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Small for gestational age babies are not related to changes in markers of adipose tissue dysfunction during reproductive age☆,☆☆

Anderson Sanches de Melo [a,*], Heloisa Bettiol [b], Antônio Augusto Moura da Silva [c], Ana Carolina Japur Sá Rosa-e-Silva [d], Viviane Cunha Cardoso [b], Rosana Maria dos Reis [d], Rui Alberto Ferriani [d], Marco Antonio Barbieri [b], Carolina Sales Vieira [d]

[a] Department of Gynecology and Obstetrics, University Hospital, Faculty of Medicine of Ribeirão Preto, FMRP-USP, Ribeirão Preto, SP, Brazil
[b] Department of Pediatrics, University Hospital, FMRP-USP, Ribeirão Preto, SP, Brazil
[c] Department of Public Health, Federal University of Maranhão, MA, Brazil
[d] Department of Gynecology and Obstetrics, University Hospital, FMRP-USP, Ribeirão Preto, SP, Brazil

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ABSTRACT

Background: Small for gestational age (SGA) birth has been associated with adipocyte dysfunction during later phases of life. Because SGA women are at a higher risk of developing polycystic ovary syndrome (PCOS), adipocyte dysfunction detected in patients with PCOS may be associated with SGA birth.
Aims: To determine whether SGA birth is related to altered serum markers of adipose tissue dysfunction during the third decade of life in Brazilian women. A secondary objective was to relate the presence of PCOS with serum markers of adipose tissue dysfunction.
Study design: Prospective cohort observational study.
Subjects: A total of 384 women born at 37 to 42 weeks of gestation from June 1, 1978 to May 31, 1979 in Ribeirão Preto, State of São Paulo, Brazil. After exclusion, 165 women participated in the study. Of these women, 43 were in the SGA group and 122 were in the adequate for gestational age group based on birth weight determined from cohort files.
Outcome measures: Body mass index (BMI), arterial systolic and diastolic pressures, abdominal circumference and serum concentrations of total testosterone, fasting glucose and insulin, lipid profile, adiponectin, leptin and necrosis factor alpha tumor (TNFα).
Results: BMI was an independent predictor of lower adiponectin (adjusted coefficient = −0.02, p = 0.01) and higher leptin (adjusted coefficient = 0.06, p = 0.01) concentrations. The serum insulin concentration was associated with higher leptin (adjusted coefficient = 0.03, p = 0.02) and TNF-α (adjusted coefficient = 0.01, p = 0.03) concentrations. Having PCOS or being born SGA did not predict any markers of adipocyte dysfunction.

1. Introduction

Small for gestational age (SGA) birth, especially when associated with intrauterine growth restriction, is a risk factor for the development of clinical and metabolic comorbidities in various phases of life (e.g., obesity, high blood pressure, glucose metabolism disorders and adipose tissue dysfunction) [1–3]. We have also demonstrated that SGA women are at two-fold higher risk of developing polycystic ovary syndrome (PCOS) during the third decade of life than women born adequate for gestational age (AGA) [4]. Because the presence of PCOS is associated with a higher frequency of metabolic changes [5,6] that are also observed in women born SGA, this association may contribute to the higher frequency of clinical and metabolic comorbidities in PCOS.

Both SGA individuals [1] and women with PCOS [7] show changes in cardiovascular risk markers, regardless of body mass index (BMI). The causes of these changes are multifactorial but may be partially explained by adipose tissue dysfunction, as indicated by imbalances in adipocyte secretion of inflammatory [e.g., tumor necrosis factor alpha (TNFα) and interleukin 6] and metabolic (e.g., leptin and adiponectin) cytokines [8,9]. These alterations in serum adipocytokine profiles arise

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* Corresponding author at: Setor de Reprodução Humana, Departamento de Ginecologia e Obstetrícia da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Av. Bandeirantes, 3900, Monte Alegre, 14049-900 Ribeirão Preto, SP, Brazil. Tel.: +55 16 3602 2821; fax: +55 16 3602 2810.
E-mail address: doctor_asmelo@yahoo.com.br (A.S. de Melo).

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when subcutaneous adipocytes exceed their maximum expansibility and show alterations in morphology and function with visceral deposition of fatty acids [8]. Even in the absence of overweight or obesity [1], adipose tissue dysfunction may be associated with inflammatory, proatherogenic and/or diabetogenic changes [10], predisposing an individual to metabolic [11] and cardiovascular [12] diseases. Despite these possible interactions between SGA birth, the increased risk of PCOS and adipose tissue dysfunction, to date, no birth cohort studies have evaluated these associations in adult women in the same age range who live under similar environmental and cultural conditions.

Evaluations of markers of adipocyte dysfunction have yielded conflicting results for women with PCOS [13–15] and those born SGA [1,16–19] because these disorders are associated with a high prevalence of obesity [20] and insulin resistance (IR) [21], which are also associated with adipocyte dysfunction [22,23]. Moreover, some of these studies included women of different ages and in different geographic areas, compromising the internal validity of the findings [13–19]. Thus, the objective of the present study was to evaluate whether SGA birth was related to altered serum markers of adipose tissue dysfunction during the third decade of life in a birth cohort of Brazilian women. A secondary objective was to determine whether PCOS is associated with serum markers of adipose tissue dysfunction.

2. Patients and methods

2.1. Patients and study design

This was an observational, prospective study of a birth cohort. The study population consisted of a sample of 384 women born at term (37–42 weeks of gestation) in the city of Ribeirão Preto, State of São Paulo, Brazil, between June 1, 1978 and May 31, 1979. All of these women have been followed since birth in the Medicine Scholl of Ribeirão Preto, University of São Paulo (FMRP-USP) and were assessed by the Department of Pediatrics at two different times, 1985–1987 and 2002–2004 [24].

