabetic group compared to the normoglycemic group, as well as the prevalence of microalbuminuria (6.3% *versus* 3.6%). Microalbuminuria in subjects with prediabetes lost significance after adjustment for age, sex and systolic blood pressure [OR 1.14 (95% CI 0.93 – 1.41)].

c. Study using combined criteria

In NHANES, 8188 American subjects aged ≥ 20 years were examined from 1999 to 2006 (Plantinga et al., 2010). Prediabetes was defined by FPG (100 – 125 mg/dL) and DKD by ACR (30 – 300 mg/g) or reduced eGFR (< 60 mL/min/1.73m^2) according to the MDRD

and the CKD-EPI (Kidney Disease Improving Global Outcomes - KDIGO, 2012, 2013). Reduced eGFR using MDRD equation was observed in 10.6% of normal tolerant subjects and in 17.7% of prediabetic ones ($p < 0.001$); similar prevalence rates were found using CKD-EPI equation (9.2% and 16.6%, respectively, $p < 0.001$), even after adjusted for age, sex and race. Among those with prediabetes and reduced eGFR approximately 20% had micro- or macroalbuminuria.

In a Chinese study including a random sample of 5584 subjects aged 20 – 79 years from Shanghai, diagnosis of prediabetes was based on FPG 100 – 125 mg/dL. The prevalence of microalbuminuria (ACR ≥ 30 mg/g) was 12.9% and reduced kidney function by MDRD (GFR < 60 mL/min/1.73m^2) was 14.1% in prediabetics subjects, while in normoglycemic patients the rates were significantly lower (8.7% and 9.2%, respectively, $p < 0.001$).

The 1988 and 2014 NHANES (Ali et al., 2018), having included 27,971 Americans aged 20 years or older, prevalence rates of ACR ≥ 30 mg/g (from 9.3% to 7.7%, $p = 0.118$) and of GFR < 60 mL/min/1.73m2 (4.7% – 4.6%, $p = 0.769$) showed non-significant decreases. In 2014, overall prevalence rates of DKD were 12.3% in prediabetic subjects and 11.3% in normoglycemic ones.

In summary, in the eleven studies in which DKD prevalence were provided, rates ranged from 4.5% to 26.0% in subjects with prediabetes. Taking into consideration two studies in which diagnosis were based on eGFR exclusively, prevalence varied from 4.5% to 21.3%, while in six in which ACR was used ranged from 6.3% to 26.0%. When both diagnostic criteria were used simultaneously the prevalence ranged from 12.3% to 17.7% (Table 1).

4. Discussion

Our study shows that few articles (Schöttker et al., 2013; Lin et al., 2017; Tapp et al., 2004; Suzuki et al., 2004; Li et al., 2009; Bahar et al., 2013; Won et al., 2015; Kim et al., 2014; Plantinga et al., 2010; Zhou et al., 2013; Ali et al., 2018) assessed DKD prevalence in prediabetic stages and they employed distinct size samples and diagnostic criteria, limiting comparison of rates among the populations investigated. DKD criteria were based

on eGFR and/or albuminuria, and prediabetes definition on WHO (Alberti and Zimmet, 1998) or ADA criteria (American Diabetes Association, 2015). Despite heterogeneity in methodological approaches, relatively high prevalence rates of DKD were reported, reaching up to one quarter of prediabetic subjects. Our review highlighted the important information that kidney damage could occur in a proportion of subjects even before overt type 2 DM, indicating that it may deserve screening and perhaps early intervention.

Some investigators have preferred GFR as the main parameter to detect glomerular injury in disturbances of glucose metabolism, considering that hyperfiltration is a high-risk condition for progressive kidney disease (De Nicola et al., 2016; Melsom et al., 2016). GFR elevation is dependent on increased plasma flow and glomerular pressure, even in the absence of systemic hypertension (Brenner et al., 1996). Hyperglycemia-related hyperfiltration (Ditzel, 1968) is attributed to afferent arteriolar vasodilation that leads to intraglomerular hypertension, increased transcapillary protein loss and tubular sodium reabsorption (Hannedouche et al., 1990). Abnormal GFR can be normalized by plasma glucose control (Jerums et al., 2010), and the microalbuminuria has been considered the hallmark of early diabetic microvascular disease in the kidney. More recently, the International Society of Nephrology has stated that prognosis to chronic kidney disease should be based on both AER and GFR (Kidney Disease Improving Global Outcomes - KDIGO, 2012, 2013). Therefore, for the purpose of the present review, microalbuminuria and reduced GFR were taken as the major search terms for DKD in prediabetes.

Hyperfiltration is hypothesized to be a precursor of intraglomerular hypertension leading to albuminuria and gradual decrease in GFR. How early hyperfiltration occurs during establishment of hyperglycemia is unknown. Studies have shown that the frequency of hyperfiltration increases with the glucose levels (Okada et al., 2012; Jones et al., 1991) and that is associated with prediabetes (Magee et al., 2009). Other factors contribute to elevate GFR such as high body index mass, oxidative stress, hyperinsulinemia and inflammatory cytokines (Tomaszewski et al., 2007; Li et al., 2011; Stefansson et al., 2016).

The deleterious effect of mild elevations in plasma glucose for the microvasculature is corroborated by the identification of retinopathy in 8.1 to 20.9% of prediabetic individuals (Sokolowska-Oracs et al., 2016; Lamparter et al., 2014; Chen et al., 2012). Such findings

reinforced our hypothesis that microangiopathy at the renal level should also be present in mild disturbances of glucose metabolism. Other studies verified that individuals in early stages of hyperglycemia as prediabetes had neurodegeneration and/or microvascular alterations (Yazgan et al., 2017; Al Shafaee et al., 2011). As far as pathology is concerned, one study performed renal biopsy in prediabetic condition and found isolated thickening of glomerular capillary basement membrane (Lai et al., 2004). Authors called attention that this abnormality should be differentiated from minimal change nephropathy due to therapeutic implications.

Among the studies reviewed, the range of DKD prevalence rates in prediabetes were wide, varying from a minimum of 4.5% to a maximum of 26.0%. Taking into consideration two studies in which diagnosis was based on eGFR or six studies that used ACR, rate variation was similar. Presuming that microalbuminuria should precede the reduction of GFR in the natural history of DKD, higher prevalence rates could be expected using AER rather than GFR. However, wide ranges were observed independent of the parameter used, and, when both were simultaneously considered, the rate found was in the mid-range. Several factors could be contributing to these findings. It is known that renal function is dependent on age, ethnicity, genetic susceptibility, degree and duration of the hyperglycemic excursions, presence of comorbidities such as obesity, hypertension, smoking and others (Levey et al., 2003). We point as main reasons for such variability differences in age range, sample size and genetic predisposition to renal diseases. It is known that the CKD prevalence is higher in Japanese population than in other Asian countries and the United States; according to the Japanese Society for Dialysis Therapy, the prevalence of subjects under dialysis reaches more than 2000 per million inhabitants (Iseki, 2008). Contrastingly, low prevalence was reported in China although rates have increased over the years (Zhang et al., 2012). The finding of our review regarding the frequencies of DKD markers in prediabetes is in agreement with a meta-analysis performed with nine cohort studies indicating that this condition is associated with an increased risk of progressing to clinical nephropathy (Echouffo-Tcheugui et al., 2016).

In ten out of eleven articles, IFG, IGT or increased HbA1c was associated with DKD even after adjusting for cardiovascular risk factors (Lin et al., 2017; Tapp et al., 2004; Suzuki et al., 2004; Li et al., 2009; Bahar et al., 2013; Won et al., 2015; Kim et al., 2014; Plantinga

et al., 2010; Zhou et al., 2013; Ali et al., 2018). Only one article reported no significant association after adjustments, suggesting that prediabetes per se would not deteriorate renal function independent of other renal factors (Schöttker et al., 2013). Despite not providing DKD prevalence data in the subset with prediabetes, the Framingham Heart Study showed that associated cardiovascular risk factors, but not isolated prediabetes, explained the relationship with chronic kidney disease (Fox et al., 2005). Therefore, lack of association in some studies may suggest that glycemic levels could affect population differently depending for instance on its genetic susceptibility.

This review has limitations. Our search included articles from 2003, but it is possible that studies could have been published before this date. Since only eight studies were eligible, an ideal representative sample of world populations was not available. Different population characteristics (age, ethnicity, sex distribution, prevalence of obesity and comorbidities) were included because of the paucity of studies in literature. For the same reason, different diagnostic criteria of DKD and prediabetes were used. Since some studies included older participants, other kidney disease risk factors were present; however, the majority has made adjustments for cardiovascular risk factors. Comparisons of DKD prevalence between prediabetic and normoglycemic subjects were available for all the studies. None performed kidney biopsy to assure that abnormal exams were really attributed to diabetic nephropathy. Marked reduction of kidney function in prediabetic phases deserves thorough investigation regarding the etiology of nephropathy and rule out other causes of renal involvement.

5. Conclusion

In conclusion, using eGFR and/or ACR as proxies of DKD, our findings indicate that this diabetic microvascular complication affects subsets of prediabetic populations at variable frequencies, varying from 4.5 to 26.0%. We suggest that this should be considered a public health concern until more data is available in literature. Mild glucose metabolism disturbance seems to have a relevant impact on the incidence and prevalence of kidney disease that deserves attention from clinicians and health authorities. Since the initial stage of DKD is reversible, it is of great interest to make an early identification of prediabetic subjects with incipient renal dysfunction in order to avoid the debilitating and costly stage of

renal failure. Further studies to be conducted in representative samples in different populations are needed to estimate how kidneys are affected under prediabetic states.

Conflicts of interest

No conflicts of interest are declared.

Acknowledgment

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Table 1. Studies regarding frequency of diabetic kidney disease in prediabetes compared to normoglycemic subjects.

Reference	Study Design	Population/Sample	DKD Criteria	Prediabetes Criteria	DKD Prevalence Prediabetes *vs* Normal
Schöttker B et al 2013[20]	Cohort[#] with cross-sectional analysis	3,538 subjects aged 50-74 years	eGFR <60 mL/min/1.73 m^2	Fasting plasma glucose and HbA1c	Reduced eGFR: 18.7%[a] and 21.3%[b] *vs* 16.0% RR 1.06 (95%CI 0.71-1.32)[#]
Lin L et al 2016[21]	Cohort with cross-sectional analysis	250,752 subjects ≥40 years	eGFR <60 mL/min/1.73m^2	Fasting plasma glucose and/or oral glucose tolerance test	Reduced eGFR: 4.5% *vs* 1.7% OR for men 1.15 (95%CI 1.02-1.32)[###]
Tapp RJ et al 2004[22]	Cohort with cross-sectional analysis	11,247 adults ≥25 years	ACR 22-220 mg/g for men; 31-220 mg/g for women	Fasting plasma glucose and oral glucose tolerance test	Elevated ACR: 9.3%[e] and 11.0%[f] *vs* 5.1% OR for IFG 1.38 (95%CI 1.02-1.87)[##]
Suzuki H et al 2004[23]	Cross-sectional	154 Japanese men aged 20-70 years	ACR 30-300 mg/mg	Oral glucose tolerance test	Elevated ACR: 14%[f] and 26%[g] *vs* 9.0% (p = 0.028)
Li XY et al 2008[24]	Cross-sectional	1,776 Chinese subjects > 40 years: 506 IGT, 516 newly diagnosed DM, 754 normoglycemic	ACR 30-300 mg/g	Fasting plasma and oral glucose tolerance test	Elevated ACR: 7.0%[f] and 8.6%[e] *vs* 4.5% (p <0.0001) OR 1.28[e] and 1.32[f]
Bahar A et al 2013[25]	Cohort with cross-sectional analysis	135 subjects: 90 with prediabetes and 45 normoglycemic	ACR 30-300 mg/g	Fasting plasma glucose and oral glucose tolerance test	Elevated ACR: 15.5% *vs* 0%
Won JC et al 2014[26]	Cohort with cross-sectional analysis	5,202 subjects >19 years	ACR ≥30 mg/g	Fasting plasma glucose	Elevated ACR: 7.6%[c] and 12.3%[d] *vs* 4.1% and 6.0% OR 1,87(95% CI 1.19-2.94)[##]
Kim CH et al 2014[27]	Cohort with cross-sectional analysis	8,775 subjects aged ≥ 19 years	ACR 30-300 mg/g	Fasting plasma glucose or HbA1c	Elevated ACR: 6.3% *vs* 3.6% OR 1,14 (95% CI 0.93-1.41)[##]
Platinga LC et al 2010[28]	Cross-sectional	8,188 subjects ≥20 years: 2,272 prediabetics, 1,125 DM, 4,791 normoglycemic	ACR ≥30 mg/g and eGFR 15-59 mL/min/1.73m^2	Fasting plasma glucose (≥100-125 mg/dL)	Combined criteria: 17.7% *vs* 10.6% (p <0.001)
Zhou Y et al 2013[29]	Cross-sectional	5,584 subjects aged 20-79 years	ACR ≥30 mg/g and eGFR <60 mL/min/1.73m^2	Fasting plasma glucose (≥100-125 mg/dL)	Combined criteria: 12.9%[h] and 14.1%[i] *vs* 8.7% and 9.2% (p <0.001)
Ali MK et al 2018[30]	Cohort with cross-sectional analysis	27,971 subjects ≥20 years	ACR ≥30 mg/g and eGFR <60 mL/min/1.73m^2	Fasting plasma glucose or HbA1c	Combined criteria: 12.3% *vs* 11.3%

ACR, albumin-creatinine ratio eGFR, estimated glomerular filtration rate DM, diabetes mellitus
[#] 8-year incidence was calculated, [a] Prediabetes defined by fasting plasma glucose or HbA1c [b] Prediabetes defined by both fasting plasma glucose and HbA1c
[c] Fasting plasma glucose 100 – 109 mg/dL [d] Fasting plasma glucose 110 – 124 mg/dL, [e] Subjects with IFG, [f] Subjects with IGT, [g] Combined IGT/IFG
[h] Prevalence of microalbuminuria, [i] Prevalence of reduced GFR by MDRD [#]adjusted for age and body mass index [##]plus lifestyle and blood pressure [###]plus medications and lipids

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4.2. **PAPER 2**

Serum Cystatin C in Early Kidney Dysfunction in Prediabetic Participants of the Brazilian Longitudinal Study of Adult Health - ELSA-Brasil

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Abstract

Serum cystatin C (sCys C) was proposed as a marker of kidney function. In diabetic subjects, slight elevation of albuminuria indicates renal damage but it is unclear whether this begins in prediabetic stages. We investigated whether sCys C levels are already increased in prediabetes, contributing to early detection of kidney dysfunction. In a cross-sectional analysis of 947 participants of the Brazilian Longitudinal Study of Adult Health - ELSA, sCys C and estimated glomerular filtration rate (eGFR) were compared between normoglycemic and prediabetic subjects. Prediabetic subjects were stratified into 4 groups - G1, normal sCys C and normal albumin-to-creatinine ratio (ACR); G2, abnormal sCys C and normal ACR; G3, normal sCys C and abnormal ACR; G4, abnormal sCys C and ACR, and their eGFR compared. Prediabetic subjects had higher sCys C than normoglycemic ones [0.67 (0.41 - 0.95) vs 0.48 (0.31 - 0.81) mg/L, $p < 0.001$] and lower eGFR (96.3 ± 17.4 vs 100.6 ± 17.1 mL/min/1.73m2, $p < 0.001$). Normoglycemic hyperfiltrating subjects had lower sCys C than normofiltrating ones ($p = 0.035$). Considering prediabetic groups, eGFR gradually decreased from G1 to G4 (96.8 ± 17.4 vs 96.2 ± 16.9 vs 94.0 ± 17.2 vs 77.2 ± 25.4 mL/min/1.73m2, p-trend = 0.06) and mean eGFR in G4 was lower than in G1 ($p = 0.017$). The finding of higher sCys C levels in prediabetic subjects suggests this determination could be a helpful marker for examining kidney function in early stages of dysglycemia when albuminuria is still within the normal range. The follow-up of participants should allow testing the role of sCys C as an early marker for renal dysfunction in prediabetes.

Keywords: Prediabetes; Kidney Disease; Glomerular Filtration Rate; Cystatin C; Microalbuminuria

Introduction

Reported prevalence rates of prediabetes are alarming worldwide and in some populations this condition could affect 7.7 to 56% of adults [1-3]. Such conditions of mild hyperglycemia (named impaired fasting glucose and impaired glucose tolerance [4]) have been associated with progression to overt diabetes, as reported in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) [5] and they are already deleterious for tissues and organs. It was demonstrated that chronic kidney disease (CKD) occurs even in prediabetic stages and that its prevalence is increasing in some populations, ranging from 9.0 to 21.3% [6-8]. CKD is a major public health concern worldwide [9] and in US diabetes represents the main cause of incident cases of renal failure [10]. According to the Global Burden of Disease [11], diabetes and CKD were the eighth cause of Disability Adjusted Life Years (DALYs) worldwide and the seventh cause in Brazil.

Sustained hyperglycemia at prediabetic levels can trigger glomerular hyperfiltration and so can contribute to kidney damage [12,13]. Thus, early identification of individuals at risk seems important for preventive purposes. Diabetic kidney disease (DKD), as defined by reduced glomerular filtration rate (GFR) and/or presence of albuminuria, represents the main cause of CKD in patients who initiate renal replacement therapy [14]. Up to 40% of subjects with type 2 diabetes mellitus develop CKD which is associated with reduced quality of life and increased mortality [15].

Usually, incipient kidney dysfunction is detected by the presence of microalbuminuria [16,17]. However, type 2 diabetic subjects may have a reduced GFR before the occurrence of microalbuminuria [18]. This condition of non-albuminuric kidney disease can occur in up to one fifth of DKD [19] and such phenotype was also reported in prediabetic subjects [20]. Therefore, search for early markers of renal dysfunction in prediabetes seems of great interest.

Serum cystatin C (sCys C) is an endogenous marker of kidney function and recent studies have suggested that this may be a more sensitive marker for early kidney impairment [21]. Its determination overcomes limitations of serum creatinine,

with accuracy to assess renal damage [22,23]. Cystatin C is not influenced by age, gender, protein intake and muscle mass [24,30], although some conditions, such as hyperthyroidism and obesity, can alter its levels [29]. Special groups such as the elderly with reduced muscle mass have particular benefit of using sCys C determination [24]. A recent study of a small sample of diabetic individuals found that sCys C was a more sensitive parameter than creatinine to detect nephropathy [25]. It is unclear how sCys C performs as an earlier marker for the detection of incipient diabetic nephropathy. When sCys C was examined in normal and microalbuminuric diabetic subjects, subtle alterations predicted the severity of albuminuria even in the normoalbuminuric stage [26]. As far as we know, whether alterations of sCys C represent an opportunity to prevent and/or delay DKD particularly in prediabetes is unclear.

