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ORIGINAL ARTICLE / *ARTIGO ORIGINAL*

Fetal programming and metabolic abnormalities: design of a case-control study

Programação fetal e alterações metabólicas em escolares: metodologia de um estudo caso-controle

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ABSTRACT: ***Objective:*** To describe the methodological features of a study on the association between restricted intrauterine growth and prevalence of overweight, obesity and hypertension in school aged children. ***Methods:*** The study was conducted in two stages in two public schools in Niterói (RJ), from June through December 2010. All students aged 6 to 14 years were eligible to participate. The first stage consisted of an interview to collect information on demographic variables, diet and other variables. A sample was selected for the second stage, in order to conduct an equivalent of a case-cohort study. There was an interval of about 15 days between the two stages. Cases were overweight students, defined as a Z-score for BMI/age/sex > +1.00 in the first stage. Controls were selected by using a random schedule in which the sample frame was the whole cohort. Bioelectrical impedance analysis, carotid ultrasound to measure intimal-medial thickness, blood measurements and interviews were obtained. Gestational age and weight at birth were used to define *proxy* variables of restricted intrauterine growth. Early health information was obtained from medical registers. ***Results:*** The study participation was 76.4% (n = 795) out of 1,040 eligible to participate). 85.1% of parent's questionnaires were returned. 62.5% of the eligible children participated in the case-control study (case: control ratio = 1:1.8). Early life health information was obtained from 292 children. ***Conclusion:*** The present study has the potential to provide important information about multiple outcomes and exposures related to restricted intrauterine growth and metabolic abnormalities.

Keywords: Obesity. Fetal growth retardation. Metabolic diseases. Child. Adolescent. Birth weight.

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RESUMO: ***Objetivo:*** Descrever a metodologia de um estudo sobre associações entre crescimento intrauterino restrito (CIUR) e prevalência de sobrepeso, obesidade e hipertensão arterial em escolares. ***Métodos:*** O estudo, conduzido em dois estágios, foi desenvolvido em 2 escolas municipais de Niterói (RJ) de junho a dezembro de 2010, sendo elegíveis todos os escolares de 6 a 14 anos. O primeiro estágio consistiu em um inquérito nutricional e de potenciais fatores de risco. Foram aplicados questionários aos responsáveis e adolescentes. Concomitantemente, foram selecionados os participantes de um estudo caso-controle, com casos prevalentes definidos como todos os escolares que apresentaram excesso de peso (Z-score de índice de massa corporal —IMC/idade/sexo > +1,00). O grupo controle consistiu em uma amostra aleatória da população de estudo do inquérito, o que permitiu o cálculo de razões de prevalências. Foram realizados bioimpedância elétrica, exames de sangue, ultrassonografia da carótida e entrevistas. As variáveis *proxy* de CIUR foram definidas a partir de informações sobre peso ao nascer (PN) e idade gestacional (IG). Adicionalmente, foram coletadas informações sobre os dois primeiros anos de vida dos escolares a partir de seus prontuários médicos. ***Resultados***: Entre os 1.040 escolares elegíveis, participaram do estudo 795 escolares (76,4%). A taxa de retorno do questionário enviado para os responsáveis foi de 85,1%. Para o estudo caso-controle, 62,5% (n = 363) dos selecionados participaram, resultando em uma razão caso:controle de 1:1,8. Foram localizados 55,8% (n = 444) dos prontuários, dos quais em 65,7% (n = 292) foram coletadas informações dos primeiros anos de vida. ***Conclusão:*** O presente estudo permitirá a análise de múltiplos desfechos e exposições relacionados ao CIUR e alterações metabólicas.

Palavras-chave: Obesidade. Retardo do crescimento fetal. Doenças metabólicas. Criança. Adolescente. Peso ao nascer.

INTRODUCTION

The increasing prevalence of obesity worldwide has required continuous efforts of control and prevention because, in addition to the set of cardiovascular risk factors such as systemic arterial hypertension (SAH), dyslipidemia, and insulin resistance, it increases morbidity and mortality rates of the population[1].

