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Blood advanced glycation end products and biomarkers of inflammation in class III obese Brazilian subjects

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Metabolic syndrome is a constellation of disorders, including hypertension, dyslipidemia, hyperglycemia, and obesity, that predisposes to the development of type 2 diabetes and ultimately to an increased risk of cardiovascular death [1]. A key component, obesity is one of the main contributors to the worldwide problem of chronic diseases and disability. Obesity has reached epidemic proportions globally; in 2014 almost two billion adults were overweight, with more than 600 millions of them clinically classified as obese individuals [2]. Body mass index (BMI) – the weight in kilograms divided by the square of the height in meters (kg/m^2) – is a commonly used index to classify obesity in adults. As WHO defines the terms, "overweight" is a BMI equal to or more than 25, "obesity" is a BMI equal to or more than 30, and obesity class III is when BMI is equal to or more than 40 [2].

In order to prevent or delay the onset of obesity and/or its linked complications with a successful therapeutic intervention, much effort is being invested in establishing new blood biomarkers. For example, the risk for obese individuals to develop type 2 diabetes or a heart attack has already been linked to blood markers of inflammation [3] and of glycotoxicity [4]. Here, we investigated whether markers of inflammation, including high-sensitive c reactive protein and neopterin levels, and markers of sustained hyperglycemia, such as, glycated hemoglobin and advanced glycation end products (AGEs), correlated with HOMA-IR (homeostasis model assessment-insulin resistance), LAP (lipid accumulation product, which predicts the incidence of cardiovascular diseases; blood and the anthropometric marker) and the Framingham score (estimates risk of heart attack in ten years) in class III obese Brazilian individuals.

Subjects and methods

This is an observational and transversal study in which 30 class III obese patients from Florianopolis, Santa Catarina, Brazil agreed to participate. The control group was composed of ten overweight volunteers. The local Research Ethics Committee approved the study and informed consent was collected from the participants (Protocol # 2150/2011). Tables 1 and 2 show the clinical characteristics and biochemical data collected at the Endocrinology and Metabiology Clinic at Hospital Universitario, Universidade Federal de Santa Catarina, Florianópolis/SC, Brazil, between June, 2012 and March of 2013.

HOMA-IR, LAP and the Framingham score were calculated as previously described [5-7]. The clinical biochemical data were determined in a biochemical autoanalyser Dimension RxL (Siemens Healthcare Diagnostics Inc., EUA). Neopterin concentrations were determined by high-performance liquid chromatography coupled with fluorescence detection as previously reported by our group [8,9], and values were expressed as μmol/L. AGEs were measured by fluorimetry (Tecan, Grödig/ Salzburg, Austria), as previously reported [10].

Findings/Discussion

As depicted in Tables 1 and 2, the class III obese subjects presented insulin resistance, high probability of developing cardiovascular diseases, and also a high risk of heart attack in ten years, and almost half of this group was under pharmacological treatment with losartan (angiotensin II receptor antagonist for the treatment of hypertension) or losartan plus metformin (oral antidiabetic agent). Class III obese patients presented increased levels of high-sensitivity c-reactive protein (hsCRP) and neopterin (Table 2 and Figure 1A), pointing to an active inflammatory status.

Chronic inflammation has been linked to beta-cell dysfunction and insulin resistance, and the increased blood levels of these biomarkers may predict the development of type 2 diabetes and cardiovascular diseases [7,11,12]. Moreover, diabetes is an independent risk factor for cardiovascular disease, conferring an increased risk of morbidity and mortality from coronary artery disease, heart failure, and stroke [13]. In this context, hypertension accounts for more than 80% of diabetes-related deaths [14]. The renin-angiotensin-aldosterone system (RAAS) is one of the most important hormonal mechanisms pharmacologically targeted to regulate blood pressure. Blocking this system produced