We hypothesized that kidney damage can occur even without the elevation of albuminuria and that sCys C could be helpful as an early marker of renal dysfunction in prediabetic subjects. We investigated whether sCys C levels are increased in prediabetes, contributing to the detection of kidney dysfunction independently of albuminuria.

Methods

- **Study design and population**

This is a cross-sectional analysis of baseline data from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a multicenter cohort study designed to identify new risk factors and determinants of diabetes and cardiovascular disease [27]. The study was approved by Ethics Committee and informed consent signed by all participants. The details of the study objectives and methods were previously reported [2,27]. Briefly, from August 2008 to December 2010, ELSA-Brasil included 15,105 employees aged 35 to 74 years from six capital cities of Brazil. A convenience sample of 998 normoglycemic and prediabetic participants aged between 35 to 54 years was selected from 5,061 participants of ELSA-Brasil in São Paulo center for a sub-study [28], in which the current study was

conducted. Participants with missing data regarding sCys C or estimated GFR (eGFR) were excluded. Estimated GFR < 45 mL/min/1.73m^2 and hypertension (blood pressure ≥ 140/90 mmHg or antihypertensive treatment) were exclusion criteria, as well as thyroid dysfunctions (TSH < 0.1 and ≥ 10 µUI/mL). It was previously shown that hypothyroidism tended to decrease while hyperthyroidism to increase sCys C levels [29,30]. Body mass index (BMI) ≥ 30 kg/m2 was also excluded since obesity can affect sCys C production and overestimate eGFR when its circulating levels are employed [29,31]. One prediabetic participant with an outlier ACR value > 2,600 mg/g was excluded from the analyses, supposing that this could be due to another renal disease rather than the disturbance of glucose metabolism. A total of 51 participants were excluded.

- **Clinical and laboratory data**

Interviews and anthropometric examinations were carried out by trained personnel using standardized questionnaires and measurements [32]. BMI was calculated as weight in kilograms divided by height in meters squared. Blood pressure (BP) was measured with Omron HEM 705CPINT device (Omron Co, Kyoto, Japan) after a 5-minute rest. Three measurements were taken at 1-min intervals and mean values were calculated. After overnight fasting, blood and urine samples were obtained for several determinations [33]. Participants underwent a 2-hour 75g oral glucose tolerance test. Aliquots of biological samples were frozen at -80°C for further determinations [34].

Plasma glucose was measured by the hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA) and A1c using a high-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, California, USA), according to the National Glycohemoglobin Standardization Program. GFR was estimated using the equations proposed by the Chronic Kidney Disease Epidemiology Collaboration (eGFR CKD-EPI) [35]. Albuminuria was determined in a 12h-overnight sample by nephelometry and was expressed as albumin-to-creatinine ratio (ACR). Serum cystatin C was measured using Human Cystatin C ELISA kit (Elabscience Biotechnology, Houston, Texas, USA). Intraassay and inter-assay coefficients of variability range were 5.05 - 5.38% and 3.29 - 6.48%, respectively.

- **Definitions**

Participants with BMI ≥ 30 kg/m2 were considered obese. Prediabetes diagnosis was defined by fasting plasma glucose (100 - 125 mg/dL) or 2-hour post-load glucose (140 - 199 mg/dL) or hemoglobin A1c (5.7 - 6.4%) [4,27]. The following categories of eGFR CKD-EPI were considered: a) hyperfiltration when eGFR ≥ 125 mL/min/1.73m^2; b) normal eGFR for values ≥ 90 and < 125 mL/min/1.73m^2 and c) mildly decreased eGFR for values ≥ 60 and < 90 mL/min/1.73m^2, according to KDIGO 2012 [29]. Microalbuminuria was defined by values of ACR ≥ 30 mg/g creatinine [29]. Normal ranges of sCys C were 0.64 to 0.84 mg/L for men and 0.57 to 0.74 mg/L for women [36].

Prediabetics participants was divided into four groups combining levels of ACR and sCys C: group 1 (G1) had both normal sCys C and ACR; group 2 (G2) had abnormal sCys C and normal ACR; group 3 (G3) had normal sCys C and abnormal ACR; and group 4 (G4) had both abnormal sCys C and ACR.

- **Statistical analysis**

Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and compared using Student *t* test. Non-normal distributed parametric variables (ACR and sCys C) were expressed as median and interquartile range and compared using Wilcoxon rank test. ANOVA or Kruskall-Wallis test was used to compare variables of more than two groups of participants, complemented with Tukey test, and p for trend was obtained. Sensitivity analyses excluding obese and hypertensive participants from comparisons were performed. Chi-squared test was used to compare frequencies and 95% confidence intervals (95%CI) were provided. Correlation was tested using Pearson coefficient. Significance level was set at a p-value of 0.05. Statistical analyses were performed using the R Project for Statistical Computing (R version 3.5.2).

Results

The mean age of 947 participants (520 women, 427 men) was 45.7 ± 4.9 years. Sixty percent referred white skin color and 40% nonwhite colors; among the

latter participants 6% has Asian ancestry. The entire sample had normal mean values of systolic (117.0 ± 14.5mmHg) and diastolic BP (75.0 ± 10.4 mmHg), eGFR 97.6 ± 17.4 mL/min/1.73m^2), ACR (10.8 ± 36.8 mg/g creatinine) and sCys C (0.75 ± 0.61 mg/L). Their mean eGFR, sCys C and AER were within normal ranges and no correlation was detected between these parameters. A total of 671 participants had prediabetes and 276 had normal glucose tolerance. In average, participants were slightly overweight and a higher proportion of physically active subjects were found in the normoglycemic group (42.0% versus 33.6%, p = 0.02).

Mean values of age, BMI, BP, lipids and serum creatinine were higher and eGFR was lower in prediabetic than in normoglycemic participants (Table 1). Despite normal ACR values in both groups, median was lower in prediabetic compared to the normoglycemic one [5.8 (4.5 - 7.5) *versus* 6.6 (5.5 - 8.1) mg/g, p < 0.01]. Median sCys C was higher in prediabetic than in normoglycemic participants [0.67 (0.41 - 0.95) versus 0.48 (0.31 - 0.81) mg/L, p < 0.01], but values were within the reference range. However, proportions of participants with elevated sCys C were higher among prediabetic participants than the in normoglycemic ones (39% *versus* 28%, p < 0.002). Medians of sCys C for men and women did not differ [0.61 (0.38 - 0.92) *versus* 0.63 (0.37 - 0.89) mg/L, p = 0.562, respectively, and analyses were not stratified by sex.

Table 1. Clinical data of prediabetic and normoglycemic participants.

	Normoglycemic N = 276	**Prediabetic N = 671**	**P-value**
Age (years)	44.7 (4.9)	46.1 (4.8)	< 0.001
Body mass index (kg/m^2)	25.3 (3.8)	26.7 (4.24)	< 0.001
Systolic blood pressure (mmHg)	111.4 (12.4)	118.8 (14.8)	< 0.001
Diastolic blood pressure (mmHg)	71.2 (9.5)	76.5 (10.4)	< 0.001
Fasting plasma glucose (mg/dL)	94.3 (3.7)	105.7 (7.0)	< 0.001
2-hour plasma glucose (mg/dL)	107.8 (18.0)	126.0 (27.4)	< 0.001
Glycated hemoglobin (%)	4.9 (0.4)	5.4 (0.5)	< 0.001
Total cholesterol (mg/dL)	202.2 (32.7)	212.3 (38.2)	< 0.001
HDL-cholesterol (mg/dL)	59.5 (13.2)	53.6 (13.0)	< 0.001

LDL-cholesterol (mg/dL)	122.3 (28.6)	131.4 (33.16)	< 0.001
Triglycerides (mg/dL)	102.1 (51.2)	139.0 (83.85)	< 0.001
Creatinine (mg/dL)	0.87 (0.16)	0.96 (0.19)	< 0.001
eGFR (mL/min/1.73m^2)	100.6 (17.1)	96.3 (17.4)	< 0.001
ACR (mg/g)*	6.6 (5.5 – 8.1)	5.8 (4.5 – 7.5)	< 0.001
Serum cystatin C (mg/L)*	0.48 (0.31 – 0.81)	0.67 (0.41 – 0.95)	< 0.001
Elevated ACR, n (%)	8 (3)	21 (3)	0.999
Elevated serum cystatin C, n (%)	77 (28)	259 (39)	0.002

eGFR, estimated glomerular filtration rate ACR, albumin-creatinine ratio
Data are expressed as frequency (%), mean (SD) or median (interquartile range). P-values obtained by chi-square, Student t or *Wilcoxon test.

Comparisons between prediabetic and normoglycemic participants within eGFR category (mildly decreased, normal and increased eGFR) showed that median values of sCys C were always higher in prediabetic ones. Median ACR was within the normal range for the three categories, although statistically higher values were observed in normoglycemic compared to prediabetic participants with normal or mildly decreased eGFR (Table 2). Considering the category of hyperfiltrating participants, normoglycemic group had a significantly higher BMI [26.2 ± 4.3 *versus* 25.0 ± 5.4 kg/m2, p = 0.041] than prediabetic one.

Figure 1 depicts sCys C values in normoglycemic and prediabetic participants according to their eGFR category. Only normoglycemic participants with hyperfiltration were associated with a significant reduction in sCys C (p = 0.035). Median sCys C was unchanged across categories of eGFR in prediabetic participants (p > 0.05).

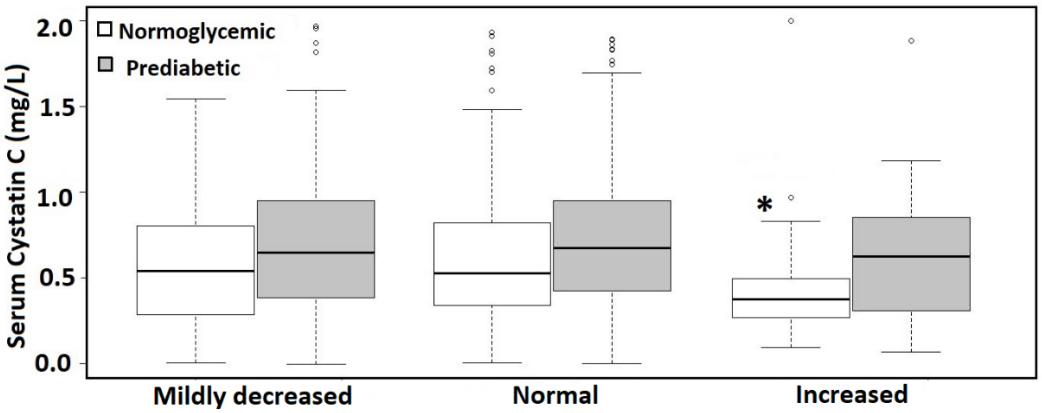

Figure 1. Median values of serum cystatin C between normoglycemic and prediabetic participants. eGFR categories: mildly decreased (eGFR ≥ 60 and < 90 mL/min/1.73m^2), normal (eGFR ≥ 90 and < 125 mL/min/1.73m^2) and increased eGFR (≥ 125 mL/min/1.73m^2). * p = 0.035 *versus* normal eGFR

Table 2. Median (interquartile interval) of renal function parameters of participants grouped according to estimated glomerular filtration rate (eGFR) categories and presence of prediabetes.

	Mildly decreased eGFR (≥ 60 - < 90 mL/min/1.73 m²) n = 330			Normal eGFR (≥ 90 - < 125 mL/min/1.73 m²) n = 544			Increased eGFR (≥ 125 mL/min/1.73 m²) n = 59		
	Normoglycemia N = 88	Prediabetes N = 242	P-value	Normoglycemia N = 163	Prediabetes N = 381	P-value	Normoglycemia N = 24	Prediabetes N = 35	P-value
Cystatin C, mg/L	0.54 (0.29-0.80)	0.66 (0.39-0.96)	0.004	0.53 (0.34-0.82)	0.68 (0.43-0.95)	0.003	0.37 (0.27-0.48)	0.62 (0.30-0.85)	0.031
ACR, mg/g	6.22 (5.07-7.25)	5.65 (4.38-7.17)	0.042	6.89 (5.79-8.30)	5.91 (4.62-7.69)	< 0.001	7.04 (6.03-8.02)	6.67 (5.20-7.65)	0.371

ACR: Albumin-Creatinine Ratio. Wilcoxon test used.

Prediabetic participants were then divided into four groups according to the combination of renal function parameters (sCys C and ACR). Figure 2 shows that eGFR gradually dropped from G1 to G4 (G1: 96.8 ± 17.4, G2: 96.2 ± 16.9, G3: 94.0 ± 17.2 and G4: 77.2 ± 25.4mL/min/1.73m^2, p-trend = 0.060). Such trend reached borderline significance but, as expected, mean eGFR in G4 was lower than in G1 (p = 0.017).

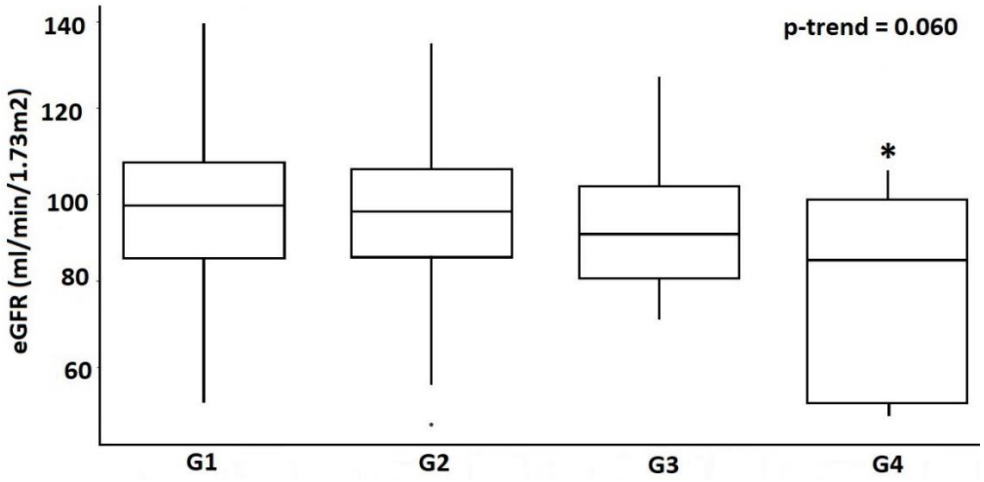

Figure 2. Box plot of eGFR in prediabetic participants among groups. Group 1: normal sCys C and normal ACR (n = 385); Group 2: abnormal sCys C and normal ACR (n = 253); Group 3: normal sCys C and abnormal ACR (n = 13) and Group 4: abnormal sCys C and abnormal ACR (n = 7). * p = 0.017 *versus* Group 1.

Discussion

Looking at sCys C levels, this study raises their potential role for early detection of renal dysfunction in prediabetic states. For the best of our knowledge, results of a higher sCys C under normoalbuminuria in prediabetic but not in normoglycemic participants, obtained in a large epidemiological study, suggest that this may be a marker of renal dysfunction in early stage of glucose metabolism disturbance, when only glomerular hyperfiltration is manifest. Since DKD has been considered a complication of overt diabetes [13], change in this marker of renal dysfunction could support a paradigm shift, indicating that slight dysglycemia is enough to cause kidney damage. This seems relevant considering that role of diabetes as a major cause of renal failure [10].

The finding of higher sCys C in the prediabetic compared to normoglycemic group, independently of eGFR categories, raises the possibility that its determination could be accurate to identify slight renal injury, before glomerular albumin loss. Considering that increased body adiposity and blood pressure could contribute to elevate albumin urinary excretion, obesity and hypertension were excluded. Median

ACR values were within the normal range, and, unexpectedly, higher levels were observed among the normoglycemic participants. Although statistically different, from a clinical point of view, such difference of ACR between groups may be considered irrelevant. In addition, a higher frequency of physically active individuals in the normoglycemic group could account in part for such difference. As a matter of fact, it is recognized an intra-individual variability in albumin excretion rate under certain conditions such as physical activity practice [29,37]. The design of our study did not allow suggesting that elevations in sCys C may be useful to detect renal damage earlier than albuminuria. This hypothesis deserves further investigation considering that some authors have already described the presence of renal injury (microalbuminuria and/or reduced GFR) in prediabetic individuals [13,38-40].

Increases in glomerular filtration are typically observed in diabetes mellitus as a consequence of sustained hyperglycemia [41], but hyperfiltration is uncommon in healthy normoglycemic subjects. Since obesity has been also associated to elevation in GFR [42], both diabetic and obese subjects were excluded from our sample. A small proportion of normoglycemic participants showed eGFR above 125mL/min/1.73m^2, which could be attributed in part to their slightly elevated mean BMI. We speculate that lower sCys C levels in those hyperfiltrating normoglycemic participants could indicate an increased renal loss of Cys C. Once circulating Cys C is filtered by glomeruli and reabsorbed and metabolized by tubular cells [43], its reabsorption may be insufficient under hyperfiltrating conditions, decreasing serum levels. This plausible mechanism to explain lower sCys C levels in hyperfiltration could not be confirmed in our study due to the absence of urinary Cys C measurement. Interestingly, in the subset of prediabetic participants with hyperfiltration higher sCys C levels were observed which could suggest that tubular cells function was impaired at the initial natural history of diabetes mellitus.

In line with our findings, previous studies had already shown that increased urinary levels of Cys C was early correlated with DKD in adults [44], also found with acute renal disease and reduced renal volume early in life such as in neonates [45,46]. This body of evidence could reinforce a possible role of Cys C as an early marker of kidney injury.

Estimated GFR and ACR are established parameters for renal function assessment. The latest update of the Kidney Disease Improving Global Outcomes (KDIGO) recommends that both, eGFR and ACR, should be used for the diagnosis of CKD [29]. According to these criteria, approximately 5% of entire population of ELSA-Brasil had CKD [2]. In the present study, we explored on the utility of sCys C in combination with ACR to test association with eGFR. As expected, participants with both abnormal ACR and sCys C values had lower eGFR. Since there is no previous report of such association, we are suggesting that ACR combined with sCys C could also be useful markers to detect kidney dysfunction in prediabetic individuals. In fact, other investigators have proposed different approaches to assess renal function such as the eGFR based on cystatin C (eGFRcys) or in combination with serum creatinine (GFRcys-creat) [29]. Several studies demonstrated that both cystatin-based eGFR formulas are more accurate in comparison with the traditional one based on serum creatinine to assess renal function in early stages of kidney injury [47]. Additionally, positive association of sCys C with the progression of prediabetes has been reported [48,49] as well as playing a role for cardiovascular risk in prediabetic individuals [50].

- **Limitation of the Study**

A main limitation of our study is related to the cross-sectional design of this study that impede inferring causality between the interest variables. It is not possible to state that the onset of sCys C elevation occurs earlier than the increase in ACR. We have chosen a definition for hyperfiltration (eGFR ≥ 125 ml/min/m^2), since there is not an internationally accepted consensus. Some studies suggest using eGFR above the 90th percentile adjusted by body area [51] and others suggest that should be used age and sex-specific cut-offs for better measure of hyperfiltration [52]. According to a meta-analysis [52], commonly used definition for hyperfiltration varied from 90 to 175 mL/min/m2. Determination of urinary cystatin C would contribute to speculate on pathophysiological mechanisms.