The hypothesis of fetal programming postulates that the environment found before birth or during childhood modulates physiological control and homeostasis that may result in increased susceptibility to chronic noncommunicable diseases (NCDs) throughout one's life[2]. Weight history in childhood is also associated with overweight and metabolic syndrome[3-6], but with differences between populations. At the beginning of the century, the hypothesis of "economic phenotype"[7] considered that malnourished children had to adapt *in utero* to save energy and, if they were to be exposed to an environment with a greater energy supply, they would be at a greater risk of developing obesity and other chronic diseases. To prevent these diseases, it is important to act at critical periods of life, so that it is possible to avoid the "programming" of future obese individuals and to prevent exposure to other risk factors that can be more harmful if the individual had intrauterine malnutrition[8].

There are gaps in knowledge about fetal programming, especially in developing countries. In addition, postnatal growth and perinatal socioeconomic context must be analyzed when studying one's life course. The study "Association between birth weight and prevalence of overweight, obesity, and hypertension in school-age children in Niterói, RJ," based on the hypothesis of fetal programming, aimed to investigate associations between the proxies of intrauterine growth restriction (IUGR), the components of the metabolic syndrome, and the thickness of carotid arteries considering the growth and socioeconomic trajectories.

The main methodological aspects of this study are presented below.

METHODOLOGY

A survey on the nutritional status and blood pressure of students aging 6 to 14 years was conducted, and their perinatal information and data from their first 2 years of age were recovered. Overweight students, sorted as cases, and a sample of all participants in the survey were selected for the case–control study. Chart 1 lists the procedures in all steps.

FIRST STEP: SURVEY

The survey was conducted from June to December 2010. For the prevalence of overweight and obesity to be expected in 15% in the age group from 6 to 14 years, with a margin of error of 5%, and a confidence level of 99.9%, the estimated sample size was 544 students. Two municipal elementary schools, all located in areas with lower risk of violence and enrolled in the Family Health Program (PMF) at least for 14 years, were selected.

Heights and weights were measured, respectively, using a portable stadiometer with 0.5 cm proximity and a calibrated digital scale with maximum capacity for 150 kg and 100 g accuracy, using the standard techniques[25]. Height was measured twice and the measurement would be taken again in case of difference greater than 0.5 cm. Based on Z-scores of body mass index (BMI: kg/height in m^2) for age and gender, calculated by WHOANTROPLUS program, students were classified as underweight ($Z < -2.00$), eutrophic ($-2.00 \leq Z \leq +1.00$), overweight ($+1.01 \leq Z \leq +2.00$), and obese ($Z > +2.00$)[26].

Blood pressure was measured twice, with a 5-minute interval, using digital equipment. If the differences between measurements of systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) was greater than or equal to 5 mmHg, a third measurement would be performed. In this situation, the measure with the less accurate value would be dismissed. The final blood pressure measurement was the value obtained by calculating the mean of both SBP and DBP measurements. BP measurements below the 90th percentile (P90) of SBP or DBP distributions were classified as normal, between P90 and P95 high normal, and P95 high above, according to gender and age[16].

Self-filling questionnaires containing 125 questions about lifestyle habits were given to students of the age 10–14 years in their class schedule (Chart 1). Caregivers were given a self-filling questionnaire developed by the researchers, containing 43 questions about pregnancy, birth, and growth of their children (Chart 1), which could be completed at school or at home.

Chart 1. Components of the questionnaires applied to the people in charge and the adolescents in the investigations of medical records and procedures conducted in the three stages of the study "Association between weight at birth and prevalence of overweight, obesity and arterial hypertension among students in the city of Niterói, RJ".

Step	Sample	Questionnaire/procedure	Variables and/or sources of information about variables
Survey	Mother/ caregiver of students*	Demographic, social, anthropometric, and reproduction characteristics of the birth mother	Current age and age at student's birth, skin color, schooling at student's birth, current body mass index of the birth mother, current height of the birth mother, parity
		Characteristics of pregnancy, delivery and student's birth	Health problems during pregnancy (diabetes, hypertension, and other), smoking during pregnancy, place of birth, type of delivery, gestational age, birth weight, size at birth
		Breast-feeding	Time of exclusive breast-feeding and breast-feeding
	Students aging 10 to 14 years*	Quality of life	KIDSCREEN 52[9]
		Physical activity	*Physical Activity 36 Questionnaire for Older Children — PAQ C*[10]
		Common mental disorders	*General Health Questionnaire — GHQ 12*[11]
		Feeding	Frequency of intake of certain food groups, meals, and food habits[12]
		Asthma	*International Study of Asthma and Allergies in Childhood — ISAAC*[13]
		Smoking	Active and passive smoking[14]
Step	Sample	Questionnaire/procedure	Variables and/or sources of information about variables
Survey	Students aging 6 to 14 years	Anthropometry	Body mass index[15] (electronic scale, capacity of 150 kg and variance of 50 g), height[15] (portable stadiometer Alturaexata®)
		Blood pressure	Measured in an electronic device (OMRON HEM 705CPINT)[16]
Case–control study	Mother/ caregivers of students (6 to 14 years)**	Sociodemographic characteristics	Criteria of Economic classification in Brazil — Associação Brasileira de Empresas de Pesquisa[17]
		Quality of life	KIDSCREEN 52[9]
		Family history	Current health status of children, parents, and grandparents
		Information about pregnancy	Complications during pregnancy, active and passive smoking during pregnancy, student's birth weight and size, gestational age