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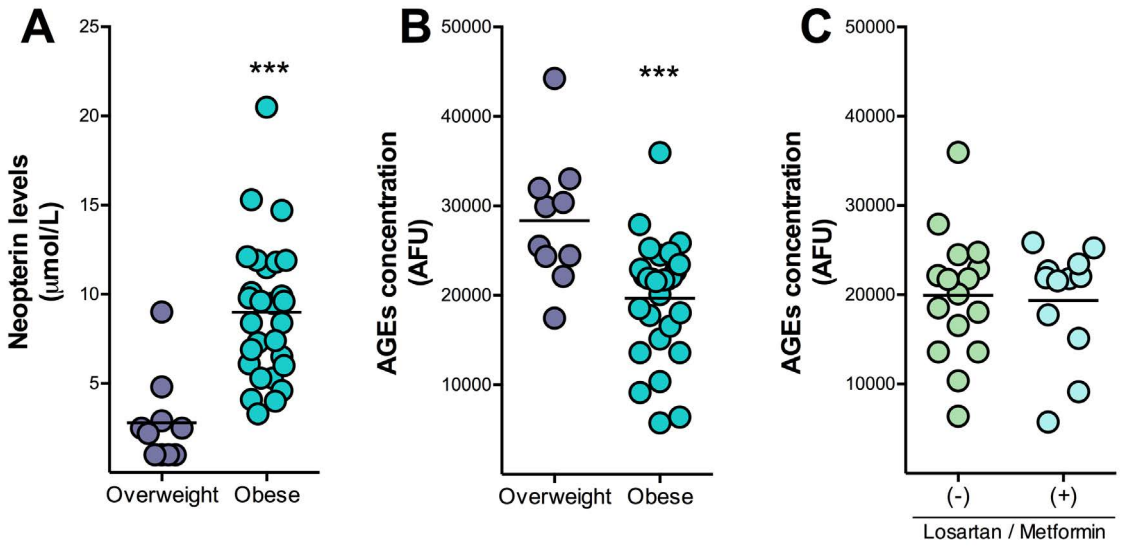

Figure 1. Blood levels of neopterin (A) and advanced glycated end products (B; AGEs) in class III obese individuals (*n=30*) and overweight subjects (*n=10*). AGEs were also measured under treatment with losartan (10 mg/day) and or plus metformine (500 mg orally twice a day) (C). Values represent the mean ± SD. $^{*}P<0.05$; $^{***}P< 0.001$, compared to the overweight group (*Student t Test).*

Table 1. Clinical characteristics of the Brazilian obese patients.

Clinical characteristics	**Class III Obese subjects** *(n = 30)*	**Overweight subjects** (*n* = 10)
Age (years)	40.6 ± 10.9	48 ± 13.0
Female (*n;* %)	23; 76.7	7; 70.0
BMI (kg/m^2) Normal range – WHO criteria: 18.5 – 24.9 [2]	49.5 ± 8.2	27.8 1.4
Weight (kg)	131.6 ± 31.1	67.8 ± 7.0
Abdominal circumference (cm)	135.3 ± 16.6	89.6 ± 8.3
Waist circumference (cm)	139.0 ± 15.9	94.8 ± 9.8
Familiar history of obesity (*n;* %)	26; 86.7	1; 10.0
Type 2 diabetes (*n;* %)	11; 36.7	1; 10.0
Under physical activity (*n;* %)	7; 23.3	5; 50
Specific diets (*n;* %)	28; 93.3	6; 60
Smokers (*n*; %)	5; 16.7	0; 0.0
HOMA-IR Homeostasis model assessment-insulin resistance: cutoff determined for the Brazilian population= 2.71 [5]	6.35 ± 0.7	ND
LAP (cm.mmol/L) Lipid accumulation product: predicts the incidence of cardiovascular diseases: [6] 25 – 49 years; 25-75th percentile: 16.4 – 57.3 > 50 years; 25-75th percentile: 31.1 – 85.3	131. 1 ± 94.4	ND
Framingham score (%) [7] Points / Female / Male 6 / 3.3 / 4.7 12 / 8.6 / 13.2 18+ / 21.6 / >30	Female: 7.3 ± 9.4 Male: 10.5 15.1	ND
Systolic blood pressure (mmHg)	132.5 ± 13.7	ND
Diastolic blood pressure (mmHg)	86.7 ± 11.5	ND
Losartan or Losartan plus Metformin (*n;* %)	14; 46.7	1; 10.0

protective effects in the kidney and heart in type 1 and 2 diabetic patients and it also stabilized blood pressure [15]. The molecular mechanisms are still not completely defined; however, it was demonstrated that angiotensin II (Ang II) is not only generated in the circulation by renin and the angiotensin converting enzymes, but it is also locally produced in diverse organs, including the kidneys, blood vessels, heart, adrenals, brain and also macrophages [16]. Most of this Ang II is not regulated by systemic hemodynamic changes, and via paracrine and autocrine signaling it mediates oxidative stress, inflammation, and fibrosis [17-19]. In this scenario, it could be suggested that hsCRP levels did not correlate with the clinical indexes - HOMA-IR, LAP or the Framingham score - of the class III obese patients (Table 2), due to the lack of anti-inflammatory effect of losartan (inhibitor of the RAAS; Ang II receptor blocker).