- **Strength of the Study**

Strengths of ELSA-Brasil [27] are its sample size and prospective design to test hypothesis raised in the present sub-study. We currently examined a relatively

novel circulating biomarker - sCys C - in a large sample to assess kidney function in early stages of glucose metabolism disturbance. We excluded participants with several factors that could interfere in sCys C assay, as thyroid disorders and obesity [29], and utility of its determination at an initial phase of kidney damage to predict DKD will be further investigated. Such approach made unnecessary adjusted analyses. This can be the largest study of sCys C data in prediabetic subjects providing subsidies to raise that this may be an early marker of renal dysfunction during glomerular hyperfiltration stage, dependent of the intermittent hyperglycemia that precedes overt diabetes.

Conclusion

In conclusion, we reinforced that sCys C levels are higher in prediabetic than in normoglycemic subjects. Serum Cys C could be a helpful marker for examining kidney function in early stages of dysglycemia when albuminuria is still within the normal range but with elevated eGFR. The follow-up of ELSA-Brasil participants should allow testing more appropriately the role of sCys C as an early marker for renal dysfunction in prediabetes, when preventive measures could be introduced.

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Abbreviations

ACR: Albumin-to-Creatinine Ratio; A1c: Glycated Hemoglobin; BMI: Body Mass Index; BP: Blood Pressure; CKD: Chronic Kidney Disease; DALYs: Disability Adjusted Life Years; DKD: Diabetic Kidney Disease; eGFR: Estimated Glomerular Filtration Rate; ELSA-Brasil: Brazilian Longitudinal Study of Adult Health; HDL-c: High Density Lipoprotein Cholesterol; KDIGO: Kidney Disease Improving Global Outcomes; LDL-c: Low Density Lipoprotein Cholesterol; sCys C: Serum Cystatin C; SD: Standard Deviation; TSH: Thyroid Stimulating Hormone.

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4.3. **PAPER 3**

Associations of prematurity and low birth weight with blood pressure and kidney function in middle-aged participants of the Brazilian Longitudinal Study of Adult Health – ELSA-Brasil

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ABSTRACT

Background: Based on the Developmental Origins of Health and Disease, adverse intrauterine environment – reflected by low birth weight (LBW) and prematurity – may induce fetal programming that favors kidney dysfunction in adulthood. We examined the association of LBW and prematurity with blood pressure (BP) and kidney function markers in non-diabetic middle-aged adults without kidney disease from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). **Methods**: A cross-sectional analysis of 768 subjects aged 35-54 years was conducted. Comparisons were performed according to birth weight: LBW (<2.5 kg) or normal birth weight (NBW) (2.5 - 4.0 kg). Associations of LBW and prematurity with BP levels and kidney function markers (glomerular filtration rate - eGFR, albumin-creatinine ratio - ACR and serum cystatin-C) were tested by multiple linear regression using adjustments based on Directed Acyclic Graphs. Propensity score matching was applied to control imbalances. **Results**: Mean age of participants was 45.5 ± 4.6 years and 56.8% were female; 64 (8.3%) participants reported LBW and 39 (5.0%) prematurity. The LBW group had higher systolic ($p = 0.015$) and diastolic BP ($p = 0.014$) and ACR values ($p = 0.031$) and lower eGFR ($p = 0.015$) than the NBW group, but no group difference for cystatin-C was found. The preterm group had higher mean levels of systolic and diastolic BP, but no difference in kidney function markers was evident. In a regression model adjusted for sex, skin color and family history of hypertension, both systolic and diastolic BP levels were associated with LBW, but this association disappeared after adding prematurity, which remained associated with BP ($p = 0.017$). Having applied propensity score matching, LBW was associated with ACR values ($p=0.003$), but not with eGFR or BP levels. **Conclusion**: The study findings of independent associations of prematurity with higher BP levels, and of LBW with markers of kidney function, in adulthood support that early life events predict risk for hypertension and kidney dysfunction in adulthood. The study design precluded the inferring of causality, and prospective studies are needed to further investigate the hypothesis raised.

Keywords: low birth weight, prematurity, reduced kidney function, blood pressure.

INTRODUCTION

Based on the Developmental Origins of Health and Disease (DOHaD) theory, early life events can increase the risk of noncommunicable diseases in adulthood[1]. Nutrition-related and other stressors during pregnancy may result in low birth weight (LBW), considered a proxy of an adverse intrauterine environment[2] and commonly the result of intrauterine growth restriction or prematurity[3]. Given the short- and long-term adverse outcomes in LBW neonates, this condition remains a major public health concern. The World Health Organization (WHO) estimates that LBW occurs in 15 - 20% of all births, with rates varying widely according to country income level[4]. Compelling evidence has indicated that LBW may predict cardiometabolic and renal disorders[5,6].

Perinatal distress has been associated with blood pressure level elevation in adulthood[7]. Underlying mechanisms for the association of early life events with blood pressure elevation have been proposed in animal and human models[8,9]. Changes in RAS gene expression, activity of the angiotensin-converting enzyme and sympathetic nervous system, may contribute to development of hypertension in adult life[10-12]. Sustained increased blood pressure levels with gradual glomerular damage may precipitate chronic renal failure.

Nephrogenesis begins at early stages of embryo life with an increase in glomeruli number in the last weeks of gestation[13]. Insults during intrauterine life (undernutrition, toxic exposures and/or placental abnormalities) [14] can negatively impact renal functional reserve. These adverse events can induce fetal programming with resultant reduction in nephron formation, hypertrophy of the remaining nephrons, leading to adaptive hyperfiltration and increased risk of kidney dysfunction later in life[15,16]. An early sign of hypertension-dependent glomerular damage is urinary protein loss[17].

Issues regarding whether LBW adults born at full-term or prematurely are prone to exhibit different long-term consequences, and whether these changes can be detected early in the natural history of hypertension and chronic kidney disease, remain understudied.

The Brazilian Longitudinal Study of Adult Health (ELSA-Brasil) is a large cohort that provides an opportunity to investigate the relationship of exposures with several outcomes in adulthood[18,19]. In particular, this allows the testing of associations of early life events with blood pressure levels and renal function markers in adults without overt kidney diseases. We examined the association of LBW and prematurity with blood pressure levels and kidney function markers in middle-aged non-diabetic participants of ELSA-Brasil without kidney disease.

METHODS

- **Study design and population**

A cross-sectional analysis was carried out of the baseline cohort from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil), a multicenter cohort study aimed at investigating biological, behavioral, environmental, occupational, psychological, and social factors related to incidence and progression of diabetes and cardiovascular disease[19]. Methodological details have been reported elsewhere[18,19]. Briefly, from August 2008 to December 2010, ELSA-Brasil included employees aged 35-74 years working in universities and research institutions located in the Northeast, Southeast and South regions of Brazil, with ample socioeconomic conditions and skin color distribution. Sample size of ELSA-Brasil was calculated based on estimations of incidence of type 2 diabetes and myocardial infarction for the Brazilian population. The current study involved a random sample of 998 without diabetes and cardiovascular disease aged 35-54 years, drawn from 5,061 participants at the São Paulo center of the ELSA-Brasil for whom information on biomarkers of inflammation and serum Cystatin C (sCys C) was available[20]. Such sample was showed the same demographic and socioeconomic distributions. The study was approved by the local Ethics Committee and informed consent was obtained from all participants.

- **Eligibility criteria**

Of the initial 998 participants, 17 with newly diagnosed diabetes and 16 with low estimated glomerular filtration rate (eGFR < 60 mL/min/1.73m^2) were not

included. Fifty individuals born with macrosomia, 18 with thyroid dysfunctions (TSH < 0.1 or ≥ 10.0 μUI/mL), 8 underweight individuals due to chronic consumptive diseases and 2 with liver dysfunctions (ALT or AST levels three-fold the normal range) were excluded. Since macrosomia confers potential risk of metabolic disorders and our aim was to analyze specifically low birth weight and prematurity, macrosomia was an exclusion criterium. Participants with missing data regarding exposure (birth weight) or outcomes (sCys C, eGFR and ACR) were also excluded (n = 119), as shown in Figure 1. Excluded participants did not change in the main composition of the final sample. A total of 768 participants were included in the present analysis.

- **Clinical and laboratory data**

Interview and anthropometric data were collected by trained personnel using standardized questionnaires[21]. Prematurity (yes or no) and birth weight were self-reported. Participants were asked to recall their weight at birth and the variable was classified into “< 2.5 kg”, “2.5-4.0 kg”, “> 4.0 kg” or “unknown”. All participants were asked to provide their specific birth weight and to recall their body weight at 20 years of age.

Sociodemographic and health factors of interest for this study were: age (years), sex (male or female), self-reported skin color (black, white, brown, yellow and indigenous), parental history of diabetes and hypertension (yes or no) and educational levels of the participant and their mother. For the purpose of the present study, skin colors were grouped into white and non-white categories.

Blood pressure was measured using an Omron HEM 705CPINT device (Omron Co, Kyoto, Japan) after a 5-minute rest in a sitting position. Three measurements were taken at 1-min intervals and mean values calculated. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. After overnight fasting, blood and urine samples were obtained and participants then underwent a 2-hour 75 g oral glucose tolerance test. Aliquots of biological samples were frozen at -80^{o}C for further analyses[22,23].

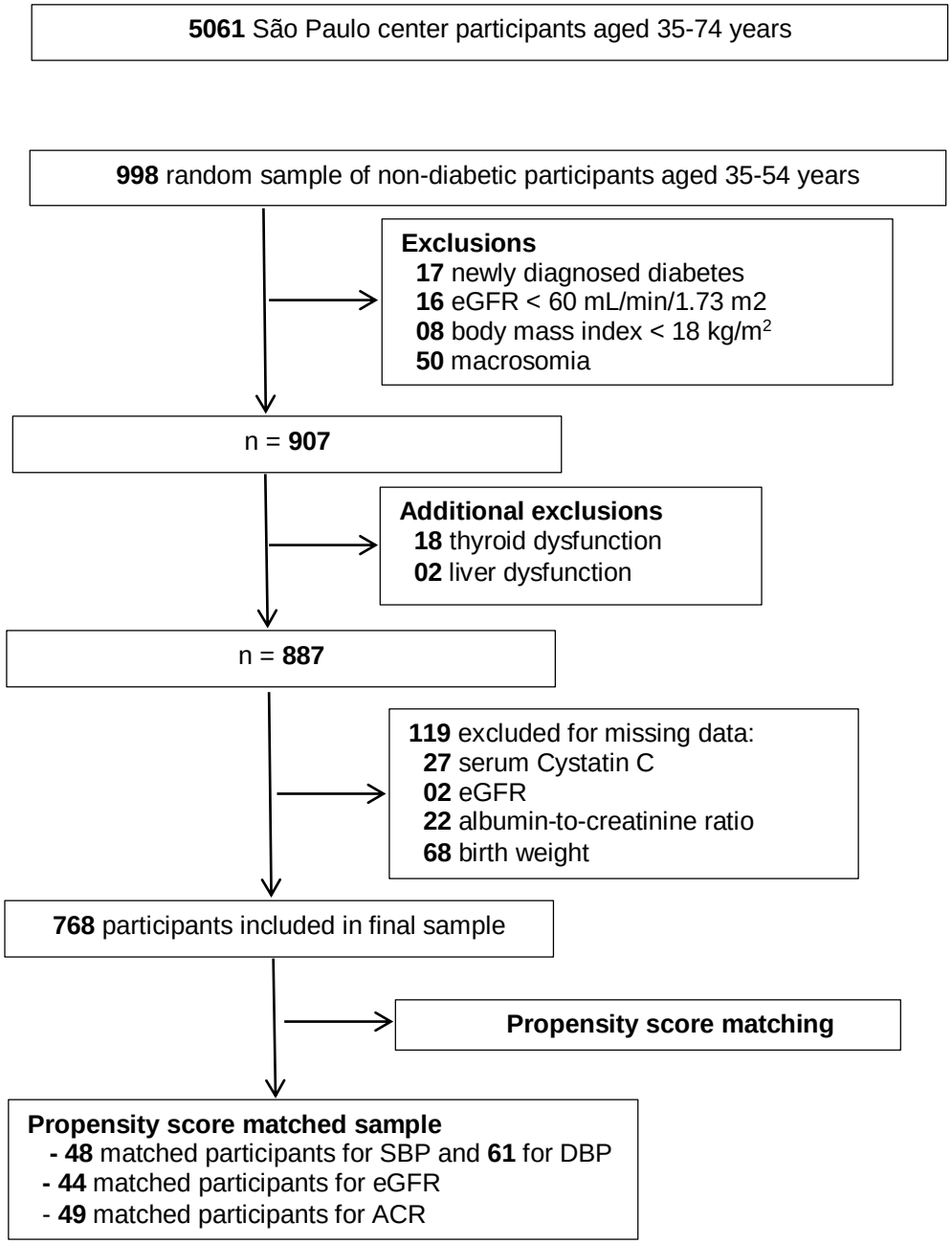

Figure 1. Flowchart of selection process of ELSA-Brasil participants in present study.

Fasting and 2-hour post-load plasma glucose were measured using the hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA) and glycated hemoglobin by high-pressure liquid chromatography (HPLC) (Bio-Rad

Laboratories, Hercules, California, USA), according to the National Glycohemoglobin Standardization Program (NGSP) certified method. Glomerular filtration rate was estimated using the equations proposed by the Chronic Kidney Disease Epidemiology Collaboration (eGFR CKD-EPI)[24]. Albuminuria was determined in a 12-h overnight sample by nephelometry and was expressed as albumin-to-creatinine ratio (ACR). Serum cystatin C was measured using a human cystatin C ELISA kit (Elabscience Biotechnology, Houston, Texas, USA). Intra-assay and inter-assay coefficient of variability ranges were 5.05 – 5.38% and 3.29 – 6.48%, respectively.

- **Definitions**

Prematurity was defined by an affirmative answer to the question: "*Were you a premature baby, that is, were you born earlier than expected*?". Birth weight was classified into 3 categories: low birth weight (< 2.5 kg), normal birth weight (2.5 – 4.0 kg) and macrosomia (> 4.0 kg). Outcomes were blood pressure (BP) levels, eGFR, ACR and sCys C and were analyzed as continuous variables.

Hypertension was diagnosed when systolic or diastolic blood pressure levels were ≥ 140 or 90 mmHg, respectively, or when individual was in use of antihypertensive drugs. Kidney function was considered altered for eGFR < 60 mL/min/1.73m^2 or urinary albumin excretion > 1,000 mg/g creatinine, according to KDIGO 2012[25]. Normal ranges of sCys C were 0.64- 0.84 mg/L for men and 0.57-0.74 mg/L for women[26].

Diabetes diagnosis was established when fasting plasma glucose ≥ 126 mg/dL or 2-hour post 75-g glucose load > 200 mg/dL or glycated hemoglobin ≥ 6.5%. Other variables were categorized as following: prediabetes (yes or no) defined as fasting plasma glucose between 100 - 125 mg/dL or 2-hour post 75-g glucose load between 140 – 199 mg/dL or glycated hemoglobin 5.7 – 6.4%; nutritional status was stratified by BMI into underweight (< 18.5 kg/m^2), normal weight (18.5 – 24.9 kg/m^2), overweight (25 – 29.9 kg/m^2) and obese (> 30 kg/m^2). Insulin sensitivity was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and considered a continuous variable.

- **Statistical analysis**

Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and compared using Student´s *t*-test. Non-normal distributed parametric variables were expressed as median and interquartile range and compared using the Wilcoxon rank test. Categorical variables were expressed as absolute and relative frequencies and compared using the chi-squared test.

Associations of LBW (exposure) with outcomes (blood pressure levels and kidney function parameters) were initially analyzed by simple linear regression. Direct Acyclic Graphs (DAG) were used to build theoretical models to analyze independent associations of exposure with outcomes in multiple linear regression analyses. The DAG is a causal diagram which allows the input of scientific evidence regarding the relationships among variables in graphics software to reach the ideal set of covariables (minimum sufficient adjustment) for the model to prevent bias and overadjustments[27,28]. For the construction of the DAG for blood pressure and kidney function, several confounder variables were considered such as age, sex, skin color, weight at age 20, maternal education level, prematurity, income, current BMI, smoking and others as shown in the Figure S1 from Supplementary material (Panels A and B). The figures were created using DAGitty software, version 3.0 (www.dagitty.net).

Based on DAGs, linear regression models for the association of LBW with blood pressure levels were adjusted for race/skin color, sex, parental history of hypertension, and prematurity. Linear regression models for the associations of LBW with kidney function parameters (eGFR, ACR and sCys C) were adjusted for skin color and prematurity.

Considering the contrasting sample sizes of the groups with normal and LBW, as well as possible selection bias due to the observational nature of the study, propensity score matching was employed to create more comparable groups[29,30]. The nearest neighbor-matching algorithm, within a caliper of 0.1 SD of logit function of propensity score was used. Firstly, to apply the propensity score matching, a multiple logistic regression model was used, adjusted for DAG-based covariates (blood pressure was adjusted for skin color, sex, parental history of hypertension and

prematurity; while kidney function markers were adjusted for skin color and prematurity), and the probability of each participant being LBW versus normal birth weight was estimated. Balance between the groups was assessed by comparing each covariate. When standardized mean difference lay in the -0.1 to 0.1 range, groups were considered balanced. This matching was able to reduce all covariate imbalance in the sample. Using the matched sample, multiple linear regression analyses were then performed to test associations of LBW with blood pressure and kidney function markers (outcomes) adjusted for the same DAG-based covariates. Tests were performed using “MatchIt”, “rbounds”, “Matching”, “twang” and “survey” packages in the R statistical environment.

Additionally, sensitivity analyses were performed by selecting the participants with self-reported preterm birth (prematurity). Also, when blood pressure levels were outcomes, sensitivity analyses were carried out excluding participants who reported use of antihypertensive agents (Supplementary Table S1). Since similar results were obtained in both analyses, preterm and hypertensive participants were retained for subsequent analysis.

All analyses were performed using R Project for Statistical Computing (R version 3.5.2) and statistical significance was set at a p-value of 0.05.

RESULTS

In the sample of 768 participants, mean age was 45.5 ± 4.6 years, 56.8% were female, and 60% reported white skin color. A total of 64 (8.3%) participants reported being LBW and 39 (5.0%) born preterm. The LBW group comprised participants that were predominantly female (54.6%), white skinned (56.0%) and had mothers with low educational level (71.9%). These characteristics showed a similar pattern in the preterm subgroup (67.0% female and 61.0% white skin color).

