Abstract

Aim. This study was conducted to ascertain if inflammation markers also correlate with parameters related to insulin resistance and the metabolic syndrome in a group of adolescents.

Background. Obesity is now considered a chronic low-grade inflammatory process, characterized by alterations in the systemic concentrations of some inflammation markers. Adiponectin, leptin and other inflammatory proteins, have been shown to correlate with insulin resistance and the metabolic syndrome in adults.

Design. Cross-sectional study in in two groups of obese and normal-weight adolescents.

Methods. Serum levels of adiponectin, leptin, ceruloplasmin and insulin levels were determined and correlated among them and with anthropometric parameters, blood pressure BMI and BMI z-score

Results. Waist circumference, BMI and blood pressure values correlated significantly with both homeostasis model assessment for insulin resistance (HOMA-IR) and insulin levels. Ceruloplasmin also correlated with both parameters with a high level of significance. However, leptin levels did not correlate with either HOMA-IR or insulin and adiponectin correlated with HOMA-IR but not insulin. All inflammation markers studied correlated with the BMI z-score. These correlations were stronger in the group of obese individuals compared to lean ones.

Conclusions. We found a relationship between insulin resistance and some inflammation in adolescents, which was particularly strong in obese individuals and was associated with the development of metabolic syndrome. Among the inflammation markers studied, ceruloplasmin revealed as a potential string marker of insulin resistance in obese adolescents.

Relevance to clinical practice. The results obtained in the present study imply a significant advance in the field of clinical practice of nursing. The adequate understanding by nursing personnel of the inflammatory processes inherent to obesity constitutes a key factor for the prevention of the disease and its complications in adolescents.

Key words: insulin resistance, inflammation, obesity, adolescents, HOMA2-IR.
Introduction

Excessive body weight is one of the global challenges of public health concern, especially for the European Region, in which the prevalence of obesity has tripled in the past two decades (Branca et al., 2007). According to the International Obesity Task Force (James et al., 2004), the prevalence of overweight of European children is 10-35% and in adolescents is 9-23%, with the highest infant rate (40%) in the Mediterranean countries. A high body mass index (BMI) in the adolescence may predict a high mortality in the adulthood due to high rates of cardiovascular disease, even if the excess of weight is lost (Dietz, 1998). Actually, many obesity-related diseases that were thought to apply only to adults, can be now diagnosed in children, such as insulin resistance and the metabolic syndrome (McGinnis et al., 2006).

Obesity is considered as a chronic low-grade inflammatory process, characterized by an increase in some inflammation markers (Trayhurn et al., 2006). Adipose tissue secretes a variety of bioactive substances, such as C-reactive protein, tumor necrosis factor-α, interleukin-6, adiponectin and leptin (Hotamisligil et al., 1993; Aronson et al., 2004). The circulating concentrations of adiponectin and leptin are reduced and increased, respectively, in obese and/or diabetic subjects and have been regarded as independent determinants of increased insulin resistance over and above lifestyle factors, anthropometric indexes, and inflammatory markers (Mente et al., 2010). Both proteins have been shown to correlate with insulin resistance and the metabolic syndrome (Kajikawa et al., 2011; Krakoff et al., 2003).

In fact, the homeostasis model assessment for insulin resistance (HOMA2-IR) score has been negatively associated with adiponectin (Goropashnaya et al., 2009) and positively with leptin (Esteghamati et al., 2009). Ceruloplasmin is released by the liver and is the main copper-carrying protein, which as an acute-phase protein, is also related to systemic inflammation. Ceruloplasmin levels have been independently associated with intraabdominal fat thickness (Cignarelli et al., 1996) and type-2 diabetes mellitus (Daimon et al., 1998) and has been found to be elevated in subjects with metabolic syndrome (Kim et al., 2002).