Continue...

Chart 1. Continuation.

Step	Sample	Questionnaire/procedure	Variables and/or sources of information about variables
Case–control study	Mother/ caregivers of students (6 to 14 years)**	Feeding in the first 2 years of life	Based on orientations by the World Health Organization for studies on this theme[18]
		Anthropometry	Body mass index[15] (electronic scale, capacity of 150 kg and variance of 50 g), height[15] (portable stadiometer Alturaexata®)
	Mother/ caregiver of students (6 to 9 years)**	Asthma	*International Study of Asthma and Allergies in Childhood — ISAAC*[13]
		Current food habits of the child	Frequency of intake of certain food groups, meals, and food habits[12]
Step	**Sample**	**Questionnaire/procedure**	**Variables and/or sources of information about variables**
Case–control study	Students aging 10 to 14 years	Quality of life	KIDSCREEN 52[9]
		Feeding	Questionnaire about feeding habits[19]
		Sexual maturation	Self-applied questionnaire[20]
	Students aging 6 to 14 years	Anthropometry	Body mass index[15] (electronic scale, capacity of 150 kg and variance of 50 g), height[15] (portable stadiometer Alturaexata®), waist circumference (the smallest curvature between the chest and the hips[15] or mean between lower curvature of the last fixed rib and the upper curvature or immediately above the iliac crest)[21], body composition[22] (bioimpedance—Biodinamycs 450®)
		Blood pressure	Measured in an electronic device (OMRON HEM 705CPINT)[16]
		Abdominal ultrasound	Assessment of nonalcoholic fatty liver disease (NAFLD)—linear transducer 10-MHz, Medison, Sonoace Pico[23]
		Carotid thickness	linear transducer 10-MHz, Medison, Sonoace Pico[2324]
		Blood test	Glucose, complete blood count, lipid profile (total cholesterol, LDL, HDL, VLDL, triglycerides), C-reactive protein, insulin, thyroid stimulating hormone (TSH), alkaline phosphatase, gamma glutamyl transferase (GGT), glutamic oxaloacetic transaminase (SGOT) glutamic pyruvic transaminase (SGPT), serum iron, ferritin, albumin, creatinine, von Willebrand factor, thrombomodulin alpha fibrinogen (Fga), and cytokines (interleukin 1β and interleukin 6)
Secondary data	Students aging 6 to 14 years	Transcription of data from medical records	Pregnancy, delivery, breast-feeding, weight, and feeding in the first 2 years of life

*Self-filling; **interview.

SECOND STEP: CASE–CONTROL STUDY

Concomitantly to the survey, the case–control study sample was selected, as the control group was a random sample of participants, with a maximum interval of 15 days between the two steps.

Cases were students who were classified as overweight ($Z > +1.00$) in the survey. To determine prevalence ratios, the control group consisted of a random sample of students participating in the survey, including cases stratified by age (6 to 9/10 to 14) and school. Case control ratio observed was of 1:2. When the population forming the sample is the total study population, odds ratio results in the prevalence ratio, namely:

The chance in total study population — $(a/c) \div [(a + b)/(c + d)]$ — was estimated by a random[27] sample, as shown in Chart 2.

Students were scheduled to attend meetings preferably accompanied by their mother, and they should fast for 12 hours, with physical inactivity, and, for girls, they should not be in their menstrual period. New anthropometric and BP measurements were taken without knowing those collected from the survey. Caregivers also had their weight and height measured. Two electric bioimpedance measurements were taken.

Blood samples were collected and processed on the Clinical Research Unit (UPC), and also stocked up as two plasma aliquots in freezer.