Neopterin, a pteridine mainly synthesized by activated macrophages in the periphery, is a sensitive marker of inflammation, immune system activation and an active participant in cardiovascular diseases [20]. Increased levels of neopterin are elevated in patients with coronary and peripheral artery disease and seem to be a prognostic marker for major adverse cardiovascular events. In particular, neopterin levels predict future major cardiac and vascular adverse events in patients presenting chronic coronary artery disease, and acute coronary syndromes [21-23]. However, this biomarker did not correlate with the clinical scores, even when the class III obese patients displayed peripheral inflammation and high risk for developing type 2 diabetes and heart diseases (Table 1). However, it is known that high levels of hsCRP and neopterin are associated with high risk for cardiovascular diseases. Here, the biochemical data did not correlate with the clinical scores, suggesting that there could exist a clinical threshold from which these biomarkers may predict the development of the disease. Accordingly, Uribarri et al. [4] suggested that more specific and sensitive biomarkers are needed to identify healthy from unhealthy obese subjects.

Figure 1 B and C show that AGEs levels were unexpectedly reduced in the blood from class III obese subjects, and the losartan/metformin therapy did not induce any alterations. AGEs are a group of pro-oxidant, cytotoxic compounds that contribute to chronic inflammation and diabetic complications [24-26]. AGEs are mainly formed because of the accumulation of the highly reactive methylglyoxal during hyperglycemic conditions [27]. Increased AGEs levels promote inflammation and oxidative stress through the activation of the cell surface receptors of AGE (RAGE), as well as directly modify the

Table 2. Altered blood biomarkers in type III obese Brazilian individuals.

Biomarker concentration	Class III Obese subjects *(Range reference values is given in parenthesis)* [7] Values are X ± SD
Glucose (mg/dL)	117.7 ± 40.3 *(70 – 99)*
Insulin (µIU/mL)	19.9 ± 13.8 *(5 – 20)*
HbA1C (%)	6.82 ± 1.4 *(4 – 6)*
Triglycerides (mg/dL)	157.4 ± 115.4 *(< 100)*
Total cholesterol (mg/dL)	175.7 ± 41.0 *(< 200)*
High-density lipoprotein-cholesterol (mg/dL)	41.5 ± 16.7 *(> 40)*
Low-density lipoprotein-cholesterol (mg/dL)	109.0 ± 32.0 *(< 130)*
Aspartate aminotransferase (U/L)	46.1 ± 17.5 *(20 – 48)*
Alanine transaminase (U/L)	27.4 ± 17.3 *(10 – 40)*
Gamma-glutamyl transferase (U/L)	48.1 ± 26.2 *(30 – 50)*
High-sensitivity c-reactive protein (mg/L)	7.5 ± 3.4 *(< 3.0)* (overweight subjects: 1.6 ± 0.8) (*P*<0.001; effect size: 4.11) Losartan/metformin (n=14): 7.3 ± 3.3 No losartan/metformin (n=16): 7.3 ± 3.7 (Losartan/metformin vs. no drugs; *P<0.05*)

extracellular matrix and action of hormones [28]. Here, we found reduced AGES levels in class III obese subjects, when comparing with the control group composed by overweight individuals. As glycated hemoglobin (HbA1C) levels positively and significantly correlated with blood AGEs in class III obese subjects ($P< 0.05$), it could be suggested AGEs in obese individuals are originated from the glucose metabolism. Unfortunately, HbA1C in overweight individuals was not measured; therefore, it cannot be suggested that the higher levels of AGEs in this group was linked mainly to dietary factors, as consistently pointed out by many researchers [26,28-30]. Nonetheless, AGEs levels in obese subjects did not correlate with higher risk of cardiovascular diseases.

Conclusions

hsCRP, neopterin, glycated hemoglobin and AGEs levels did not correlate with the clinical scores, HOMA-IR, LAP and the Framingham score. Considering the vast literature demonstrating the intricate relationship between the biomarkers and the prediction of heart diseases development, we suggest that in these class III obese patients there is an unknown modifiable factor which is mascaraing the clinical / biochemical associations, or as suggested it is needed more specific and sensitive biomarkers to identify healthy from unhealthy obese subjects, who are at risk for cardiovascular disorders.

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