An important issue is how adiposity regulates inflammation during childhood and adolescent obesity, but it has not been sufficiently investigated. Gil-Campos and
colleagues found alterations in the adiponectin (Gil-Campos et al., 2011) and leptin (Gil-Campos et al., 2010) plasma concentrations in obese children, and Wärnberg et al., (2006) found significant correlations between BMI and waist circumference values with ceruloplasmin levels in a group of 224 adolescent females. However, it remains to be clarified whether insulin resistance, alike obesity measurement parameters, may be considered a predictor of inflammation in adolescents. Therefore, the aim of the present study was to search for correlations between these obesity-related inflammatory markers and insulin resistance in obese adolescents.

Materials and Methods

Subjects

24 Caucasian subjects (12-16 years old, 9 females, 15 males), who were part of a study designed to evaluate the clinical parameters that characterize the prevalence of obesity among children and adolescents in the area of the province of Granada (Spain) and who did not present a history of digestive or metabolic disorders, were recruited from a school in the city of Guadix (Spain). All participants were recruited randomly from the same high-school and had similar dietary and cultural habits, physical activity and socioeconomic level.

All procedures were in accordance with Institutional and national ethical standards for human experimentation and with the Helsinki declaration of 1975 as revised in 2000. The volunteers' parents or tutors gave written informed consent to a protocol approved by the Institutional Committee on Human Research (Hospital San Cecilio, Granada, Spain). For all individuals, the following parameters were recorded: age, height, weight, BMI, waist circumference, hip circumference, waist-to-hip ratio, systolic and diastolic blood pressure (Table 1). Subjects were divided into two groups, according to their BMI. Children were classified as normal-weight or obese using the Cole et al., (2006) age- and gender-specific obese cut-offs of BMI for children.

Biochemical determinations

After an overnight fast, a baseline blood sample was taken from subjects via a catheter in the cubital vein. Insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, UK) and the Homeostasis Model Assessment (HOMA2-IR) score was
calculated using the HOMA Calculator software (Diabetes Trial Unit, Churchill Hospital, Oxford, UK).

**Inflammatory markers**

Assays of adiponectin, leptin and ceruloplasmin were carried out in serum by conventional ELISAs, following the manufacturer's recommendations (RayBiotech, Inc., Norcross GA, USA for adiponectin and leptin and Assaypro St. Charles, MO, USA for ceruloplasmin).

**Statistical analysis**

Data are expressed as mean values and standard deviations. Correlations between variables were assessed using Pearson’s correlation coefficients. The statistical significance was assessed by an unpaired Student’s t-test. An associated probability (P value) of <5% was considered significant. Analyses were performed using GraphPad Prism 4.03 (GraphPad Software, Inc., San Diego, CA, USA).

**Results**

**Correlations between parameters of insulin resistance and anthropometric values**

Figure 1 shows the correlations between insulin resistance indexes and anthropometric parameters that are related with obesity. All anthropometric parameters correlated positively with both the HOMA2-IR values and the insulin concentration, except for the waist circumference and the waist-to-hip ratio, which correlated only with the HOMA2-IR score (Figure 2D). In contrast, the waist-to-hip ratio correlated with the insulin concentrations in both obese and normal-weight groups (p=0.032 and p=0.036, respectively).

The level of significance was extremely high for the correlation between the BMI and the HOMA2-IR values (p<0.001, Figure 1A) and insulin concentrations (p=0.001, Figure 1B). Correlations with the BMI z-score were also significant, but the level of significance was not as high as that observed for BMI. These correlations were maintained in the group of obese subjects but not in normal-weight adolescents (data not shown).
Both systolic and diastolic pressures correlated well with insulin concentrations (Figure 1F), but the level of significance was higher when the HOMA2-IR score (Figure 1E) was included. Only the correlation of diastolic pressure and insulin was maintained in the normal-weight group but not in the obese group.