The thickness of intima and media layers of the carotid (in millimeters) performed bilaterally were calculated as the mean measurement in three locations: internal carotid artery, common carotid artery, and carotid bifurcation[24].

Ultrasound diagnosis of hepatic steatosis was based on the presence of hyperechoic image of the hepatic parenchyma compared to the renal parenchyma in the medium axillary line[23].

Later, interviews were conducted separately, addressing lifestyle habits with both caregivers and students aged 10 to 14 years (Chart 1). The self-filling questionnaires and interviews took 40 minutes on an average.

The students participated in two meetings (one at the school and another at the UPC), and the caregivers attended only the meeting at the UPC.

Chart 2. Contingency table of a case–control study and respective measures of association.

Variable	Cases	Non-cases	Total
Present	a	b	a + b
Absent	c	d	c + d
Prevalence odds ratio	(a/c) ÷ (b/d)		
Prevalence ratios	(a/c) ÷ [(a + b)/(c + d)] = (a/a + b) ÷ (c/c + d)		

THIRD STEP: SECONDARY DATA

Information about pregnancy, childbirth, breast-feeding, food introduction, and growth until 2 years of age of the participants was obtained from medical records at the health units in Niterói and from the lists of the Information System Database on Live births (SINASC), from 1996 to 2004. At this stage, weight data from the first 2 years of life were obtained to evaluate the association between growth recovery (catch-up) of participants, who were small for gestational age (GA), and their nutritional status and metabolic markers in the current age.

Intrauterine malnutrition proxies

Data on birth weight (BW) and GA were obtained from different sources to ensure the quality of information.

When BW and accurate GA were available, IUGR was set as BW below the 10th percentile expected for GA, specific for each gender[28]. When categorical GA was available, regardless of presentation of BW, the simplified definition of IUGR was used, restricted to non-premature: BW less than 2.500 g at 37 weeks or more of pregnancy[29].

Alternatively, BW controlled by GA (both continuous variables) may be used.

The choice of intrauterine malnutrition proxy depends on completeness and reliability of the information about BW and GA, the type of respective variables (categorical or continuous), and the study population chosen for the analysis, survey or case–control study.

QUALITY CONTROL

We conducted a pilot study in June and July 2010. No protocol changes were necessary or significant differences were found between the population of the pilot and the main studies, so we opted by adding the sample to the pilot study, thus increasing statistical power.

The field team was composed of health professionals trained, monitored, and evaluated regularly by leading researchers. The quality of anthropometric measurements was evaluated according to the protocol by Norton and Olds[30] and through concordance analysis between measurements made in the first two steps. Imaging tests were performed according to the procedures of the study Metabolic Alterations Reggio Calabria Adolescents (M.A.RE.A.).

Data were processed by double input and stored in databases developed at the Census and Survey Processing System Program[31]. Stata software, version 12.0, was used.

ETHICAL CONSIDERATIONS

The study was approved by the Ethics Committee of Institute for Collective Health Studies (77-2008).