Overall, cardiometabolic risk profile and kidney function of the total sample were within normal ranges, except for being generally overweight (mean 26.0 kg/m^2 ± 4.15) and having borderline fasting plasma glucose (102.0 mg/dL ± 7.5). Mean values of systolic (116.7 ± 14.7 mmHg) and diastolic (74.9 ± 10.5 mmHg) blood

pressure, eGFR (89.6 ± 13.8 mL/min/1.73 m^2), ACR (11.0 ± 39.0 mg/g creatinine) and sCys C (0.74 ± 0.61 mg/L) were within normal ranges.

Only 133 participants fulfilled the diagnostic criteria for hypertension. Among the hypertensive participants, 8.1 % and 14 % were receiving antihypertensive treatment in the normal and LBW groups, respectively. Hypertension rates differed between normal and LBW groups (16.2% versus 29.7%, p = 0.024).

Low birth weight participants were, on average, older than normal birth weight subjects (47.0 ± 5.5 years versus 45.3 ± 4.8 years, p = 0.027) and had higher prevalence of low educational level (6.3 versus 3.7 %, p = 0.025). Mean BMI values were lower at age 20 (21.2 ± 2.81 versus 20.7 ± 3.0 kg/m^2, p = 0.196) than at current age (26.2 ± 4.1 versus 26.0 ± 4.4 kg/m^2, p = 0.688) for both normal and LBW participants, respectively, with no difference between subgroups.

Participants with LBW had higher mean blood pressure and ACR, and lower eGFR, than individuals with normal birth weight, but there were no group differences for sCys C (Table 1).

The preterm subgroup also had significantly higher mean systolic and diastolic blood pressure levels, but no difference in kidney function markers was detected (Supplementary Table S2).

In multiple linear regression analysis, LBW was associated with blood pressure levels, but this association did not persist after adjustment for prematurity, which remained associated with systolic and diastolic blood pressure (Table 2). Associations of LBW with eGFR and ACR in the fully adjusted models had borderline significance (p = 0.05) (Table 3). In a separate analysis of only preterm participants, prematurity was independently associated with both systolic and diastolic blood pressure (Supplementary Table S3).

- ***Imbalance of variables***

Initially, the sample had 64 participants with LBW. Propensity score matching was applied, yielding sub-samples with matched participants (normal and LBW) as follows: (a) 48 matched participants for systolic and 61 matched participants for diastolic blood pressure; (b) 44 matched participants for eGFR; and (c) 49 matched

participants for ACR with the same proportion of normal and LBW participants in each sub-sample. The matching approach rendered all covariates appropriately balanced (standardized mean difference of between -0.1 and 0.1) for further analyses. Imbalance for all variables was assessed before and after matching to compare improvement by matching (Supplementary Table S4).

Table 1. Main characteristics of ELSA-Brasil participants born with normal and low birth weight.

	Normal Birth Weight n = 704	**Low Birth Weight n = 64**	***P*-value**
Age (years)	45.4 (4.8)	46.9 (5.5)	0.027
Body mass index at age 20 (kg/m^2)	21.2 (2.8)	20.7 (3.0)	0.196
Body mass index (kg/m^2)	26.2 (4.1)	26.0 (4.4)	0.688
Systolic blood pressure (mmHg)	116.3 (14.6)	121.0 (14.8)	0.015
Diastolic blood pressure (mmHg)	74.6 (10.5)	77.6 (10.8)	0.040
Hypertension n (%)	114 (16.2)	19 (29.7)	0.024
Antihypertensive treatment n (%)	57 (8.1)	9 (14)	0.162
LDL-cholesterol (mg/dL)	128.3 (32.6)	132.6 (32.1)	0.314
HDL-cholesterol (mg/dL)	55.2 (13.2)	58.2 (14.0)	0.114
Tryglicerides (mg/dL)	127.7 (76.3)	124.1 (58.5)	0.646
Fasting plasma glucose (mg/dL)	101.9 (8.0)	102.2 (7.9)	0.792
HOMA-IR*	2.32 (1.65-3.38)	2.52 (1.56-3.65)	0.765
Creatinine (mg/dL)	0.92 (0.18)	0.94 (0.17)	0.254
eGFR (ml/min/1.73m^2)	98.9 (16.9)	94.2 (13.9)	0.015
Albumin-creatinine ratio (mg/g)*	6.2 (4.9-7.7)	6.9 (5.2-8.9)	0.031
Cystatin C (mg/L)*	0.62 (0.37-0.91)	0.58 (0.34-0.81)	0.313

eGFR, estimated glomerular filtration rate; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance
Data are expressed as mean (SD) or median (interquartile range). Student t or *Wilcoxon test was used.

Table 2. Association of low birth weight with systolic and diastolic blood pressure.

	B	95% CI	*P*-value
Systolic blood pressure			
Model 1	4.34	0.85 – 7.83	0.015
Model 2	4.90	1.37 – 8.43	0.007
Model 3	2.54	- 1.52 – 6.61	0.220
Sex- male	10.78	8.77 – 12.78	< 0.001
Skin color – non-white	4.16	2.16 – 6.17	< 0.001
Parental history of hypertension	3.20	0.95 – 5.45	0.005
Prematurity	6.16	1.09 – 11.23	0.017
Diastolic blood pressure			
Model 1	2.63	0.070 – 5.19	0.044
Model 2	2.93	0.34 – 5.52	0.026
Model 3	1.20	-1.78 – 4.18	0.430
Sex- male	6.62	5.15 – 8.09	< 0.001
Skin color – non-white	3.09	1.62 – 4.55	< 0.001
Parental history of hypertension	2.82	1.17 – 4.47	< 0.001
Prematurity	4.52	0.81 – 8.24	0.017

Model 1: adjusted for sex and skin color.
Model 2: adjusted for sex, skin color and parental history of hypertension.
Model 3: adjusted for sex, skin color, parental history of hypertension, and prematurity.

Table 3. Association of low birth weight with estimated glomerular filtration rate and albumin-to-creatinine ratio.

	B	95% CI	*P*-value
Estimated glomerular filtration rate			
Model 1	-4.22	-6.47; - 0.001	0.049
Model 2	-4.17	-6.39 - 0.059	0.051
Albumin-to-creatinine ratio			
Model 1	14.22	4.52 – 24.49	0.004
Model 2	11.08	-0.29 – 22.88	0.056

Model 1: adjusted for skin color.
Model 2: adjusted for skin color and prematurity.

- ***Associations of LBW with blood pressure and kidney function markers after propensity score matching***

The multiple linear regression model, adjusted for skin color and prematurity, showed that being born with low weight was directly associated with ACR values (ß 1.34; 95%CI 0.47 – 2.20; p = 0.003). Even when reaching adequate balance, LBW was not associated with eGFR or with systolic and diastolic blood pressure levels (Table 4).

Table 4. Estimates of associations of LBW with blood pressure and kidney function markers after propensity score matching in ELSA-Brasil participants.

	Propensity score matching		
	Coefficient (ß)	**95%CI**	***P*-value**
Albumin-to-creatinine ratio (mg/g)	1.34	0.47 – 2.20	0.003
eGFR (ml/min/1.73m^2)	-0.62	-3.57 – 2.32	0.672
Systolic blood pressure (mmHg)	0.03	-4.70 – 4.77	0.989
Diastolic blood pressure (mmHg)	-1.97	-6.24 – 2.31	0.365

CI, confidence interval; eGFR, estimated glomerular filtration rate

DISCUSSION

We hypothesized that being born full-term or preterm, with LBW, is associated with altered blood pressure levels and kidney function markers in adulthood, indicating an insult during intrauterine life. The findings showing both LBW and preterm participants had higher mean blood pressure levels, and that the LBW group also exhibited lower eGFR and higher ACR, than individuals with normal birth weight corroborated this hypothesis. Enhancing our analysis by propensity score matching and DAG-based adjustments on multiple linear regression boosted confidence in the associations found between early-life events and outcomes in adulthood. We suggest that a subset of individuals with LBW may be at increased risk of renal abnormalities even when routine parameters are within normal values.

Hypertension is a major cause of chronic kidney disease (CKD) and both are prevalent disorders globally[31,32]. Additionally, these conditions increase the risk for

cardiovascular events and deaths[33]. Therefore, studies have sought to identify predictors for prevention of these diseases. There is evidence that the pathophysiological process starts in early life, congruent with the DOHaD theory[34,35]. Our main results are in line with the DOHaD, which holds that insults in fetal life induce programming and that LBW is a surrogate of this condition. An interesting finding of the present study was that prematurity was associated with blood pressure levels, yet LBW was not. Previous studies have reported an association of prematurity with elevated blood pressure levels[36,37]. Proposed mechanisms linking prematurity to higher blood pressure levels in adult life involve pre- and postnatal factors. Premature birth has been associated with increased vascular resistance[38,39], endothelial dysfunction[40], immature autonomic blood pressure regulation[41] and high sympathetic nervous system activity[12]. The extent of these abnormalities are dependent on the fetal adaptations and cardiovascular system programming in response to an adverse intrauterine environment during a critical period of development[42]. It is known that the full number of nephrons is achieved at between 32 and 36 weeks of gestation and that glomerular filtration starts during intrauterine life[13,43]. Therefore, prematurity may cause morphological and functional renal changes that lead to hemodynamic disturbances and hypertension[44]. Most preterm infants have accelerated postnatal growth during the first years of life[45], where this compensatory catch-up growth is commonly associated with rapid body weight gain and obesity in childhood[46]. We speculate that the overweight participants in the present study have an increased risk of hypertension later in life.

Several nutritional, toxic or emotional determinants of LBW – regardless of gestational age at delivery – can impact adult kidney function[47-49], assessed by a number of parameters such as GFR, serum creatinine, sCys C and albuminuria in clinical practice. Elevated ACR is a recognized biomarker of kidney dysfunction, even when eGFR is within normal ranges[50]. In the present study, mean values of eGFR and ACR were significantly worse in the LBW group compared to the other groups.

Furthermore, having employed the propensity score matching, a strong association of LBW was found with albuminuria, but not with eGFR or sCys C. We speculate that ACR might be a useful marker for early detection of kidney dysfunction

in adults born with LBW. The higher albuminuria seen in LBW individuals might have been due, in part, to fetal glomeruli morphological alterations and prenatal programming. This notion is supported by evidence demonstrating that LBW individuals have fewer nephrons[51], lower glomerular volume at birth[52], abnormal glomeruli structure with enlarged Bowman's capsule and altered glomerular tufts[53,54], factors that, in the long-term, could result in increased glomerular permeability and albuminuria. The present sample of LBW individuals without overt nephropathy may have had glomerular hypertrophy of remaining nephrons and hyperfiltration. The current mean eGFR of this group was within the normal range, as was albuminuria, but remained significantly higher than the group with normal birth weight. Whether ACR, periodically measured, serves as a suitable marker of early kidney dysfunction in LBW individuals requires investigation by studies with the appropriate design. This is relevant considering the synergistic impact of the nephron mass on the development of hypertension favoring CKD in later life.

We elected to measure sCys C based on previous reports suggesting its greater accuracy than creatinine for detecting decreased renal function[55-57]. SCys C determination, combined with creatinine, can be used in GFR estimation (KDIGO 2012)[25]. However, in the present sample with normal eGFR, sCys C failed to detect kidney dysfunction in LBW individuals.

This study has limitations in relation to recall bias, given that data about early life events were collected retrospectively. However, our sample was middle-aged and previous studies have shown that perinatal-related events can be accurately reported during adult life[58-60]. We also used the exposure variable (LBW or prematurity) as a categorical variable, where this may have reduced the statistical power of the analysis. This variable was chosen so as to minimize information bias from participants unable to accurately remember their birth weight. The frequency of self-reported prematurity in our sample was comparable to the rate reported in the Brazilian population at large[61]. Despite non-representative of the general Brazilian population, employees enrolled in ELSA-Brasil represent a heterogeneous sample regarding socioeconomic and ethnic-racial aspects[18]. A strength of the present study was its methodological approach. DAG was employed to avoid overadjustments[27,28] when performing the regression analysis.

However, although several covariates were incorporated into our theoretical model, including body weight at age 20, other exposures throughout the participants' life course were unavailable. Size difference of the groups with normal birthweight and LBW was also a concern; therefore, propensity score matching was applied to reduce potential selection confounders from observational studies[29,30] and sufficiently balanced groups was achieved.

In conclusion, prematurity was found to be associated with higher blood pressure levels and LBW with albuminuria in adulthood. Considering the magnitude of the ELSA-Brasil and potentialities of its cohort[19,23], our findings are important to raise awareness of early life events and to be alert to a subset of heathy adults born with LBW at increased risk for hypertension and kidney dysfunction. Long birth cohort studies could help confirm the hypothesis raised in this study.

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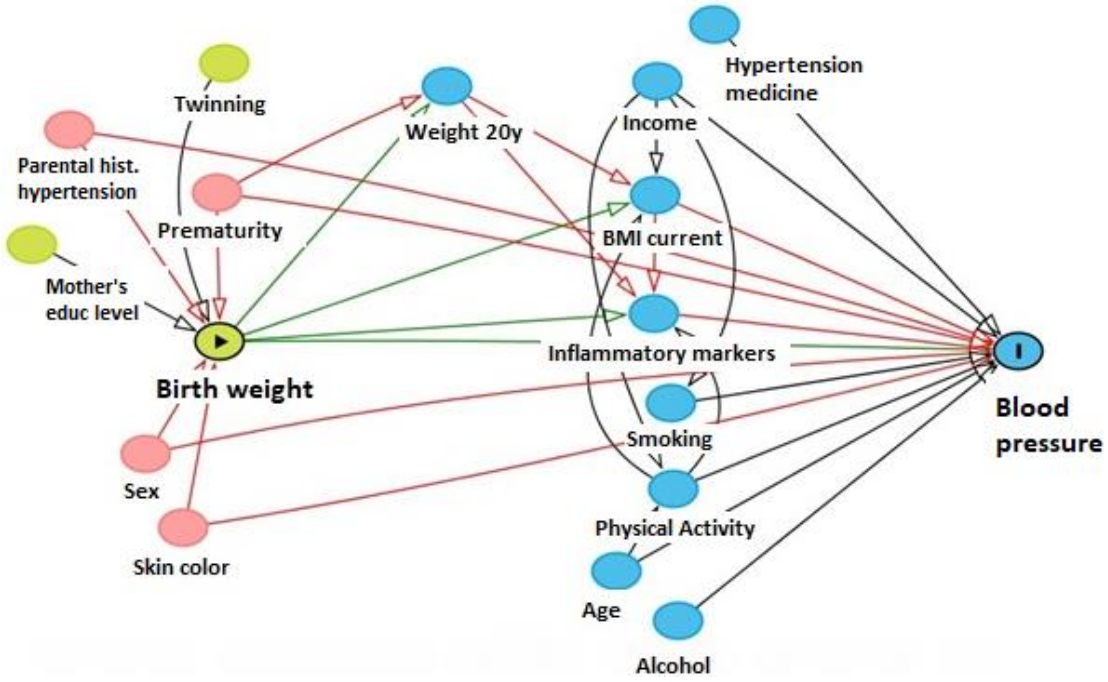
SUPPLEMENTARY MATERIAL

Figure S1. Direct acyclic graph for blood pressure (panel A) and estimated glomerular filtration rate and albumin-to-creatinine ratio (panel B).

A



B

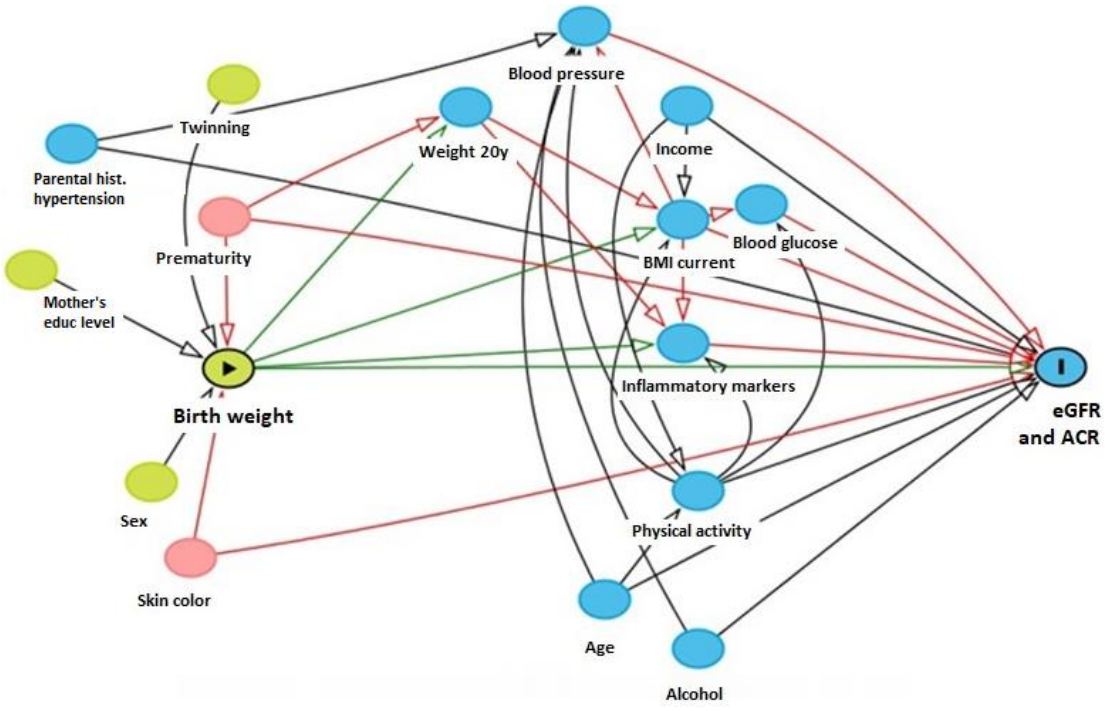


Table S1. Blood pressure levels in normal and low birth weight participants excluding those in use of antihypertensive agents (n = 66).

	Normal Birth Weight n = 647	Low Birth Weight n = 55	*P*-value
Systolic blood pressure (mmHg)	115.3 (14.3)	119.4 (14.8)	**0.031**
Diastolic blood pressure (mmHg)	73.9 (10.3)	76.6 (10.7)	**0.046**

Student´s *t*-test was used, and data expressed as mean (SD).

Table S2. Baseline characteristics of full-term and preterm born participants.

	Full-term (n = 729)	Preterm (n = 39)	*P*-value
Systolic blood pressure (mmHg)	116.3 (14.7)	122.2 (14.2)	0.015
Diastolic blood pressure (mmHg)	74.7 (10.5)	78.9 (11.0)	0.022
Creatinine (mg/dL)	0.89 (0.18)	0.87 (0.15)	0.288
eGFR (ml/min/1.73m^2)	89.6 (13.9)	90.0 (12.4)	0.842
Albumin-creatinine ratio (mg/g) *	6.4 (4.9-7.9)	7.1 (5.4-8.8)	0.149
Cystatin C (mg/L)*	0.62 (0.37-0.92)	0.52 (0.37-0.77)	0.169

eGFR, estimated glomerular filtration rate.
Student´s *t-test* or *Wilcoxon´s test was used, and data expressed as mean (SD) or median (interquartile range).