**Correlations between insulin resistance parameters and markers of inflammation**

Plasma adiponectin, leptin and ceruloplasmin concentrations, as well as the leptin to adiponectin ratio, from all participants were subjected to correlation with insulin resistance parameters (Figure 2). Adiponectin concentrations (negatively) and the leptin to adiponectin ratio (positively) correlated with HOMA2-IR values (Figure 2A and Figure 2C) but not with insulin concentrations (Figure 2B and Figure 2D). Leptin concentrations did not correlate with either the HOMA2-IR score or insulin concentrations. The highest levels of significance for correlations were found for ceruloplasmin, with \( p<0.001 \) for the correlation with HOMA2-IR values and \( p=0.001 \) with insulin concentrations. In addition, ceruloplasmin concentration was the only parameter that maintained the correlation with insulin concentrations and HOMA2-IR score when participants were divided into groups and the correlation was found for the group of obese adolescents only (data not shown).

**Correlations between BMI and markers of inflammation**

The correlations between the BMI z-score and inflammation markers are depicted on Figure 3. All markers correlated well but the best levels of significance were achieved for adiponectin (\( p<0.001 \), negatively) and ceruloplasmin (\( p<0.001 \), positively). However, when groups were divided according to the presence of obesity the only correlation maintained was that of ceruloplasmin in both the obese (\( p<0.001 \)) and the normal-weight (\( p=0.047 \)) groups (Table 2).

**Discussion**

Obesity is considered as a chronic low-grade inflammatory process, characterized by alterations in the systemic concentrations of some inflammation markers, like adiponectin and leptin, which also correlate with insulin resistance and the metabolic syndrome (Mente et al., 2010; Kajikawa et al., 2011; Krakoff et al., 2003). In the present study we assess if this relationship is also found in obese adolescents.
Unlike adults, in whom BMI values can be compared directly, in children it must be
adjusted for age and gender. An alternative is to use the age- and gender-specific BMI z-
score, which provides a continuous variable. In our study, both BMI and BMI z-score
correlated positively with both the HOMA2-IR values and insulin concentrations with a
high level of significance. The relationship of obesity and insulin resistance is well
known, as both conditions are considered for the diagnosis of the metabolic syndrome
(Grundy et al., 2004). This correlation has also been found in children (Garcés et al.,
2005; Liu et al., 2010) and adolescents (Lee et al., 2006). Total fat mass is thought to
represent the critical risk factor for IR in children and adolescents. Lee et al. (2006)
evaluated the association of adiposity with IR based on HOMA2-IR levels in a larger
population-based sample, finding a direct correlation of BMI with HOMA2-IR. The
presence of insulin resistance and the metabolic syndrome worsens with increasing
obesity and the consequence is the presence of cardiovascular risk biomarkers (Weiss et
al., 2004). BMI and the z-score resulted to be a more precise indicator of insulin
resistance in adolescents than waist circumference and the waist-to-hip ratio, which
confirms the results of others in adults (Rueda-Clausen et al., 2010). In the present
study, the other anthropometric parameters related to the metabolic syndrome that were
measured, waist circumference, waist-to-hip ratio, systolic pressure and diastolic
pressure, also correlated well with insulin levels and the HOMA2-IR score. This is in
agreement with what has been previously reported for children (Abdullah et al., 2009;
Bitsori et al., 2009; Zeelie et al., 2010).