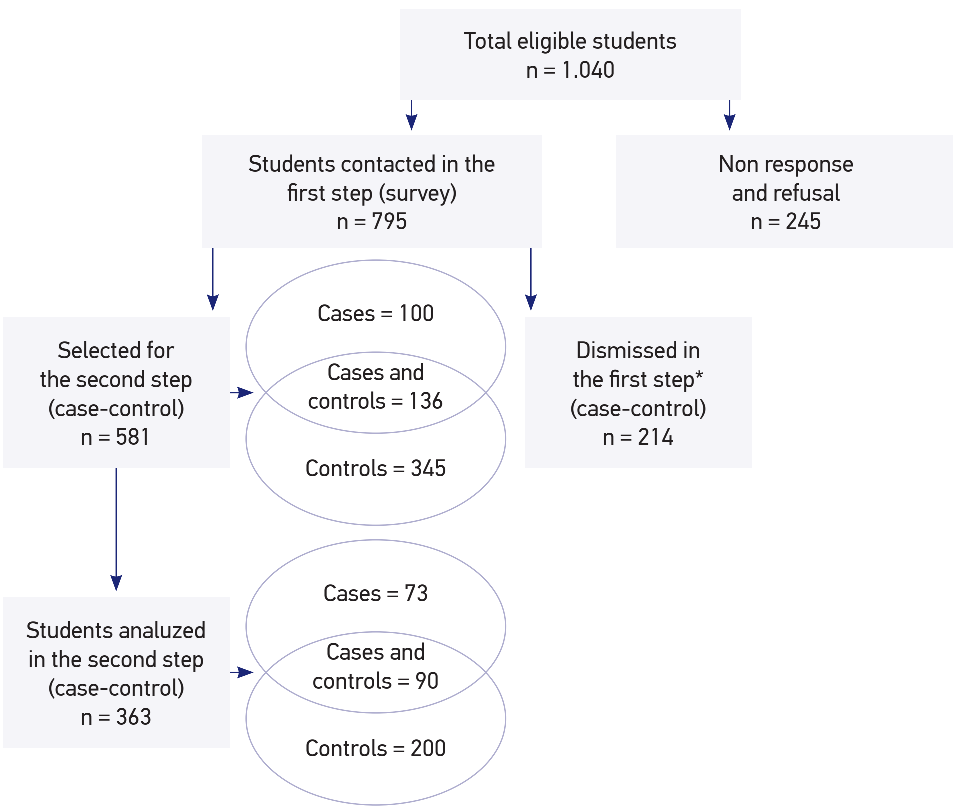
RESULTS

In total, 1,040 schoolchildren enrolled in the school for the year 2010 were eligible for the sample. The study included 795 children (76.4%) whose caregivers signed the informed consent form (Figure 1).

The questionnaire return rate sent to children's caregivers was 85.1% (n = 676).

Of the 581 schoolchildren selected for the case–control study, 62.5% (n = 363) went to the University Hospital Antonio Pedro, resulting in a case: control ratio of 1:1.8 (Figure 1).

Records of 55.8% (n = 444) of schoolchildren measured in schools were located, of which 65.7% (n = 292) had their information about the first years of life collected.



*Not selected for the second step.

Figure 1. Flowchart with number of participants in each step of the study "Association between birth weight and prevalence of overweight, obesity, and hypertension in schoolchildren from Niterói, RJ".

DISCUSSION

In studies on fetal programming, the long interval between exposure and outcomes in the course of life increases the likelihood of loss to follow-up and confounding problems. Another limitation is the lack of information about both exposure and potential confounding factors[32], outlined in this study to obtain the data of perinatal and up to 2 years of age available in medical records, in addition to information obtained through caregivers.

Sample size is one of the limitations of this study, which reduces the statistical power of the associations evaluated. As this is a sample composed of two schools, their representativeness is possibly restricted, particularly as to prevalence estimates, but not necessarily as to measures of association[27].

Given the gap between the main data (IUGR) and outcomes (6 – 14 years), one must consider the possibility of reporting bias, particularly with regard to validity of information concerning the early life of students. Medical records and data recovery from SINASC, both promising strategies for studies about fetal programming, aim to mitigate such problems.

Over the years, SINASC has proven to be an important epidemiological tool to improve the coverage and quality of information they provide. Registration of BW in SINASC features high reliability when compared with medical records data (intraclass correlation coefficient = 0.97), being an excellent source to identify babies with low BW[33]. This study used different sources of proxy IUGR variables, with possibility of using markers such as SGA, restricted or not to those born at term[29]. The choice of markers entails greater or lesser error of measurements of the main data, in view of the different stages of information collection, that is, at birth (SINASC) and at the current time. Using only information about BW is another option[34].

Data such as maternal education at birth and at present and BW, postnatal growth in childhood, or adolescence allow assessing relevant socioeconomic and nutritional pathways in the course of their life.

The methodology in the selection of controls in the second step should be highlighted, configuring a sample of the population recruited in the survey, which allows the calculation of prevalence ratios rather than odds ratio prevalence[27]. It is known that the odds ratio estimated when the outcome has a high prevalence (as in the case of overweight) can lead to an incorrect interpretation of association of magnitude because of built-in bias[35].

Storage of blood samples enables the evaluation of risk factors related to obesity and other NCDs not considered initially.

CONCLUSION

Facing the global scenario of high load of NCDs-related disorders, early prevention measures are necessary. This study is expected to provide a basis for further research with larger samples and/or other designs in which associations will be evaluated between sub-clinical atherosclerosis measures and IUGR with metabolic factors. In short, this study will allow analysis of multiple exposures and outcomes related to IUGR, fetal programming, metabolic disorders, and risk factors for NCDs in an age group that is studied less in developing countries.

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