Table S3. Association of prematurity with systolic and diastolic blood pressure levels.

	β	95% CI	*P*-value
Systolic blood pressure			
Model 1	5.94	1.26 – 10.62	0.013
Model 2	6.42	1.68 – 11.15	0.008
Model 3	7.70	3.28 – 12.14	< 0.001
Diastolic blood pressure			
Model 1	4.33	0.97 – 7.68	0.012
Model 2	4.46	1.05 – 7.86	0.010
Model 3	5.25	2.01 – 8.49	0.002

Model 1: adjusted for skin color.
Model 2: adjusted for skin color and parental history of hypertension.
Model 3: adjusted for skin color, parental history of hypertension, and sex.

Table S4. Propensity score. Standardized mean differences in adjustment variables to estimate association of LBW with blood pressure and kidney function markers in ELSA-Brasil participants, 2008 - 2010.

Variables	Summary of balance		
	Crude balance	Balance for matched data	% Balance improvement
Systolic blood pressure (n = 48)			
SBP	0.332	0.002	99.38
Skin color	0.122	0.042	65.80
Parental history of hypertension	-0.083	0.000	100.0
Sex	-0.0097	0.018	99.78
Prematurity	0.788	0.000	100.0
Diastolic blood pressure (n = 61)			
DBP	0.272	-0.018	93.44
Skin color	0.122	0.042	65.80
Parental history of hypertension	-0.083	-0.045	45.44
Sex	-0.009	0.041	97.80
Prematurity	0.788	0.000	100.0
Albumin-to-creatinine ratio (n = 49)			
ACR	0.149	0.012	91.60
Skin color	-0.129	0.000	100.0
Prematurity	0.794	0.000	100.0
Estimated glomerular filtration rate (n = 44)			
eGFR	-0.240	-0.054	77.54
Skin color	0.122	0.000	100.0
Prematurity	0.794	0.000	100.0

SBP, systolic blood pressure; DBP, diastolic blood pressure; ACR, albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

4.4. **PAPER 4**

Low birth weight, β cell function and insulin resistance in adults: the Brazilian Longitudinal Study of Adult Health

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ABSTRACT

Background: Adverse intrauterine environment — reflected by low birth weight (LBW) — has been linked to insulin resistance and type 2 diabetes later in life. Whether β-cell function reduction and insulin resistance could be detected even in middle-aged adults without overt diabetes is less investigated. We examined the association of LBW with β -cell function and insulin sensitivity in non-diabetic middle-aged adults from the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil).

Methods: This is a cross-sectional analysis of 2,634 ELSA-Brasil participants aged between 34 and 59 years, without diabetes. Participants were stratified according to LBW defined as < 2.5 kg and their clinical data were compared. HOMA-IR, HOMA-β, HOMA-adiponectin, TyG index, QUICKI and TG/HDL were calculated and their association with LBW were tested using multiple linear regression including adjustments suggested by Directed Acyclic Graphs and propensity score matching was applied.

Results: The sample (47.4 ± 6.3 years) was composed of 57.5% of women and 9% had LBW. Subjects with LBW and normal weight reported similar BMI values at the age of 20 years and current BMI was slightly lower in the LBW group. In average, cardiometabolic risk profile and also indexes of β -cell function and insulin sensitivity were within normal ranges. In regression analysis, log-transformed HOMA- β — but not with the other indexes — was associated with LBW ($p = 0.014$) independent of sex, skin color, prematurity, and family history of diabetes. After applying propensity-score matching in a well-balanced sample, HOMA-AD and TG/HDL indexes were associated with LBW.

Conclusion: The association between LBW and insulin sensitivity markers may occur in healthy middle-aged adults before overt glucose metabolism disturbances. Our data are coherent with the detection of early life events consequent with insulin resistance markers that could contribute to the risk of glucose metabolism disturbances.

Keywords: low birth weight, early life events, beta cell function, insulin sensitivity, prediabetes

INTRODUCTION

Diabetes mellitus remains one of the most relevant public health concerns worldwide due to its micro and macrovascular complications (1). The etiology of type 2 diabetes mellitus (T2DM) is multifactorial, involving genetic, environmental, and lifestyle factors (2) and is commonly accompanied by excess body adiposity.

Based on the Developmental Origins of Health and Disease (DOHaD) theory, cardiometabolic disorders in adulthood might have their origins early in life stemming from intrauterine insults, namely, maternal and fetal undernutrition (3), maternal smoking, alcohol consumption or health conditions in perinatal life (4). As an adaptation to survive under adverse gestational conditions, fetal programming occurs, resulting in structural and functional changes in body organs and systems (5,6).

Low birth weight (LBW), a proxy of intrauterine adversity, has been associated with adult-onset diseases, namely, obesity, T2DM and the metabolic syndrome (7,8). It has been reported that LBW is associated with decreased b-cell mass and reduced function, resulting in a low insulin response to glucose levels (9,10). Additional underlying mechanisms have been related to evidence of decreased insulin sensitivity in the genesis of glucose metabolism disturbance, concomitant with progressive β-cell dysfunction during adulthood (11,12). Studies show that perinatal stress may affect insulin action in peripheral organs with reduced glucose uptake, and decreased expression of GLUT4 glucose transport by muscle and adipose cells (13–15). This condition becomes particularly worrisome considering the tendency of weight gain associated with our current environment and lifestyle. In this context, greater awareness of glucose metabolism abnormalities is important for early identification of risk later in adult life.

A number of studies have associated LBW with T2DM (16,17), although identifying an association of birth weight with impaired insulin sensitivity and β-cell function before the onset of diabetes, the focus of interest of the present study, would be more opportune for preventive measures.

The Brazilian Longitudinal Study of Adult Health (ELSA- Brasil) is a large cohort study of adult health in Brazil, designed to investigate risk factors associated with diabetes and cardiovascular disease (18,19). Therefore, the ELSA-Brasil represents an opportunity to investigate associations of early life events with outcomes in adulthood. The present study examined the association of LBW with parameters of β-cell function and insulin sensitivity in non-diabetic participants of the ELSA-Brasil.

METHODS

- **Study Design and Population**

A cross-sectional analysis was carried out of baseline data from the multicenter ELSA-Brasil study, whose methodological details have been reported elsewhere (18,19). The baseline assessment was conducted from August 2008 to December 2010 and included 15,105 employees aged 35 – 74 years from six Brazilian universities and research institutions. The present analysis drew on the baseline data of 5,061 participants of both sexes, aged 35 – 74 years from the São Paulo center. The study was approved by the local Ethics Committee and informed consent was obtained from all participants.

- **Eligibility Criteria**

To be eligible for the present study, participants had to be aged < 60 years (to reduce recall bias), non-diabetic and have preserved renal function. Of the 5,061 participants, the following subjects were excluded: 1,036 with diabetes (self-reported or in use of antidiabetic medications or newly diagnosed), 210 with glomerular filtration rate < 60 mL/min/1.73 m2 or macroproteinuria, and 623 aged ≥ 60 years (20). Thirty-nine participants were subsequently excluded for being underweight (BMI < 18.5 kg/m2) and 186 because they were born with macrosomia (birth weight > 4.0 kg). A further 333 participants were excluded for missing data on exposure (birth weight) or outcome (plasma glucose, insulin and lipids) variables. Therefore, a total of 2,634 participants were included in the present study (Figure 1).

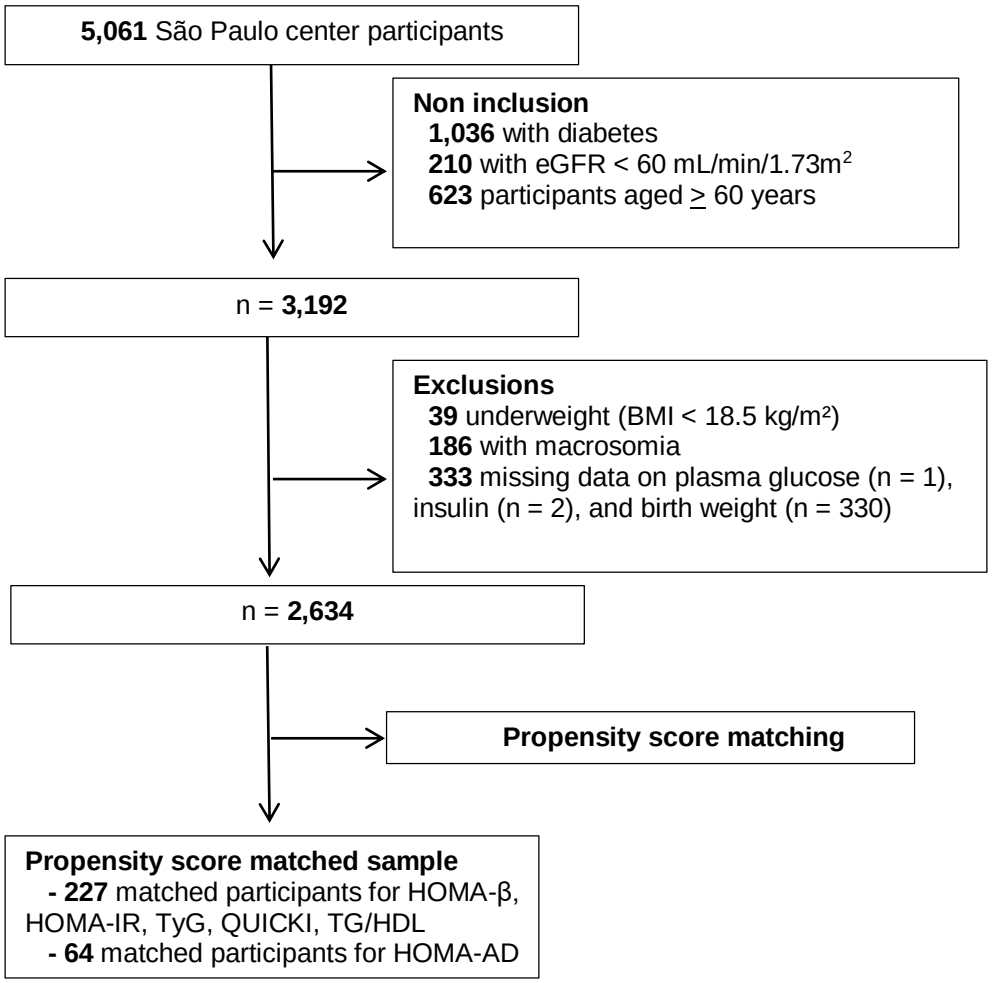

Figure 1. Flowchart of selection process of ELSA-Brasil participants in present study.

- **Clinical and Laboratory Data**

Participants were interviewed using standardized questionnaires (21). Self-reported data regarding demographics, socioeconomic status and health conditions were obtained. Variables of interest were age (years), sex (male, female), skin color (black, white, brown, yellow or indigenous, further stratified into white and non-white categories), family history of diabetes and hypertension (yes or no) and maternal educational level of participant.

Prematurity (yes or no) and birth weight (kg) were self-reported when possible. Birth weight was also categorized into “< 2.5 kg”, “2.5 – 4.0 kg”, “> 4.0 kg”

or “unknown”. All participants were also asked to provide their body weight at 20 years of age.

Weight and height were measured and body mass index (BMI) then calculated as weight in kilograms divided by height in meters squared to express nutritional status. Waist circumference was measured at the midpoint between the last rib and the iliac crest using an inelastic tape. Blood pressure was measured using an Omron HEM 705CPINT device (Omron Co, Kyoto, Japan) after a 5-minute rest in a sitting position. Three measurements were taken at 1-min intervals and mean values calculated. After overnight fasting, blood samples were collected and participants then underwent a 2-hour 75 g oral glucose tolerance test. Fasting and 2-hour plasma glucose and insulin were determined. Aliquots were frozen at −80°C for further determinations (22,23).

Plasma glucose was measured by the hexokinase method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA), and glycated hemoglobin determined by high-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, California, USA) according to the National Glycohemoglobin Standardization Program certified method. Insulin (Siemens, Tarrytown, USA) and adiponectin (Enzo Life Sciences, Farmingdale, NY, USA) were determined using enzyme-linked immunoenzymatic assays.

The HOMA- β and HOMA-IR indexes were used to assess β-cell function and insulin sensitivity, respectively, and were calculated using the equations:

HOMA- β = [20 x fasting insulin (μUI/ml)] / [fasting glucose (mmol/L) – 3.5]

HOMA-IR = [fasting insulin (μUI/ml) x fasting glucose (mmol/L)] / 22.5

Additionally, insulin sensitivity was evaluated by HOMA-adiponectin (HOMA-AD), the Triglycerides–glucose index (TyG index), QUICKI (Quantitative Insulin SensitivityCheck Index) and the Triglyceride-to-HDL-c ratio (TG/HDL-c), using the following equations: HOMA-AD = fasting glucose(mmol/L) × fasting insulin (mU/L) / 22.5 × adiponectin (μg/ml); TyG index = log [fasting triglycerides (mg/dl) × fasting

glucose (mg/dl)] / 2; QUICKI = 1/(log insulin (μUI/ml) + (log fasting glucose (mg/dl) and TG/HDL-c.

Adiponectin was measured in a sub-sample of 1,000 participants. After applying exclusion criteria, 742 participants were included with available adiponectin data.

Total cholesterol was assessed using the enzymatic colorimetric method (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA). HDL-c was determined by the homogeneous colorimetric method without precipitation, and triglycerides by the glycerophosphate peroxidase method according to the Trinder assay (ADVIA Chemistry; Siemens, Deerfield, Illinois, USA). LDL-c concentrations were calculated using the Friedewald equation.

- **Definitions for Analyses**

Birthweight (exposure variable) was classified into three categories: low birth weight (< 2.5 kg), normal birth weight (2.5 – 4.0 kg) and macrosomia (> 4.0 kg). Prematurity was defined by an affirmative answer to the question: “Were you a premature baby, in other words, were you born earlier than expected?”. Outcomes were HOMA-β, HOMA-IR, HOMA-AD, TyG index, QUICKI and TG/HDL-c, analyzed as continuous variables.

Nutritional status was classified into underweight (BMI < 18.5 kg/m2), normal weight (18.5 – 24.9 kg/m2), overweight (25.0 – 29.9 kg/m2) and obesity (> 30.0 kg/m2). Hypertension was diagnosed when systolic or diastolic blood pressure levels were ≥ 140 or 90 mmHg, respectively, or when participant was in use of antihypertensive drugs.

Diabetes was diagnosed according to the American Diabetes Association criteria (24), as follows: fasting plasma glucose ≥ 126 mg/dl or 2-hour post challenge > 200 mg/dl or glycated hemoglobin ≥ 6.5%. Prediabetes (yes or no) was defined as fasting plasma glucose between 100 and 125 mg/dl or 2-hour post challenge between 140 and 199 mg/dl or glycated hemoglobin 5.7 – 6.4%.

- **Statistical Analysis**

Distribution normality was tested for continuous variables and those with non-normal distribution (HOMA-β, HOMA-IR, HOMA-AD, QUICKI, and TG/HDL-c) were log-transformed before analysis to achieve normality. Continuous variables with a normal distribution were expressed as mean ± standard deviation (SD) and compared using Student´s t-test. Non-normally distributed variables were expressed as median and interquartile range and compared using the Wilcoxon rank test. Categorical variables were expressed as absolute and relative frequencies and compared by the chi-squared test.

Associations of exposure (LBW) and outcome (HOMA-β, HOMA-IR, HOMA-AD, TyG index, QUICKI, and TG/HDL-c) variables were initially analyzed by simple linear regression. Directed Acyclic Graphs (DAG) were used to build theoretical models and analyze independent associations of exposure with outcomes in multiple linear regression analyses. The DAG is a causal diagram which allows scientific evidence regarding the relationships among variables to be incorporated in graphics software to reach the ideal set of covariables (minimum sufficient adjustment) for the model to prevent biases and overadjustments (25,26). Figures were created by DAGitty software, version 3.0 (www.dagitty.net) included in the **Supplementary Material (Figures S1A, B)**.

Based on the DAGs, the association of LBW with HOMA-β and parameters of insulin sensitivity were adjusted for sex, skin color, family history of diabetes, and prematurity.

Considering the difference in sample size between groups with normal birth weight and LBW, and potential selection bias due to the nature of the study, propensity score matching was employed to create more comparable groups (27,28). The nearest neighbor-matching algorithm within a caliper of 0.1 SD of logit function of propensity score was used. First, for the propensity score matching, a multiple logistic regression model was used, adjusted for DAG-based covariates (sex, skin color, family history of diabetes, and prematurity), and the probability of each participant having LBW versus normal birth weight was estimated. Balance between the groups was assessed by comparing each covariate. When

standardized mean difference fell in the −0.1 to 0.1 range, groups were considered balanced. This matching reduced all covariate imbalance in the sample. The matched sample was then submitted to multiple linear regression in order to analyze associations of LBW (exposure as independent variable) with β-cell function and insulin sensitivity markers (outcomes as dependent variables) adjusted for the same DAG-based covariates. Tests were performed using “MatchIt”, “rbounds”, “Matching”, “twang” and “survey” packages in the R statistical environment. All analyses were performed using the R Project for Statistical Computing software (R version 3.5.2) and statistical significance was set at a p-value of 0.05.

RESULTS

For the study sample of 2,634 participants, mean age was 47.4 ± 6.3 years, 57.5% were women and 59.3% reported white skin color. In general, the cardiometabolic parameters of the sample were within normal ranges (systolic and diastolic blood pressures of 116.6 ± 14.9 and 74.2 ± 10.4 mmHg, respectively), except for overweight (26.8 ± 4.6 kg/m2) and prediabetic (plasma glucose of 102.0 ± 7.7 mg/dl) status.

A total of 238 (9.0%) participants reported LBW and 145 (5.5%) were born preterm. LBW participants were predominantly women (61.7%), had white skin color (52.2%), and reported low maternal educational level (62.0%).

Participants with LBW had a higher rate of low educational level compared to those reporting normal birth weight (16.1% versus 10.8%, p = 0.007). LBW and normal-weight groups reported similar BMI values at the age of 20 years and current BMI was slightly lower in the LBW group, with borderline significance (p = 0.075, **Table 1**). Mean values of waist circumference were higher in the normal birth weight than the LBW group (88.2 ± 11.6 versus 86.0 ± 11.7 cm, p = 0.008), but both values were, on average, within normal ranges. Blood pressure levels and lipid metabolism variables were similar for the two groups. No differences in β-cell secretion and insulin sensitivity indexes were found between the groups.

On multiple linear regression analyses, associations of LBW with markers of beta-cell function and insulin sensitivity were tested. LBW was associated with log-transformed HOMA-β values (p = 0.014), but not with the other indexes of insulin sensitivity (**Table 2**).