Despite the strong correlations found for anthropometric parameters, not all adipokines
correlated with markers of insulin resistance. Adiponectin did not correlate with insulin
levels (although a tendency was observed, p=0.080) and leptin did not correlate with
either HOMA2-IR or insulin. However, ceruloplasmin correlated very strongly with
both parameters. Adipocytokines are signaling molecules released by adipose tissue
with numerous functions, including their participation in inflammatory processes. These
molecules are particularly interesting in childhood, considering the rising prevalence of
obesity and the linkage of this condition to inflammation. Plasma concentrations of
adiponectin and leptin are altered in obese subjects and correlate with insulin resistance
and the metabolic syndrome (Mente et al., 2010; Kajikawa et al., 2011; Krakoff et al.,
2010). Esteghamati et al., (2009) in a group of 387 Iranian adults and Goropashnaya et
al., (2009) in 669 adult Eskimos found significant correlations between HOMA2-IR
with leptin and adiponectin, respectively. However, Kajikawa et al., (2011) was unable to significantly correlate adiponectin with HOMA2-IR and leptin and adiponectin with insulin concentrations in adult patients with at least one coronary risk factor. We also found a negative correlation between adiponectin and HOMA2-IR in the group of adolescents studied, but no association with leptin. After assessing the correlations of HOMA2-IR with total and high-molecular weight adiponectin in 70 prepubertal children, Martos-Moreno et al., (2010) concluded that the impairment of adiponectin levels in childhood obesity is different to that in elder obese patients and Mangge et al., (2008) suggested that preatherosclerosis in obese juveniles and adolescents is associated with altered subfractions of adiponectin. Therefore, these studies show that there is evidence pointing out to the relationship between inflammation markers and insulin resistance in adults, but that it is not always the case in adolescents.

In our study the strongest correlation between an inflammatory marker and insulin resistance was found for ceruloplasmin. Ceruloplasmin is related to systemic inflammation and has been found to be elevated in subjects with metabolic syndrome (Kim et al., 2002). To our knowledge, this is the first study reporting a positive correlation between insulin resistance and ceruloplasmin, which was observed in the whole group of participants and in the group of obese adolescents. A significant correlation of ceruloplasmin with BMI and WHR has been reported in male adolescents (Wärnberg et al., 2006) and was confirmed recently in a mixed-gender group (Aguilar et al., 2011; Aguilar et al., 2010; Aguilar et al., 2011).

In the present study ceruloplasmin also correlated with the BMI z-score in both groups, although it was stronger among the obese participants, suggesting that ceruloplasmin can be considered not only as a good marker for the excess of body fat but also for insulin resistance in adolescents. Ceruloplasmin is expressed mainly in the liver, but also by monocytes, astrocytes and Sertoli cells and is secreted as a α2-glycoprotein in plasma (Fox et al., 2000). It also forms part of the inflammation-sensitive protein family, which that includes α1-antitrypsin, haptoglobin, orosomucoid and fibrinogen (Lee et al., 2006). High levels of ceruloplasmin have been associated with cardiovascular risk factors like hypercholesterolemia, increased body weight diabetes and hypertension (Uriu et al., 2005). It has been indicated that serum ceruloplasmin could be an independent risk factor for cardiovascular disease (Mänttäri et al., 1994; Reunanen et al., 1998), but the mechanism is not established. Ceruloplasmin can
oxidatively modify LDL in vitro (Ehrenwald et al., 1994; Lamb et al., 1994) and act synergistically with other cardiovascular risk factors in vivo (Reunanen et al., 1992). Additionally, ceruloplasmin levels are elevated in subjects with metabolic syndrome (Kim et al., 2002) and those with insulin resistance (Lee, 2001).

Therefore, our results point out to a relationship between insulin resistance and inflammation in adolescents, which is particularly strong in obese individuals. Among the inflammation markers studied, ceruloplasmin revealed as a potential strong marker of insulin resistance in obese adolescents. However, the mechanisms relating ceruloplasmin and insulin resistance need to be unveiled and the results should be confirmed in a larger group of adolescents.

Relevance to clinical practice.
The results obtained in the present study imply a significant advance in the field of clinical practice of nursing, since only by means of an adequate understanding of the associations between obesity and the inherent inflammatory processes, nursing personnel will be able to develop adequate prevention procedures of the disease and its complications in adolescents. Despite the number of studies dealing with obesity in adults there is still scarce information available on the relationship of insulin resistance and inflammation in obese infants and adolescents. The results obtained here may constitute a first step in the investigation of the inflammatory alterations related to the metabolic syndrome in the pediatric population.

