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Adipocytokines, inflammatory and oxidative stress markers of clinical relevance altered in young overweight/obese subjects

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ABSTRACT

Objective: The aim of the study was to assess the influence of overweight and obesity in youth on adipocytokines levels, inflammatory and oxidative markers.

Design and methods: Cross-sectional study of 149 young adults (54 normal weight, 27 overweight, 68 obese).Clinical and biochemical parameters, including lipid profile, fasting glucose, insulin and HOMA were determined. The levels of adipocytokines(adiponectin, retinol-binding protein 4 (RBP4), and resistin), markers of inflammation (high-sensitivity C-reactive protein (hs-CRP) adenosine deaminase (ADA), dipeptidyl peptidase-IV (DPP-IV) activities, serum IL-6 levels and oxidative stress (malondialdehyde and ferric reducing antioxidant power — FRAP) were measured.

Results: Obese subjects presented significantly lower levels of Sulfhydryl groups (SH groups), adiponectin, HDL-C and the highest levels of RBP4, hs-CRP, resistin, IL-6, ADA, DPP-IV activities, and oxidative markers than compared to those who were of normal weight. There was a positive correlation between hs-CRP, IL-6, DDP-IV activity, anthropometric measurements and biochemical parameters.

Conclusions: This analysis shows that resistin, RBP4, IL-6, ADA and DPP-IV activities and the reduction of adiponectin can promote inflammation, impairment of insulin sensitivity, and may contribute development of the pathways involved in obesity. These findings may hold promise in identifying new inflammatory markers, benchmarks that assist in the diagnosis and monitoring of patients with overweight and obese.

1. Introduction

Excess weight and obesity are two of the most complex clinical syndromes whose prevalence has reached epidemic proportions in recent decades, especially in earlier ages [1]. Current data indicate that 52.5% of Brazilians are overweight and 17.9% are obese. Carrying excess weight is more common among men (56.5%), while obesity is higher in women (18.2%), with a 23% weight increase over the last nine years [2]. Human obesity is accepted as an important risk factor for development of metabolic syndrome (MetS), diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, insulin resistance, hepatic steatosis, non-alcoholic liver disease and high morbidity and mortality [3].

Multiple mechanisms may contribute to obesity-related comorbidity development, including an abnormal production of adipocytokines, aberrant oxidative stress and dysregulated proinflammatory response in tissues such as the muscle and liver [4]. Both obesity and oxidative stress can manifest as early as the first two decades of life [5].

Adipocytokines and inflammatory markers could mediate the facilitating effect of obesity on the appearance of its comorbidities such as insulin resistance (IR), diabetes mellitus 2 (DM2) and cardiovascular disease (CVD) [5]. Adiponectin levels were previously observed to be significantly diminished with both obesity and DM2 and a significant negative correlation was detected between adiponectin and the parameters of obesity [6]. Evidence demonstrates that low adiponectin concentrations correlate with high plasma insulin and high IR, while adiponectin administration was found to suppress

Abbreviations: ADA, adenosine deaminase; Ado, adenosine; Apo B, apolipoprotein B; BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; DM2, diabetes mellitus 2; DPP-IV, dipeptidyl peptidase-IV; FRAP, ferric reducing antioxidant power; GLUT-4, glucose transporter 4; HbA1c, glycated hemoglobin A1c; HC, hip circumference; HDL-C, high density lipoprotein cholesterol; HOMA, homeostasis model assessment; hs-CRP, high-sensitivity C-reactive protein; IR, insulin resistance; LDL-C, low density lipoprotein cholesterol; MetS, metabolic syndrome; IRS-1, insulin receptor substrate 1; IL-6, interleukin 6; NC, neck circumference; NF-kB, nuclear factor kappa B; RBP4, retinol-binding protein 4; SBP, systolic blood pressure; SH groups, sulfhydryl groups; SI, insulin sensitivity; TNF-α, tumor necrosis factor-alpha; TBARS, thiobarbituric acid reactive substances; VLDL, very low density lipoprotein; WC, waist circumference.

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proinflammatory agents, e.g., tumor necrosis factor alpha (TNF-α) and IL-6, and directly ameliorate endothelial dysfunction [7]. Furthermore, resistin is an adipocytokine specifically expressed in white adipose tissue that has secretion strongly related to insulin resistance. The highest levels were observed in DM2 subjects [8], and also correlated with markers of inflammation and can be a predictor of coronary atherosclerosis [9].

Another prominent adipocytokine, the retinol-binding protein 4 (RBP4), mainly secreted by adipocytes and liver is the main protein carrier of vitamin A in the circulation [10]. Recent studies demonstrate that RBP4 levels were increased in obese and IR human and mice models. Additionally, a genetic or pharmacologic elevation of serum RBP4 causes insulin resistance in normal mice, probably by inhibiting insulin signaling and stimulating gluconeogenesis [11]. Evidences shows strong correlations of serum RBP4 levels with the severity of insulin resistance, obesity, hypertension, dyslipidemia, waist/hip ratio, cardiovascular disease, and intra-abdominal fat mass [12]. However, others results do not demonstrate these correlations, thus, this is still poorly understood [13].

It is well known that the chronic inflammatory response is characterized by increases in the production of markers of inflammation i.e., C-reactive protein (CRP), IL-6 and TNF-α [14]. In particular, in our recent studies we observed an increase in adenosine deaminase (ADA), dipeptidyl peptidase-IV (DPP-IV) activities in MetS subjects and in the activity of these enzymes in saliva of obese young adults [15]. These enzymes in inflammatory processes may influence insulin sensitivity since the ADA reduces levels of adenosine (Ado), important to the transport of glucose in the adipocytes. In addition, DPP-IV activity may be required for T-cell activation cascade together with ADA [16].