- **Propensity-Score Matching — Variable Balance**

Initially, the sample contained 238 participants with LBW. After applying the propensity-score matching, the final samples included 227 matched participants for HOMA-β, HOMA-IR, TyG, QUICKI and TG/HDL analysis and 64 matched participants for HOMA-AD analysis. The matching approach made all covariates appropriately balanced (standardized mean difference of between −0.1 and 0.1) for further analyses. Variable balance was compared before and after matching to assess the improvement of pairing (**Supplementary Table S1**).

- *Associations of LBW With β-Cell Function*

After propensity-score matching, the multiple linear regression model, adjusted for sex, skin color, family history of diabetes and prematurity, showed no association between LBW and HOMA-β (ß 0.003, 95%CI −0.038–0.045 p = 0.107) (**Table 3**).

- *Associations of LBW With Insulin Sensitivity*

The fully adjusted multiple linear regression model showed that being born with LBW was directly associated with HOMA-AD (ß 0.046, 95% CI 0.015–0.078, p = 0.005) and TG/HDL index (ß 0.021, 95% CI 0.013–0.036, p <0.001). There was no association of LBW with HOMA-IR, TyG or QUICKI (**Table 3**).

Table 1. Clinical characteristics of participants born with normal and low birth weight.

	Normal Birth Weight n = 2,582	**Low Birth Weight n = 238**	***P*-value**
Age (years)	47.4 (6.3)	47.5 (6.4)	0.657

Body mass index at age 20 (kg/m^2)	21.8 (3.4)	20.9 (3.0)	0.125
Body mass index (kg/m^2)	26.9 (4.6)	26.3 (4.5)	0.075
Waist circumference (cm)	88.2 (11.6)	86.0 (11.7)	0.008
Systolic blood pressure (mmHg)	116.5 (14.9)	117.4 (15.3)	0.417
Diastolic blood pressure (mmHg)	74.2 (10.4)	74.7 (10.6)	0.507
LDL-cholesterol (mg/dL)	129.7 (33.0)	132.5 (33.1)	0.224
HDL-cholesterol (mg/dL)	56.7 (14.4)	57.0 (13.4)	0.328
Triglycerides (mg/dL)	130.2 (91.9)	126.5 (71.3)	0.449
Fasting plasma glucose (mg/dL)	102.6 (8.4)	102.7 (7.9)	0.832
Glycated hemoglobin (%)	5.2 (0.54)	5.3 (0.57)	0.963
Fasting insulin (mg/dL)	6.0 (3.5 – 10.0)	5.9 (3.2 – 8.9)	0.181
2-h plasma glucose (mg/dL)	122.0 (24.0)	122.9 (27.5)	0.610
2-h insulinemia	43 (26.7 – 69.1)	41.2 (26.0 – 64.6)	0.434
HOMA-IR*	2.5 (1.66 – 3.5)	2.4 (1.58 – 3.4)	0.187
HOMA-β*	56.0 (32.9 – 91.9)	53.9 (30.8 – 83.8)	0.189
HOMA-AD*	0.43 (0.22 – 0.96)	0.42 (0.19 – 1.10)	0.776
TyG	2.0 (0.12)	2.1 (0.11)	0.959
QUICKI*	0.36 (0.33 – 0.39)	0.37 (0.34 – 0.40)	0.186
TG/HDL*	1.9 (1.3 – 3.2)	2.0 (1.4 – 2.9)	0.824

HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; AD, Adiponectin; TyG, Triglycerides glucose index; QUICKI, Quantitative Insulin Sensitivity Check Index; TG/HDL, Triglycerides HDL-cholesterol index, Data are expressed as mean (SD) or median (interquartile range). Student t or *Wilcoxon test was used.

Table 2. Association of low birth weight with parameters of β-cell function and insulin sensitivity.

	β	**95% CI**	***P*-value**
HOMA-β$^{\#}$			
Model 1	-0.03	-0.054 – 0.003	0.080
Model 2	-0.03	- 0.054 – 0.003	0.082

Model 3	-0.04	-0.072; - 0.008	0.014
HOMA-IR#			
Model 1	-0.02	-0.051 – 0.011	0.198
Model 2	-0.02	-0.050 – 0.012	0.233
Model 3	-0.03	-0.065 – 0.005	0.089
HOMA-AD#			
Model 1	0.000	-0.110 – 0.110	0.997
Model 2	0.006	-0.106 – 0.117	0.922
Model 3	0.027	-0.100 – 0.155	0.667
TyG			
Model 1	0.002	-0.013 – 0.017	0.754
Model 2	0.004	-0.012 – 0.019	0.636
Model 3	-0.001	-0.018 – 0.016	0.899
QUICKI#			
Model 1	0.003	-0.001 – 0.008	0.184
Model 2	0.003	-0.002 – 0.007	0.215
Model 3	0.005	-0.000 – 0.009	0.077
TG/HDL#			
Model 1	-0.001	-0.036 – 0.033	0.945
Model 2	0.002	-0.033 – 0.037	0.898
Model 3	-0.007	-0.045 – 0.032	0.725

Model 1: adjusted for sex and skin color.
Model 2: adjusted for sex, skin color and family history of diabetes.
Model 3: adjusted for sex, skin color, family history of diabetes and prematurity.
#Log-transformed values of outcomes for analyses.

Table 3. Estimates of associations of LBW with parameters of β-cell function and insulin sensitivity after propensity-score matching in ELSA-Brasil participants.

	Propensity-score pairing		
	Coefficient (ß)	**95%CI**	***P*-value**
HOMA-β#	0.003	-0.038 – 0.045	0.107
HOMA-IR#	0.0004	-0.00037 – 0.003	0.818
HOMA-AD#	0.046	0.015 – 0.078	0.005
TyG	0.009	-0.017 – 0.018	0.991
QUICKI#	0.0008	-0.0009 – 0.0092	0.986
TG/HDL#	0.021	0.013 – 0.036	< 0.001

HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; AD, Adiponectin; QUICKI, Quantitative Insulin Sensitivity Check Index; TG/HDL, Triglycerides HDL-c; CI, confidence interval
[#] Log-transformed values for analyses

DISCUSSION

We found evidence further supporting the hypothesis that LBW is associated with decreased β-cell function and with insulin resistance in middle-aged non-diabetic participants from the ELSA-Brasil (18,19). The study findings are strengthened by the facts that several indexes of insulin secretion and sensitivity were used and DAG applied for adjustments and propensity score matching analysis. An association was found of LBW with HOMA-AD and TG/HDL indexes, after adjustment and propensity-score matching. No association between low birth weight and HOMA-β was found. The results reinforced the possible role of early life events in insulin sensitivity, even with marker values within the normal range in adults born with LBW.

Considering the magnitude of T2DM as a public health concern, causing morbidity and mortality worldwide (29,30), initiatives to improve prediction and prevention are timely. The present study was prompted by evidence that population-attributable risk of T2DM is associated with increased mortality in adults born with LBW compared to those with normal birth weight (16). Additionally, LBW has been associated with hyperinsulinemia and increased risk of diabetes later in childhood (31,32). We hypothesized that these abnormalities can affect pancreatic function during the life course, justifying the assessment of β-cell secretion capacity, and peripheral insulin sensitivity before glucose metabolism disturbances emerged. In this context, our study evaluated traditional and novel indexes of β-cell function and insulin sensitivity/resistance.

HOMA-β and HOMA-IR are the most common indexes for estimating insulin secretion and resistance (33). In the present study, HOMA-AD was also calculated to assess insulin sensitivity. This index is a modified version of HOMA-β which incorporates the total serum adiponectin level in the denominator to indirectly adjust

to degree of body adiposity. Adiponectin is a protein involved in the pathophysiology of obesity and low levels tend to be observed in obese individuals with ectopic adipose tissue deposition (34). Hypoadiponectinemia has been considered an independent risk factor for the development of T2DM (35). To the best of our knowledge, the present study is the first to assess insulin sensitivity in overweight adults with LBW. HOMA-AD has been evaluated in the pediatric population, individuals with chronic kidney disease and chronic liver disease (36–38). We also calculated TG/HDL ratio, an alternative, low-cost, useful index for clinical practice. These lipid parameters are typically altered in individuals with the metabolic syndrome, in which insulin resistance is the main pathophysiological event. Both HOMA-AD and TG/HDL were associated with LBW in the well-balanced sample after applying propensity-score matching. Considering that most people are born with normal birth weight, as was the case in the present sample, this analysis was valid for improving the reliability of comparisons of subgroups stratified according to birth weight. Several studies have shown that HOMA-AD offers greater accuracy than HOMA-IR for assessing insulin resistance in overweight non-diabetic individuals (39,40). Given that diabetic individuals were excluded from the study sample, the findings regarding the HOMA-AD and TG/HDL indexes suggest their utility for early detection of insulin resistance in middle-aged adults.

In the natural history of T2DM, insulin resistance precedes the decline of β-cell function and is associated with ectopic fat deposition in the liver, muscles and pancreas (41). In turn, weight loss can prevent this condition by improving insulin sensitivity. Our results are consistent with insulin resistance preceding β-cell dysfunction in overweight adults who have not developed a glucose metabolism disturbance. LBW was initially associated with HOMA-β on multiple linear regression. However, after applying propensity-score matching in a well-balanced sample, including for the adiposity parameter (BMI), this association no longer persisted. Therefore, these results revealed an association of LBW with insulin sensitivity markers in middle-aged adults, where an association with HOMA-β can be expected in the long term if a weight loss intervention is not pursued. To our knowledge, no previous studies have reported the use of HOMA-AD and TG/HDL

indexes as early markers of insulin sensitivity in adults who still have preserved β-cell function.

Explanations for these findings are based on the reported associations of LBW and glucose metabolism dysfunction, and particularly when these infants also experience catch-up growth in childhood. These individuals are prone to developing obesity, increased visceral adiposity and insulin resistance (42,43). An elevated number of insulin receptors in their adipocytes and abnormal signaling by phosphorylation of insulin-receptor substrate 1 may result in an anti-lipolysis state (44,45). Also, it has been shown that each tertile decrease in birth weight was associated with a 1.72 times greater risk of insulin resistance in adults (46). Concordantly, our data favor the hypothesis that once insulin resistance is installed, insulin production will increase and can progress to β-cell failure over time. Other studies support the possibility that decreased β-cell function can occur without insulin resistance. This was observed in individuals with intrauterine growth restriction who had a marked reduction in number of β-cells (47–49). Another study confirmed that adults born with LBW had a 30% reduction in insulin secretion (10). Animal models involving intrauterine energy restriction showed similar results, with a reduction in β-cell mass of up to 35% (48). Despite uncertainties over the underlying mechanisms, the present results support the occurrence of early onset of insulin resistance in adults without diabetes.

Other indexes could have been useful to assess β-cell function such as the OGIS (50) and Matsuda index (51) that require several determinations of plasma glucose and insulin during glucose tolerance tests. More recently, insulin clearance was raised as an important aspect of glucose metabolism and its impairment has been related to the risk of developing T2DM (52–54). Although this method would enhance the β-cell function evaluation, measurements for its estimation were not available in the ELSA-Brasil.

The present study has limitations related to recall bias, given that retrospective data were collected regarding early life events. This bias can be reduced by using a sample of middle-aged participants, under 60 years of age. Some

studies have shown that perinatal-related events are reliably reported during adult life (55–57). We also use the exposure (LBW) as a categorical variable, possibly reducing the statistical power of the analysis. This approach was chosen to minimize information inaccuracy from participants who were unable to accurately recall their birth weight in kilograms. The use of the propensity-score method decreased the sample size, limiting the ability to find valid associations. Therefore, future studies investigating the association between LBW and HOMA-β and other indexes in larger samples are needed. A cross-sectional analysis of the ELSA- Brasil data was conducted. Further analyses of the follow-up of the sample can allow causality between LBW and the occurrence of glucose metabolism disturbances to be explored.

A strength of this study was the methodological approach employed, including the Directed Acyclic Graph method to identify confounding variables, avoiding over adjustments in the regression models constructed (25,26). Although a variety of covariates were controlled for, other exposures which occurred during the life course of participants were not included. However, we collected body weight at age 20 in an attempt to define participant body weight trajectory. Another strength was the use of propensity-score matching to reduce potential selection confounders seen in observational studies (27,28), achieving sufficiently balanced groups in the analysis. Furthermore, the frequency of self-reported LBW found in the sample was comparable to that reported in the Brazilian population at large (58).

In conclusion, LBW was found to be associated with insulin sensitivity markers in adulthood before overt glucose metabolism disturbances emerged. HOMA-AD and TG/HDL indexes appeared to be useful for detecting insulin resistance in overweight adults who had LBW. These findings are relevant in reinforcing the hypothesis that early life events affect glucose metabolism during the life course. Thus, identifying the subset of individuals at risk may be important to allow early implementation of preventive measures.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study was approved by the National Commission on Ethics Research (CONEP) and the local ethics committee, the Research Ethics Committee (CEP) under registration number 76 of the University of São Paulo (HU-USP). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Study design, analysis, interpretation and preparation of the manuscript: JB, BA-P and SF. Acquisition of data and critical revision for the manuscript content: PL and IB. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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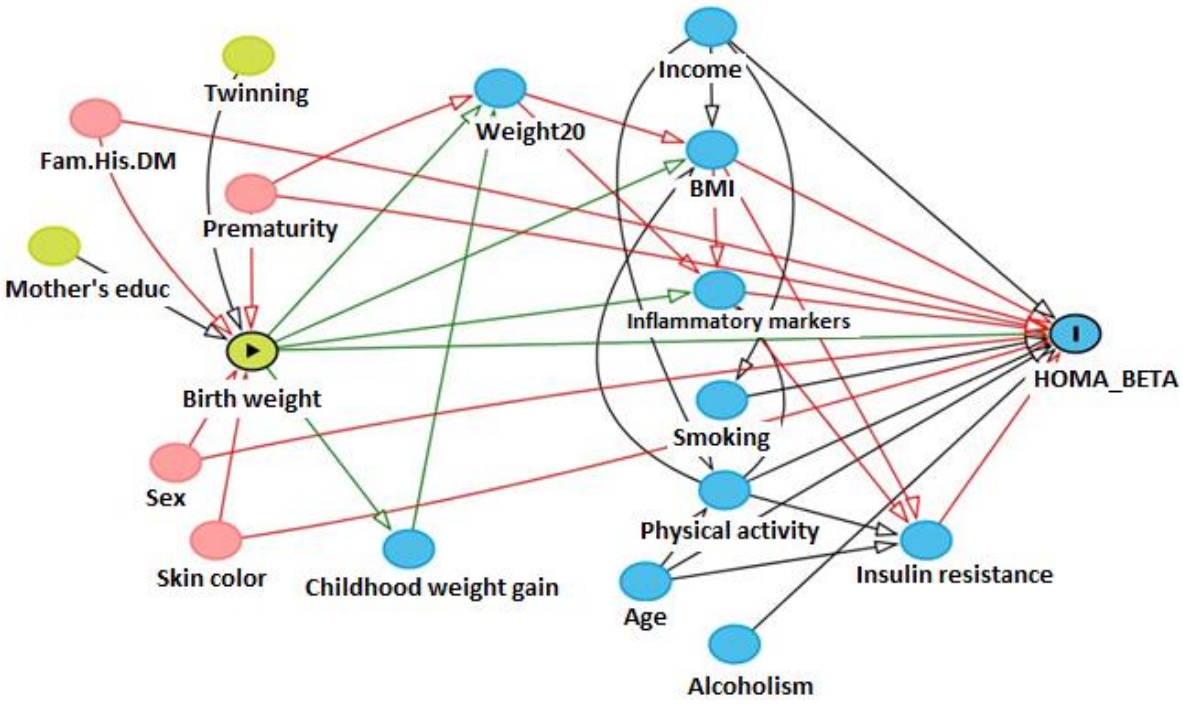
SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.842233/full#supplementary-material.

Figure S1. Directed acyclic graph for β-cell function (panel A) and insulin sensitivity (panel B).

A



B

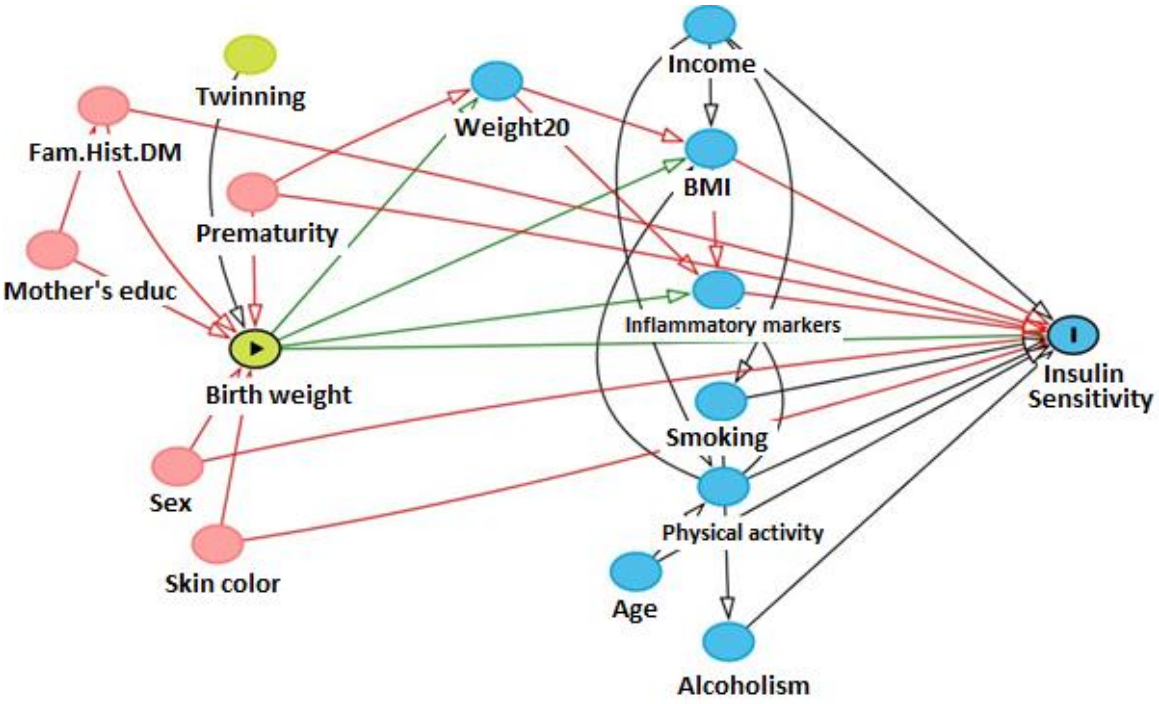


Table S1. Results of propensity-score matching: standardized mean differences of adjusted variables for association of LBW with β-cell function and insulin sensitivity markers of ELSA-Brasil participants.