Acknowledgements
We would like to thank the individual volunteers, their tutors and nurses who generously gave up their time to participate in this study. This work was supported by the “Ayuntamiento de Guadix” (Spain) and was funded, in part, by a grant of the Spanish Ministry of Science and Innovation (AGL2011-23810) and Hospital San Cecilio.

Conflicts of interest
The authors declare no conflict of interest
Contributions
Study design: MJA, EG; data collection: MJA, EG, AA; data analysis: JS; and manuscript preparation: MJA, EG, JS.

References


Table 1. Anthropometric characteristics of the volunteers enrolled in the study.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Non-obese</th>
<th>Obese</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td>Mean  SD</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>14.0 1.1</td>
<td>13.9 1.0</td>
<td>14.1 1.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.4 19.8</td>
<td>57.5 11.4</td>
<td>88.5 13.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.2 6.4</td>
<td>21.5 2.9</td>
<td>33.1 2.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI z-score</td>
<td>1.47 1.14</td>
<td>0.49 1.02</td>
<td>2.28 0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic Pressure (mmHg)</td>
<td>129.6 15.3</td>
<td>113.5 7.8</td>
<td>141.5 4.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic Pressure (mmHg)</td>
<td>70.0  8.8</td>
<td>60.3  2.0</td>
<td>77.2  2.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.8  15.3</td>
<td>78.3 14.3</td>
<td>98.2  9.5</td>
<td>0.0003</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>98.9 12.3</td>
<td>90.2 11.1</td>
<td>105.3 8.9</td>
<td>0.0005</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.90 0.07</td>
<td>0.86 0.07</td>
<td>0.93 0.05</td>
<td>0.0114</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation (SD) for the whole group of volunteers (All, n=24), the group non-obese volunteers (n = 12) and the group of obese volunteers (n = 12). p: statistical significance obese vs. non-obese groups (Student's t-test). n.s.: not significant.
Table 2. Correlations between inflammation markers and BMI z-score in the obese and non-obese groups.

<table>
<thead>
<tr>
<th></th>
<th>Adiponectin</th>
<th>Leptin</th>
<th>Lep/Adip Ratio</th>
<th>Ceruloplasmin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-obese</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12) Pearson's r</td>
<td>-0.2206</td>
<td>0.5128</td>
<td>0.4733</td>
<td>0.6393</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>0.5402</td>
<td>0.1296</td>
<td>0.1671</td>
<td>0.0466</td>
</tr>
<tr>
<td><strong>Obese</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=12) Pearson's r</td>
<td>-0.2535</td>
<td>-0.4862</td>
<td>-0.1328</td>
<td>0.8553</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>0.4266</td>
<td>0.1090</td>
<td>0.6808</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; P, statistical significance of the correlation; Lep/Adip ratio, leptin-to-adiponectin ratio.
Figure 1. Correlations between anthropometric parameters and markers of insulin resistance in the whole group of volunteers (n=24), regardless of BMI. Abbreviations: HOMA2-IR, homeostasis model assessment for insulin resistance; P, statistical significance of the correlation according to Pearson’s; BMI, body mass index.

59x40mm (300 x 300 DPI)
Figure 2. Correlations between inflammation markers and insulin resistance in the whole group of volunteers (n=24), regardless of BMI. Abbreviations: HOMA2-IR, homeostasis model assessment for insulin resistance; P, statistical significance of the correlation according to Pearson's; Lep/Adip ratio, leptin-to-adiponectin ratio.

67x62mm (300 x 300 DPI)
Figure 3. Correlations between inflammation markers and BMI z-score in the whole group of volunteers (n=24), regardless of BMI. Abbreviations: HOMA2-IR, homeostasis model assessment for insulin resistance; P, statistical significance of the correlation according to Pearson's; BMI, body mass index; Lep/Adip ratio, leptin-to-adiponectin ratio.

46x24mm (300 x 300 DPI)