This way, considering that systemic inflammation could be the causative link between obesity and various diseases, as well as obesity is associated with a chronic inflammatory response characterized by abnormal adipocytokine production, and the activation of several proinflammatory signalling pathways resulting in the induction of several biological markers of inflammation [7], the purpose of the present study was to evaluate the adipocytokines (adiponectin, RBP4 and resistin) levels, inflammatory markers (IL-6, hs-CRP, ADA and DPP-IV activities) and oxidative markers (TBARS, FRAP and SH groups), in order to clarify the influence of adiposity on the metabolic risk profile and the metabolic consequences of weight change in early adulthood in overweight and obese young adults.

2. Materials and methods

2.1. Study design and study population

These cross-sectional study and measurements were taken in a single moment. Participants were recruited from January to August 2013 in São Miguel do Oeste city Santa Catarina State located in southern Brazilian Region. The patients were from basic health units. The protocol of the study was approved by the Ethics Committee of the University of West Santa Catarina (UNOESC — no 219.091) and all participants provided written informed consent. The groups studied included 149 young subjects with ages between 18 and 30 years: 54 normal weight subjects and gender-matched healthy volunteers served as control group (32 females and 22 males). The subjects with increased weight were divided in two subgroups, matched for sex, age, and body mass index, and were enrolled as follows: 1) 27 overweight subjects (17 females and 10 males); 2) 68 obese young subjects (41 females and 27 males). The participants were non-smokers and were not taking any medications. The group studied did not present previous diseases such as DM, coronary, stroke, neoplasias, other diseases or dysfunctions that could influence the obesity status.

2.2. Anthropometric measurements

All measures were taken in the Anthropometry Laboratory at UNOESC. Standing height (H, cm) using a wall-mounted stadiometer (Charder, model HM-210D). Weight (W, kg) was measured using a calibrated electronic scale (Toledo, model 2124). Body Mass Index (BMI) was calculated as W/H^2 (kg/m^2). Waist circumference (WC), neck circumference (NC) and hip circumference (HC) were measured in centimeters with a flexible tape. For WC the tape was applied above the iliac crest with the subject standing with the abdomen relaxed and arms at the sides and the feet together (feet close in the same position and facing forward fully supported on the platform). NC for the participant remained in the same position and tape was placed on half of the neck on the hyoid bone. The percentages of fat and fat weight were determined by bioimpedance (Biodynamics Model 450). All measurements were taken on the left side of the body, according to standardized procedures by Weiner and Lourie, 1981 [17]. During the anthropometric measurements, all participants were barefoot and clothed appropriately.

2.3. Indices and classifications

According to the World Health Organization, underweight was defined as BMI <18.5 kg/m^2, normal weight as BMI 18.5–24.9 kg/m^2, overweight as BMI 25–29.9 kg/m^2, and obesity as BMI ≥30 kg/m^2 [18], all without comorbidities. According to Gallagher et al., 2000 [19] % fat ≥20% (males) and % fat ≥33% (females) are the cut-points adopted to define over fatness, corresponding to overweight classification using BMI in a population of young adults. According to the National Institute for Health and Clinical Excellence guidelines, WC ≥102 cm for men and ≥88 cm for women are prerequisite risk factors for the diagnosis of the MetS, as WSR ≥0.5 for both males and females [20].

2.4. Laboratory measurements

Blood samples containing liquid EDTA and serum samples were obtained from collected blood samples from participants after an overnight fast of at least 12 h. Total blood cholesterol, HDL-C, triglyceride, glucose and uric acid were measured enzymatically using a commercial assay kit (Labtest Diagnostics® - Brazil). LDL-c was subsequently calculated using the Friedewald formula [21].

The insulin and hs-CRP were determined by electrochemiluminescent immunoassay using an Elecsys 2010 analyzer (Roche diagnostics®). Insulin resistance index was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) as (fasting insulin mIU/L) × (fasting glucose mg/dL)/22.5 and evaluation of insulin sensitivity (SI), the index QUICKI (Quantitative Insulin Sensitivity Check Index) was used. HbA_{1c} was measured by high performance liquid chromatography and expressed as %.

The serum adiponectin, resistin and RBP4 concentrations were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA), according to manufacturer (EMD Millipore Corporation, Billerica, MA, EUA) in the Luminex 100 IS Analyzer System (Luminex Corp, Austin, TX, USA). The resistin showed sensitivity of 0.16 ng/mL, accuracy of 90–108%, inter-assay precision was 7.1–7.7% and intra-assay 3.2–7.0% and the curve range: 0.16–10 ng/mL. Adiponectin showed a sensitivity of 1.5 ng/mL, accuracy of 92–102%, inter-assay precision was 2.4–8.4% and intra-assay 1.0–7.4% and the curve range: 1.5–100 ng/mL. RBP4 showed a sensitivity of 0.78 ng/mL, accuracy of 76–113%, inter-assay precision was 3.8% and intra-assay 4.8% and the curve range: 0.14–100 ng/mL.

Serum IL-6 levels were determined by ELISA using commercial kits from R&D Systems (Minneapolis, MN, USA) according to the manufacturer's instructions. The detection limits of the assays were: 0.09 pg/mL, the sensitivity of 2 pg/mL and the curve range: 23.3 to 2560 pg/mL.