Variables	Summary of balance		
	Crude	**Balance for matched data**	**% Balance improvement**
HOMA-β[#] (n = 227)			
Skin color	-0.221	-0.070	68.48
Prematurity	0.699	0.000	100.0
Sex	-0.118	-0.051	56.27
Family history of diabetes	-0.106	-0.021	80.41
HOMA-IR[#] (n = 227)			
Skin color	-0.221	-0.040	81.81
Prematurity	0.699	0.000	100.0
Sex	-0.118	-0.073	38.16
Family history of diabetes	-0.106	0.032	70.32
HOMA-AD[#] (n = 64)			

Skin color	-0.101	0.160	-58.26
Prematurity	0.757	0.000	100.0
Sex	-0.005	-0.080	-146.0
Family history of diabetes	-0.277	-0.177	36.22
TyG (n = 227)			
Skin color	-0.221	0.000	100.0
Prematurity	0.699	0.000	100.0
Sex	-0.118	-0.102	12.97
Family history of diabetes	-0.106	-0.010	90.25
QUICKI[#] (n = 227)			
Skin color	-0.221	0.040	81.99
Prematurity	0.699	0.000	100.0
Sex	-0.118	-0.010	91.25
Family history of diabetes	-0.106	-0.031	70.61
TG/HDL[#] (n = 227)			
Skin color	-0.221	0.000	100.0
Prematurity	0.699	0.000	100.0
Sex	-0.118	-0.103	12.54
Family history of diabetes	-0.106	-0.042	60.82

HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; AD, Adiponectin; QUICKI, Quantitative Insulin Sensitivity Check Index; TG/HDL, Triglycerides HDL-cholesterol index. CI, confidence interval.
[#] Log-transformed values for analyses

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5. SUMMARY AND CONCLUSIONS

The focus of the present thesis was on possible risk factors or markers that could indicate early renal and endocrine-pancreatic dysfunction, before their clinical manifestations. In Brazil, such investigation based on a large epidemiological study is uncommon. The ELSA-Brasil provided data to address this issue among others.

In the first paper, our findings showed that diabetic kidney disease could affect individuals in prediabetes stages, at a variable frequency, from 4.5% to 26.0% according to sociodemographic characteristics. The prompt identification of individuals with prediabetes is relevant to monitor their kidney function, to prevent comorbidities and expensive interventions and to preserve quality of life. Since the initial stage of kidney injury is reversible, studies are needed to investigate how renal function is affected in early stages of prediabetes, in populations with different genetic background and environmental conditions.

In paper 2, higher serum cystatin C concentrations were found in prediabetic than in normoglycemic individuals. Our findings indicated that this biomarker could be useful for examining kidney function in early stages of dysglycemia even when albuminuria is still normal range in individuals with elevated eGFR. Considering the relevance of the incidence and prevalence of diabetic kidney disease for clinical practice as well as for public health system, serum cystatin C measurement could be recommended in prediabetic hyperfiltrating individuals to follow their kidney function in the long term.

In our third paper, we tested the association of LBW with blood pressure levels and kidney function. LBW was associated with blood pressure, but this association did not persist when prematurity was added to the model. Prematurity *per se* showed to be independently associated with higher blood pressure levels. As far as kidney function is concerned, LBW was associated with albuminuria in adulthood after optimizing the analyses with the propensity score-matching test. Whether there was causal relationship between these early-life events requires investigations in long birth cohort studies.

In paper 4, we proposed to evaluate associations of LBW with β-cell function and insulin sensitivity markers. LBW was independently associated with some insulin sensitivity markers (HOMA-AD and TG/HDL) in adulthood after applying the propensity score-matching. These findings were seen in individuals with increased BMI who did not present glucose metabolism disturbance. This reinforces that insulin resistance should be an initial mechanism in the natural history of type 2 DM. LBW appears to be a risk factor for altered glucose metabolism in adulthood. Therefore, facing the identification of individuals born with low weight, preventive measures should be considered.

With the present thesis, we conclude that early risk factors can be present in apparently healthy individuals. Attention should be directed to those reporting LBW who can be at increased risk for certain diseases, considering that adverse intrauterine conditions can compromised functional reserve. The present study brings innovative knowledge based on the DOHaD theory, showing a potential usefulness of renal and endocrine-pancreatic biomarkers investigated in ELSA-Brasil participants. Follow-up is needed to examine a causal relationship. Meanwhile, we reinforce the importance of early intervention measures in pre-diabetic individuals and the attention to those born with low weight regarding the risk of NCCD.

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7. APPENDIX

7.1. **Attachment 1: Consent form of ELSA-Brasil**

ID NUMERO:							

Código Formulário: TCL
Versão: 10/06/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

Termo de Consentimento Livre e Esclarecido (TCLE)

a. Declarou que compreendeu as informações apresentadas no TCLE e deu consentimento para participação no estudo

[ ] Não

[ ] Sim

b. Declarou concordar que amostras de sangue sejam armazenadas para análises futuras sobre as doenças crônicas em estudo.

[ ] Não

[ ] Sim

7.2. **Attachment 2: Baseline questionnaire of the ELSA-Brasil**

ID NUMERO:							

Código Formulário: HMP
Versão: 10/06/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

Informações Administrativas:

0a. Data da entrevista: ☐☐/☐☐/☐☐☐☐ **0b. Nº Entrevistador(a):** ☐☐☐

HISTÓRIA MÉDICA PREGRESSA (HMP)

Para começar, faremos algumas perguntas sobre seu estado de saúde e alguns problemas de saúde que o(a) senhor(a) teve ou tenha.

01. De um modo geral, em comparação a pessoas da sua idade, como o(a) senhor(a) considera o seu estado de saúde? (LEIA AS ALTERNATIVAS)
[ ] Muito bom [ ] Bom [ ] Regular [ ] Ruim [ ] Muito ruim [ ] NÃO SABE/ NÃO QUER RESPONDER

Alguma vez um <u>médico</u> lhe informou que o (a) senhor(a) teve ou tem alguma das seguintes doenças?

02. Hipertensão (pressão alta)? (LEIA AS ALTERNATIVAS SE PARTICIPANTE FOR MULHER)	
[ ] Não [ ] NÃO SABE/ NÃO QUER RESPONDER	**(PULE PARA A QUESTÃO 04)**
[ ] Sim, somente durante a gravidez **(PULE PARA A QUESTÃO 04)**	
[ ] Sim ----------→	03. Com que idade um médico lhe informou, pela primeira vez, que o(a) senhor(a) teve ou tem <u>hipertensão</u> (pressão alta)?
	|__|__| anos de idade [ ] NÃO SABE/ NÃO QUER RESPONDER

ID NUMERO:							

Código Formulário: HMP
Versão: 10/06/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

04. Diabetes? (LEIA AS ALTERNATIVAS SE PARTICIPANTE FOR MULHER)	
[ ] Não [ ] NÃO SABE/ NÃO QUER RESPONDER	**(PULE PARA A QUESTÃO 08)**
[ ] Sim, somente durante a gravidez **(PULE PARA A QUESTÃO 08)**	
[ ] Sim ----------→	05. Com que idade um médico lhe informou, pela primeira vez, que o(a) senhor(a) teve ou tem diabetes?
	|___|___| anos de idade [ ] NÃO SABE/ NÃO QUER RESPONDER
	06. O(a) senhor(a) faz uso de insulina?
	[ ] Não [ ] NÃO SABE/ NÃO QUER RESPONDER — **(PULE PARA A QUESTÃO 08)**
	[ ] Sim
	07. A insulina foi o primeiro medicamento usado para tratar seu diabetes?
	[ ] Não [ ] NÃO SABE/ NÃO QUER RESPONDER [ ] Sim
08. Colesterol alto (gordura no sangue)?	
[ ] Não [ ] NÃO SABE/ NÃO QUER RESPONDER	**(PULE PARA A QUESTÃO 10)**
[ ] Sim ----------→	09. Com que idade um médico lhe informou, pela primeira vez, que o(a) senhor(a) teve ou tem colesterol alto (gordura no sangue)?
	|___|___| anos de idade [ ] NÃO SABE/ NÃO QUER RESPONDER

ID NUMERO:							

Código Formulário: PSE
Versão: 10/06/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

Informações Administrativas:

0a. Data da entrevista: □□/□□/□□□□ **0b. Nº Entrevistador(a):** □□□

POSIÇÃO SÓCIO-ECONÔMICA (PSE)

As próximas perguntas se referem à sua história pessoal ou suas condições de vida.

<table>
<tr><td colspan="2">01. Qual seu grau de instrução?</td></tr>
<tr><td>[ ] Nunca freqüentou escola</td><td>(PULE PARA A QUESTÃO 04)</td></tr>
<tr><td rowspan="2">[ ] 1º grau incompleto ---------→</td><td>02. Qual a última série cursada com aprovação?</td></tr>
<tr><td>[ ] 1ª série [ ] 5ª série
[ ] 2ª série [ ] 6ª série
[ ] 3ª série [ ] 7ª série
[ ] 4ª série
(PULE PARA A QUESTÃO 04)</td></tr>
<tr><td>[ ] 1º grau completo
[ ] 2º grau incompleto</td><td>(PULE PARA A QUESTÃO 04)</td></tr>
<tr><td colspan="2">[ ] 2º grau completo
[ ] Universitário incompleto
[ ] Universitário completo
[ ] Pós-graduação</td></tr>
<tr><td colspan="2">03. Com que idade o(a) senhor(a) terminou o 2º grau?</td></tr>
<tr><td colspan="2">|___|___| anos de idade Não aceitar número < 14
[ ] NÃO SABE/ NÃO QUER RESPONDER</td></tr>
</table>

ID NUMERO:							

Código Formulário: PSE
Versão: 10/06/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

04. Qual é o grau de instrução de sua mãe?	
[ ] Nunca freqüentou escola [ ] 1º grau incompleto [ ] 1º grau completo [ ] 2º grau incompleto [ ] 2º grau completo [ ] Universitário incompleto [ ] Universitário completo [ ] Pós-graduação [ ] NÃO SABE INFORMAR	
05. Em sua opinião qual é sua cor ou raça?	
[ ] NÃO SABE/ NÃO QUER RESPONDER	
06. Em sua casa trabalha algum empregado ou empregada doméstica mensalista?	
[ ] Não [ ] NÃO SABE/ NÃO QUER RESPONDER	**(PULE PARA A QUESTÃO 08)**
[ ] Sim ----------→	07. Quanto(a)s? |___|___| empregado(s) ou empregadas domésticas mensalistas [ ] NÃO SABE/ NÃO QUER RESPONDER

ID NUMERO:							

Código Formulário: PCO
Versão: 09/07/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

Informações Administrativas:

0a. Data da entrevista: ☐☐/☐☐/☐☐☐☐ **0b. Nº Entrevistador(a): ☐☐☐**

PESO CORPORAL (PCO)

O peso corporal influencia vários aspectos da saúde. Gostaríamos de saber um pouco sobre sua história de peso.

Vamos começar pelo tempo em que o(a) Sr(a) era criança.

01. O(a) Sr(a) foi um bebê prematuro, isso é, nasceu antes do previsto?	
[ ] Não	
[ ] Sim	
[ ] NÃO SABE INFORMAR	
02. De acordo com a informação que o(a) Sr(a) tem, qual foi o seu peso ao nascer? LEIA AS ALTERNATIVAS	
[ ] Abaixo de 2,5 kg	
[ ] Entre 2,5 kg e 4 kg	
[ ] Acima de 4 kg	
[ ] NÃO SABE INFORMAR -----→ **PULE PARA A QUESTÃO 04**	
03. O(a) Sr(a) sabe informar de maneira mais precisa qual era o seu peso ao nascer?	
|__|,|__|__|__| kg ---------→	**PULE PARA A QUESTÃO 05; ANTES DA PERGUNTA LEIA O CABEÇALHO**
[ ] NÃO SABE INFORMAR	
04. O(a) Sr(a) tem como obter essa informação?	
[ ] NÃO SABE INFORMAR	
[ ] Não	
[ ] Sim ----------→	**REGISTRE A FORMA COMBINADA PARA OBTER ESSA INFORMAÇÃO NO DIÁRIO DE CAMPO**

ID NUMERO:							

Código Formulário: PCO
Versão: 09/07/2009

Estudo Longitudinal de Saúde do Adulto ELSA BRASIL

Agora vou lhe mostrar uma série de figuras. **Entrevistador(a):** APRESENTE OS 15 CARTÕES (PCO) CONFORME O MANUAL
05. Qual a figura que melhor representa o seu corpo hoje?
Figura |___|___| [ ] NÃO SABE/NÃO QUER RESPONDER
06. Qual a figura que melhor representa o corpo que gostaria de ter?
Figura |___|___| [ ] NÃO SABE/NÃO QUER RESPONDER **Entrevistador(a)**: RECOLHA OS 15 CARTÕES (PCO) CONFORME O MANUAL
07. Aproximadamente, quanto o(a) Sr(a) pesava aos 20 anos de idade [excluindo períodos de gravidez, no caso das mulheres]?
|___|___|___| kg [ ] NÃO SABE INFORMAR

7.3. **Attachment 3:** Meeting presentations

79th Scientific Sessions American Diabetes Association 2019

Abstract published at Diabetes 2019; 68(Supplement_1):509-P

Published online June 01, 2019: https://doi.org/10.2337/db19-509-P

509-P: Utility of Serum Cystatin C in Early Kidney Dysfunction in Prediabetics

JULIA I.F. BRANDA[1,3]; BIANCA ALMEIDA[2,3]; SANDRA R.G. VIVOLO[1,3]

[1]Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil, [2]Department of Preventive Medicine, Federal University of São Paulo, São Paulo, Brazil; [3]Center of Clinical and Epidemiological Research at University of São Paulo, São Paulo, Brazil

Background: Serum cystatin C (sCys C) has been proposed as a marker of kidney function with advantages compared to creatinine. It is unclear whether renal damage begin in prediabetes (PDM), when sCys C could be a promisor marker of early kidney dysfunction. We investigated whether sCys C differed between normoglycemic (NG) and PDM subjects and contributed to the detection of kidney dysfunction.

Methods: A cross-sectional analysis of 947 nondiabetic participants (mean age 45.7 years) from the Brazilian Longitudinal Study of Adult Health was performed. sCys C (determined by ELISA) and CKD-EPI eGFR were compared between NG and PDM (fasting plasma glucose: 100-125 mg/dL or 2-hour post-load: 140-199 mg/dL or A1c: 5.7-6.4%). PDM were stratified in 4 groups according to altered sCys C and albumin excretion rate (AER): normal sCys C and AER (G1), abnormal sCys C and normal AER (G2), normal sCys C and abnormal AER (G3) and abnormal sCys C and AER (G4) were compared using ANOVA.

Results: PDM subjects (n = 671) had anthropometric and biochemical data higher than NG (n=276), higher sCys C levels [0.67 (0.41-0.95) vs. 0.48 (0.31-0.81), p<0.001] and lower eGFR (96.3±17.4 vs. 100.6±17.1 mL/min/1.73m^2, p<0.001). In eGFR categories (60-<90, 90-<125 and ≥125 mL/min/1.73m^2), sCys C were always higher in PDM subjects than in NG. Comparing NG individuals, hyperfiltrants had lower sCys C than normofiltrants (p=0.03) but not than mildly reduced eGFR individuals (p=0.12). Considering the PDM groups, eGFR gradually dropped from G1 to G4 (96.8±17.4 vs.

96.2±16.9 vs. 94.0±17.2 vs. 77.2±25.4 mL/min/1.73m^2, p-trend = 0.06) and, as expected, mean eGFR in G4 was lower than in G1 (p=0.017).

Conclusion: Our data confirmed that sCys C is increased in PDM. Reduced sCys C only in NG hyperfiltrant subjects could suggest an inability of hyperfiltrant PDM to excrete Cys C. No statistical evidence that sCys C could be an early marker of kidney dysfunction in prediabetes was found; prospective studies should address its utility to detect dysfunction before AER elevation.

Disclosures: J.I.F. Branda: None. **B. Almeida:** None. **S.R.G. Vivolo:** None.

80th Scientific Sessions American Diabetes Association 2020

Abstract published at Diabetes 2020; 69(Supplement_1):1474-P

Published online June 01, 2020: https://doi.org/10.2337/db20-1474-P

474-P: Association between Low Birth Weight and ß-Cell Function in Adults without Diabetes from the Brazilian Longitudinal Study of Adult Health

JULIA I. BRANDA[1,4]; BIANCA ALMEIDA PITITTO[2,4]; ISABELA M. BENSENOR[3,4]; PAULO A. LOTUFO[3,4]; SANDRA ROBERTA GOUVEA F.G. VIVOLO[1,4]

[1]Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil, [2]Department of Preventive Medicine, Federal University of São Paulo, São Paulo, Brazil; [3]Center of Clinical and Epidemiological Research at University of São Paulo, São Paulo, Brazil; [4]Center of Clinical and Epidemiological Research at University of São Paulo, São Paulo, Brazil

Based on the fetal origins of diseases hypothesis, adverse intrauterine environment - reflected by low birth weight (LBW) - has been linked to insulin resistance and type 2 diabetes later in life. Whether β cell function reduction could be detected even in middle-aged adults is less investigated. We examined the association of LBW with β cell function and insulin sensitivity. This is a cross-sectional analysis of 2634 ELSA-Brasil participants aged between 34 and 59 years. Exclusion criteria were diabetes, glomerular filtration rate <60 ml/min/m2, thyroid dysfunction, liver disease, BMI <18.5 kg/m2 and macrosomia. Participants were stratified according to LBW defined as <2.5 kg and their clinical data were compared. HOMA-IR, HOMA-β, HOMA-adiponectin, TyG index and QUICKI were calculated. Associations of LBW with β cell function and insulin sensitivity indexes were tested using multiple linear regression including

adjustments suggested by Directed Acyclic Graphs. The sample (47.0±6.3 years) was composed of 58% of women and 9% had LBW. Subjects with LBW had lower mean body weight at age 20 but similar current BMI than the normal weight born ones. In average, cardiometabolic risk profile as well as indexes of β cell function and insulin sensitivity were within normal ranges. Lower median HOMA-β values in the subset with LBW did not differ statistically from normal birth weight ones. In regression analysis, log-transformed HOMA-β - but not with the other indexes - was associated with LBW (p=0.016) independent of sex, skin color, prematurity and family history of diabetes. The association between HOMA-β and LBW suggests that decrease in insulin secretion may occur in healthy middle-aged adults. Our data are coherent with a relatively reduced β cell mass in low weight born subjects that could contribute to the risk of glucose metabolism disturbances.

Disclosures: J.I. Branda: none. B. Almeida Pititto: none. I.M. Bensenor: none. P.A. Lotufo: none. S.F.G. Vivolo: none.