The total birth cohort consisted of 6827 newborns (3316 females and 3511 males). Of the 3316 females, 3095 were born at term, and the data for 1064 females were updated when they were assessed by the Department of Pediatrics at FMRP-USP in 2002–2004. Of these 1064 subjects, 1002 were born at term: 101 were SGA, 849 were AGA and 52 were large for gestational age (LGA). LGA women (i.e., females with birth weight above p90 for gestational age) were not considered, and thus, 950 subjects were eligible for recruitment in 2007–2008 by the Department of Gynecology and Obstetrics to assess the prevalence of PCOS [4].

The sample size calculation for the study was based on the difference in the prevalence of PCOS. To demonstrate an absolute difference of 15% in PCOS prevalence between SGA females and AGA females, 99 patients per group were necessary (a total of 198 women), given an alpha value of 5% and a power of 80%. Thus, from November 2007 to October 2008, all 101 women born SGA and a random subsample of 283 women born AGA (i.e., 1 in 3 of the 829 AGA females) were selected by a computer program (www.randomizer.org) for a total subsample of 384 individuals. Of these, 21 women did not agree to participate in the study and 48 could not be located after five search phases: making an initial telephone contact; updating the telephone number according to the address in the follow-up files of the cohort; sending written correspondence; posting notices in print, radio and television media; and actively searching the municipal health registry. In addition, some women were excluded: users of a hormonal contraceptive who did not want to do a wash-out for at least 12 weeks even when offered a non-hormonal contraceptive as an alternative (n = 76) and women who were pregnant (n = 63) or nursing (n = 11). Thus, the included participants (n = 165) were divided into two groups based on birth weight in relation to gestational age [25]: 1) the SGA group consisted of newborns with a birth weight below the tenth percentile (n = 43), and 2) the AGA group consisted of newborns with a birth weight between p10 and p90 (n = 122). PCOS was diagnosed based on the Rotterdam Consensus [26]; 13 SGA women (30.2%) and 17 AGA women (13.9%) were diagnosed with this condition [4]. The minimum and maximum birth weights of the study population were 2000 g and 3870 g, respectively.

The present study is a secondary evaluation of the principal study, which was performed to assess the prevalence of PCOS among women born SGA [4]. Thus, in the present study, 16 patients per group were required to reach 80% power to demonstrate a difference of one or more standard deviations in serum adipocytokine concentrations between the SGA and AGA groups, with an alpha of 5%.

The flowchart is illustrated in Fig. 1. The maternal data were recently published in the study of PCOS prevalence [4]. The study was approved by the Research Ethics Committee of the Hospital of FMRP-USP, and all of the women signed the written informed consent form.

2.2. Clinical and laboratory evaluation

All of the participants were evaluated in the Laboratory of Gynecology of the Academic Hospital of FMRP-USP during a single visit after a 12-hour fast. The evaluations were performed between the third and fifth days of the menstrual cycle or, in subjects with amenorrhea, on any day of the menstrual cycle, as long as a corpus luteus and a follicular image larger than 10 mm were ruled out by ultrasound examination [4]. We evaluated age, weight (W) and height (H), birth weight, BMI (defined as W (kg)/H $(m)^2$), systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and abdominal circumference (AC).

After a 15-minute rest, 20 mL of venous blood was collected from each participant. One 10-mL sample was centrifuged at 2500 rpm (1600 *g*) at room temperature for 10 min, and the serum was stored at −70 °C so that the main variables could be evaluated at a single time, whereas the other 10 mL of serum was subjected to biochemical analysis within a maximum of 2 h of collection. The serum of the patients was analyzed for all variables.

Fasting serum insulin and TNFα concentrations were determined by chemiluminescence using a DPC Immulite® 2000 apparatus (Diagnostic Products Corporation, Los Angeles, CA, USA®). Total testosterone was determined with a Tri Carb 2100 TR scintillator (Packard® Instrument Company, Downers Grove, IL, USA). Fasting glycemia was determined using the oxidation method with a Konelab 60i kit and a Wiener Lab® apparatus (Rosario, Argentina). The intra- and inter-assay coefficients of variation were 4.2% and 6.8% for TNFα and 3.3% and 4.1% for total testosterone, respectively.

IR was analyzed using the homeostasis model assessment-insulin resistance index (HOMA-IR), i.e., HOMA-IR = fasting glucose (mg/dL) × 0.05551 × fasting insulin (μU/mL)/22.5 [27].

The adipocytokines (adiponectin and leptin) were analyzed by ELISA using a Biotek apparatus (model ELx808, Winooski, VT, USA), the Millipore® kit (Billerica, MA, USA) and GEN5 software (BioTek Instruments, Inc.). The intra- and inter-assay coefficients of variation were 3.4% and 5.7% for adiponectin and 3.7% and 4.2% for leptin, respectively.

With regard to the lipid profile, total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides (TG) were determined with an enzymatic method using the BT 3000 plus apparatus (Wiener lab®, Rosario, Argentina). Serum low-density lipoprotein (LDL) was calculated using the Friedewald formula [LDL-cholesterol = total cholesterol − (HDL + TG / 5)] because none of the subjects had a TG concentration greater than 400 mg/dL [28].

2.3. Statistical analysis

The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to determine the normality of the sample distribution. Because some variables did not follow a normal distribution, for uniformity, we opted to perform each univariable comparison with the Mann–Whitney test.