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo - FAPESP; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES); CNPq - National Research Council

7.4. **Attachment 4: Oral presentation at the Latin American DOHaD Congress 2020 – Honorable Mention Award**

Oral Presentation Certificate

4th Meeting of DOHaD Latin American Chapter
4th International Symposium of DOHaD and Stress
2nd Colloquium Prof. Cesar Timo-Iaria

October 13 – 16 • 2020 • On the Web

We certify that **JULIA INES FLEITAS BRANDA** presented the study entitled "Low birth weight or prematurity associated with reduced kidney function in adults from the brazilian longitudinal study of adult health – ELSA-Brasil" at the virtual congress 4th Meeting of DOHaD Latin American Chapter 4th International Symposium of DOHaD and Stress 2nd Colloquium Prof. Cesar Timo-Iaria, from 13th – 16th October 2020.

Authors: Julia Ines F. Branda, Bianca de Almeida-Pititto, Isabela Bensenor, Paulo A. Lotufo, Sandra Roberta G. Ferreira

Prof. Egberto Gaspar de Moura, PhD
Universidade do Estado do Rio de Janeiro – Brazil
President of the Congress

Prof. Paulo Cezar de Freitas Mathias, PhD
Universidade Estadual de Maringá – Brazil
President of Latin American DOHaD Chapter

Cesar Victora Award

4th Meeting of DOHaD Latin American Chapter
4th International Symposium of DOHaD and Stress
2nd Colloquium Prof. Cesar Timo-Iaria

October 13 – 16 • 2020 • On the Web

We certify that **JULIA INES FLEITAS BRANDA** was awarded with **honorable mention for oral presentation** titled "Low birth weight or prematurity associated with reduced kidney function in adults from the brazilian longitudinal study of adult health – ELSA-Brasil" at the virtual congress 4th Meeting of DOHaD Latin American Chapter 4th International Symposium of DOHaD and Stress 2nd Colloquium Prof. Cesar Timo-Iaria, from 13th – 16th October 2020.

Authors: Julia Ines F. Branda, Bianca de Almeida-Pititto, Isabela Bensenor, Paulo A. Lotufo, Sandra Roberta G. Ferreira

Prof. Egberto Gaspar de Moura, PhD
Universidade do Estado do Rio de Janeiro – Brazil
President of the Congress

Prof. Paulo Cezar de Freitas Mathias, PhD
Universidade Estadual de Maringá – Brazil
President of Latin American DOHaD Chapter

7.5. **Attachment 5: Paper 1 published. Available at: https://www.sciencedirect.com/science/article/abs/pii/S2451847619300259?via%3Dihub**

ELSEVIER

Obesity Medicine
Volume 15, September 2019, 100105

Review

Prevalence of diabetic kidney disease in prediabetes

Julia Ines F Branda [a], Bianca de Almeida-Pititto [b], Sandra Roberta G. Ferreira [c]

[a] School of Public Health, University of São Paulo, Brazil
[b] Department of Preventive Medicine, Federal University of São Paulo, Brazil
[c] Department of Epidemiology, School of Public Health, University of São Paulo, Brazil

Received 19 March 2019, Revised 18 May 2019, Accepted 22 May 2019, Available online 25 May 2019, Version of Record 3 June 2019.

7.6. **Attachment 6: Paper 2 published. Available at: https://www.ecronicon.com/ecdmr/ECDMR-04-00071.php**

ECRONICON OPEN ACCESS

EC DIABETES AND METABOLIC RESEARCH
Research Article

Serum Cystatin C in Early Kidney Dysfunction in Prediabetic Participants of the Brazilian Longitudinal Study of Adult Health - ELSA-Brasil

Julia Ines F Branda[1,4], Bianca de Almeida-Pititto[2,4], Isabela Bensenor[3,4], Paulo A Lotufo[3,4] and Sandra Roberta G Ferreira[1,4]*

[1]Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil
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[3]Department of Internal Medicine, Medical School, University of São Paulo, Brazil
[4]Center of Clinical and Epidemiological Research at University of São Paulo, São Paulo, Brazil

***Corresponding Author:** Sandra Roberta G Ferreira, Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil.

Received: January 23, 2020; **Published:** February 08, 2020

7.7. **Attachment 7: Paper 3 submitted to BMC Nephrology Journal**

Your submissions

Track your submissions

Associations of prematurity and low birth weight with blood pressure and kidney function in middle-aged participants of the Brazilian Longitudinal Study of Adult Health – ELSA-Brasil

9 Reviewer(s) invited

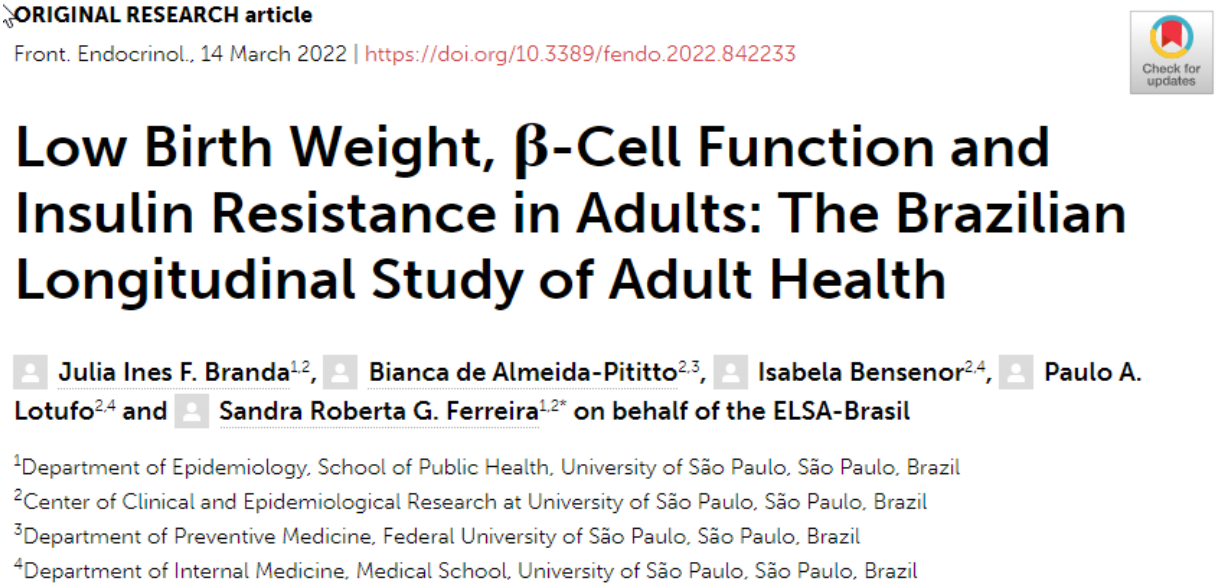
Corresponding Author: Sandra Roberta G. Ferreira

BMC Nephrology

69553832-edc9-4ee9-908f-bdfd1cfa6a63 | v.1.1

7.8. **Attachment 8: Paper 4 published. Available at:**

https://www.frontiersin.org/articles/10.3389/fendo.2022.842233/full



ORIGINAL RESEARCH article

Front. Endocrinol., 14 March 2022 | https://doi.org/10.3389/fendo.2022.842233

Check for updates

Low Birth Weight, β-Cell Function and Insulin Resistance in Adults: The Brazilian Longitudinal Study of Adult Health

Julia Ines F. Branda[1,2], Bianca de Almeida-Pititto[2,3], Isabela Bensenor[2,4], Paulo A. Lotufo[2,4] and Sandra Roberta G. Ferreira[1,2*] on behalf of the ELSA-Brasil

[1]Department of Epidemiology, School of Public Health, University of São Paulo, São Paulo, Brazil
[2]Center of Clinical and Epidemiological Research at University of São Paulo, São Paulo, Brazil
[3]Department of Preventive Medicine, Federal University of São Paulo, São Paulo, Brazil
[4]Department of Internal Medicine, Medical School, University of São Paulo, São Paulo, Brazil

7.9. **Attachment 9: University of São Paulo records of the PhD candidate (Janus System)**

NUSP: 10715483 Nome: Julia Ines Fleitas Branda

Ficha do Aluno

	Curso	Área	Nº Sequencial	Situação	Visualizar
	Doutorado Direto	Nutrição em Saúde Pública (6138)	1	Transferido de Área	
➔	Doutorado Direto	Epidemiologia (6141)	1	Matrícula de Acompanhamento	

Preparar para imprimir

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Saúde Pública
FICHA DO ALUNO

6141 - 10715483/1 - Julia Ines Fleitas Branda

Email:	julia_ines_f@hotmail.com (favor indicar um email usp.br o mais rápido possível)
Data de Nascimento:	21/01/1984
Cédula de Identidade:	RNE - V411070-P - SP
Local de Nascimento:	Paraguai
Nacionalidade:	Paraguaia
Graduação:	Médica - Universidade Federal do Triângulo Mineiro - Minas Gerais - Brasil - 2010

Curso:	Doutorado Direto
Programa:	Epidemiologia
Data de Matrícula:	22/01/2018
Início da Contagem de Prazo:	22/01/2018
Data Limite para o Depósito:	22/11/2022
Co-orientador:	Prof(a). Dr(a). Bianca de Almeida Pititto - 10/10/2019 até o presente. Email: bapititto@unifesp.br
Orientador:	Prof(a). Dr(a). Sandra Roberta Gouvea Ferreira Vivolo - 10/10/2019 até o presente. Email: sandrafv@usp.br
Proficiência em Línguas:	Inglês, 22/01/2018
Data de Aprovação no Exame de Qualificação:	Aprovado em 29/05/2019
Data do Depósito do Trabalho:	
Título do Trabalho:	
Data Máxima para Aprovação da Banca:	
Data de Aprovação da Banca:	
Data Máxima para Defesa:	
Data da Defesa:	
Resultado da Defesa:	
Histórico de Ocorrências:	Primeira Matrícula em 22/01/2018

Aluno matriculado no Regimento da Pós-Graduação USP (Resolução nº 7493 em vigor a partir de 29/03/2018).
Última ocorrência: Matrícula de Acompanhamento em 21/03/2022
Impresso em: 20/05/2022 12:15:34

Janus - Sistema Administrativo da Pós-Graduação

Janus - Sistema Administrativo da Pós-Graduação

Universidade de São Paulo
Faculdade de Saúde Pública
FICHA DO ALUNO

6141 - 10715483/1 - Julia Ines Fleitas Branda

Sigla	Nome da Disciplina	Início	Término	Carga Horária	Cred.	Freq.	Conc.	Exc.	Situação
PSP5121-1/5	Bioestatística	20/02/2018	01/05/2018	90	6	100	A	N	Concluída
HNT5758-3/1	Síndrome Metabólica: Fisiopatologia, Epidemiologia e Controle	03/04/2018	08/05/2018	30	2	100	A	N	Concluída
PSP5118-1/3	Delineamento e Introdução a Análise Epidemiológica	15/05/2018	26/06/2018	60	4	100	A	N	Concluída
EPI5713-2/1	Introdução ao R para a Análise de Dados	04/06/2018	09/07/2018	30	2	100	A	N	Concluída
MCM5909-2/4	Bioestatística II: Aplicações na Clínica Médica usando R (Faculdade de Medicina - Universidade de São Paulo)	21/08/2018	15/10/2018	120	8	100	A	N	Concluída
HNT5772-1/2	Estudos Epidemiológicos Multicêntricos em Doenças Crônicas não Transmissíveis relacionada à Nutrição e Estilo de Vida	27/08/2018	02/09/2018	30	2	100	A	N	Concluída
PSP5515-1/3	Aspectos Pedagógicos do Ensino Superior em Saúde	14/09/2018	15/11/2018	45	3	100	A	N	Concluída
MCM5893-2/5	Endocrinologia Aplicada I (Faculdade de Medicina - Universidade de São Paulo)	07/03/2019	15/05/2019	120	8	100	A	N	Concluída
FBA5897-3/3	Nutrigenômica do Câncer (Faculdade de Ciências Farmacêuticas - Universidade de São Paulo)	25/03/2019	31/03/2019	30	2	100	A	N	Concluída
PSP5103-1/4	Modelos de Regressão Aplicados em Epidemiologia I	06/08/2019	09/09/2019	60	4	100	A	N	Concluída
MPR5729-7/1	Análise de Estudos Epidemiológicos I (Faculdade de Medicina - Universidade de São Paulo)	02/09/2019	13/10/2019	90	6	90	A	N	Concluída
PSP5104-1/4	Modelos de Regressão Aplicados em Epidemiologia II	10/09/2019	21/10/2019	60	4	100	B	N	Concluída
PSP5105-1/4	Modelos de Regressão Aplicados em Epidemiologia III	22/10/2019	25/11/2019	60	4	100	B	N	Concluída
HNT5768-3/2	Modelos Lineares Generalizados	18/11/2019	24/11/2019	30	0	-	-	N	Matrícula cancelada

	Créditos mínimos exigidos		Créditos obtidos
	Para exame de qualificação	Para depósito de tese	
Disciplinas:	0	40	55
Estágios:			
Total:	0	40	55

Créditos Atribuídos à Tese: 152

Conceito a partir de 02/01/1997:
A - Excelente, com direito a crédito; B - Bom, com direito a crédito; C - Regular, com direito a crédito; R - Reprovado; T - Transferência.
Um(1) crédito equivale a 15 horas de atividade programada.

7.10. **Attachment 10**: Curriculum Lattes of the PhD candidate

Julia Inés Fleitas Branda

Endereço para acessar este CV: http://lattes.cnpq.br/6925304754714179

ID Lattes: **6925304754714179**

Última atualização do currículo em 24/02/2021

Possui graduação em Medicina pela Universidade Federal do Triângulo Mineiro (2010). Especialização em Clínica Médica pelo Hospital Beneficência Portuguesa de São Paulo (2014). Título de Especialista em Clínica Médica pela Sociedade Brasileira de Clínica Médica (2015). Especialização em Endocrinologia e Metabologia pelo Hospital Ipiranga (2016). Título de Especialista em Endocrinologia e Metabologia pela Sociedade Brasileira de Endocrinologia e Metabologia (2016). Doutoranda na Faculdade de Saúde Pública da USP com o projeto de pesquisa "Associação de baixo peso ao nascer com pressão arterial sistêmica e com biomarcadores de função pancreática e renal em adultos de idade média, sem diabetes e nefropatia". **(Texto informado pelo autor)**

Identificação

Nome Julia Inés Fleitas Branda

Nome em citações bibliográficas BRANDA, J. I. F.;FLEITAS, J.I.;F BRANDA, JULIA INES;BRANDA, JULIA I.F.;BRANDA, JULIA I;BRANDA, JULIA I.

Lattes iD http://lattes.cnpq.br/6925304754714179

7.11. **Attachment 11**: Curriculum Lattes of the counselor

Sandra Roberta Gouvea Ferreira Vivolo

Bolsista de Produtividade em Pesquisa do CNPq - Nível 1B

Endereço para acessar este CV: http://lattes.cnpq.br/6633883139386818

ID Lattes: **6633883139386818**

Última atualização do currículo em 25/03/2022

Possui graduação em Medicina pela Pontifícia Universidade Católica de Campinas (1981), mestrado em Medicina (Endocrinologia Clínica) pela Universidade Federal de São Paulo (1986) e doutorado em Medicina (Endocrinologia Clínica) pela Universidade Federal de São Paulo (1988). É professora titular na Faculdade de Saúde Pública da Universidade de São Paulo, onde ocupou cargos de chefia de departamentos e presidente de Comissão de Pós-graduação. Tem bolsa de produtividade em pesquisa do CNPq, nível 1-B. Tem experiência na área de Medicina, com ênfase em Epidemiologia e Nutrição, atuando principalmente nos seguintes temas: diabetes mellitus, síndrome metabólica, hipertensão arterial, obesidade, nutrição e microbiota intestinal. **(Texto informado pelo autor)**

Identificação

Nome Sandra Roberta Gouvea Ferreira Vivolo

Nome em citações bibliográficas FERREIRA, S. R. G.;Ferreira, Sandra R.;Ferreira, Sandra;Ferreira, Sandra R.G.;Ferreira, Sandra Roberta G.;Ferreira Vivolo, SRG;FERREIRA, Sandra Roberta Gouveia;FERREIRA, Sandra Roberta Gouvea;FERREIRA, Sandra R G;Ferreira, S.R.G.;FERREIRA, SANDRA R;FERREIRA, SANDRA R. G.;FERREIRA, SANDRA RG;VÍVOLO, SANDRA ROBERTA GOUVEA FERREIRA;Sandra Roberta Gouvea Ferreira;Sandra R G Vivolo;VIVOLO, SANDRA R. G.;VIVOLO, SANDRA R.G.;VIVOLO, SANDRA ROBERTA GOUVEA FERREIRA

Lattes iD http://lattes.cnpq.br/6633883139386818

Orcid iD ? https://orcid.org/0000-0002-7015-7391

Endereço

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Ramal: 218
Fax: (11) 30616601

7.12. **Attachment 12**: Curriculum Lattes of the co-advisor

Bianca de Almeida Pititto

Endereço para acessar este CV: http://lattes.cnpq.br/8433932854107690

ID Lattes: **8433932854107690**

Última atualização do currículo em 19/05/2022

Possui formação em Clínica Médica, mestrado em fatores de risco cardiovascular pela Endocrinologia da Universidade Federal de São Paulo - UNIFESP (2003) e doutorado em intervenção para prevenção de diabetes mellitus e impacto em fatores de risco cardiovascular pela Faculdade de Saúde Pública-USP (2009), tendo sido "visiting student" no MRC Epidemiology Unit of Cambridge University-UK (2007). Finalizou pós-doutorado pela Faculdade de Saúde Pública ? USP (2013), estudando fatores de risco cardiovascular não tradicionais em coorte de estudo epidemiológico, ELSA-SP. Curso de pós-graduação Latu Senso em Geriatria na UNIFESP e na USP. É médica concursada do Departamento de Medicina Preventiva-UNIFESP, atuando na graduação e residência médica e orientação de pós-graduação pelo Programa de Pós Graduação em Endocrinologia, na área de epidemiologia das doenças crônicas não transmissíveis e fatores de risco cardiometabólico.s Áreas de atuação: epidemiologia, diabetes mellitus, obesidade, fatores de risco cardiovascular, prevenção e epidemiologia do ciclo vital (eventos precoces da vida). **(Texto informado pelo autor)**

Identificação

Nome Bianca de Almeida Pititto

Nome em citações bibliográficas Almeida-Pititto B;Almeida-Pititto, Bianca de;Almeida B;Almeida-Pititto Bianca;B. Almeida-Pititto;ALMEIDA-PITITTO, B.;ALMEIDA-PITITTO, BIANCA;DE ALMEIDA-PITITTO, BIANCA;DE ALMEIDA-PITITTO, B.;ALMEIDA, BIANCA;PITITTO, BIANCA ALMEIDA;Bianca de Almeida-Pititto;Almeida'Pititto, Bianca;ALMEIDA'PITITTO, BIANCA;Pititto, Bianca de Almeida;Pititto, Bianca Almeida;Bianca Almeida Pititto;PITTITO, BIANCA ALMEIDA;DE ALMEIDA PITITTO, BIANCA

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