Body fat and lipid profile of monozygotic twins discordant for insulin resistance

Gordura corporal e perfil lipídico de gêmeos monozigóticos discordantes para resistência à insulina

Timothy Gustavo Cavazzotto¹
Marcos Roberto Queiroga¹,³
Sandra Aires Ferreira¹
Eduardo Kokubun²
Maria Raquel de Oliveira Bueno¹
Helio Serassuelo Junior¹

Abstract – The study investigated alterations in body fat and metabolic profile of adolescent monozygotic twins, resulting from discordance for insulin resistance, adjusted for physical activity, physical fitness and heredity. Thirty-eight pairs of monozygotic twins were assessed for anthropometric measurements to estimate body fat. Physical fitness was estimated with treadmill test and use of ergospirometer. Daily physical activity was estimated from the daily count of steps measured by a pedometer during 3 days. Fasting blood samples were used to determine blood glucose, insulin, lipid parameters. The Homa-IR and HOMA-β indexes were calculated. Twins with measures higher than 2.5 were considered insulin resistant. When both brothers were below or above cutoffs, the pair was allocated to the concordant group. When one brother was insulin resistant and the other was not, the pair was allocated in the discordant group. Twins were compared using paired test. In the discordant group, it was observed that insulin-resistant twins had higher birth weight values, bodyweight, BMI, waist circumference, body fat percentage, body fat (sum of skinfolds), Homa-β index and lower HDL compared to their corresponding pair. Insulin-resistant twins showed higher values in anthropometry and body composition, as well as in the glycemia and insulin index and lower HDL. These events may have been unchained by metabolic alterations possibly originating from gestational stage, however, modulated by body composition.

Key words: Body composition; Monozygotic twins; Newborn; Physical fitness.

Resumo – O objetivo do estudo foi investigar alterações na composição corporal e perfil metabólico de gêmeos monozigóticos adolescentes, decorrentes da discordância para resistência à insulina, ajustados para atividade física, aptidão cardiorrespiratória e hereditariedade. Participaram do estudo 38 pares de gêmeos monozigóticos (11 a 17 anos). Foram obtidas as medidas antrópométricas de massa corporal (MC), estatura, circunferência da cintura (CC) e espessuras de dobras cutâneas (EDC). Todos os gêmeos foram submetidos a teste de esforço máximo em esteira rolante com análise direta dos gases (VO2max), avaliação da atividade física diária por meio de pedômetros, a coleta de sangue em jejum para estimativa da glicemia, insulina e perfil lipídico, e posterior estimativa do índice HOMA-RI e HOMA-β. Os pares onde os irmãos apresentavam-se ambos abaixo ou acima do ponto de corte (Homa-RI < 2,5) foram alocados no grupo concordante (GC). Quando um irmão era resistente e outro não resistente à insulina, este par foi alocado no grupo discordante (GD). Foi observado, no GD, que os gêmeos resistentes à insulina, apresentavam maiores valores de peso de nascimento, MC, IMC, CC, percentual de gordura, adiposidade corporal (soma EDC) e índice Homa-β, além de menor valor de HDL comparado aos seus pares correspondentes. Jovens resistentes à insulina apresentaram valores superiores na antropometria e composição corporal, bem como, índices glicêmicos e insulinênicos e menor HDL. Estes eventos podem ter sido desencadeados pelas alterações metabólicas possivelmente originadas na fase gestacional, porém, moduladas pela composição corporal.

Palavras-chave: Composição corporal; Gêmeos monozigóticos; Recém-Nascido; aptidão física.
INTRODUCTION

A regrettable progression in the onset of metabolic diseases among young people has been observed in the last decades\textsuperscript{1-3}. Coincidentally, this population has shown a reduction in the daily physical activity levels, reduction of hours in physical exercise and increase in hours spent with sedentary activities\textsuperscript{4}.

Insulin resistance (IR) is one of the metabolic diseases in evidence, since it is associated with obesity and compromises the metabolic glucose system to such an extent that it is considered a precursor of type 2 diabetes mellitus (DM2) and metabolic syndrome\textsuperscript{5,6}.

Some studies have shown excessive inflammatory markers expressed in obese adolescents, which hinders the interaction between insulin and insulin receptors in the muscle tissue\textsuperscript{5-7}. Consequently, there is an overload in the release of this hormone into the bloodstream\textsuperscript{5-7}. Nevertheless, glucose uptake appears to remain normalized for some years. However, there is a reduction of glucose uptake by insulin pathways over time in this state and the development of DM2 is considered the next step. Insulin resistance rarely have symptoms, and some studies have indicated that the hyperinsulin status may occur for years\textsuperscript{8,9}.

Currently, the prevalence of DM2 has advanced at increasingly younger ages, so it is acceptable to indicate that these diseases, especially insulin resistance, begin in childhood and adolescence and can be a reflex of the gestational phase\textsuperscript{10,11}.

Subjects with history of DM2 presented lower glucose uptake through insulin pathways; however, the low cardiorespiratory fitness observed in subjects with history of the disease does not allow confirming the hereditary effect\textsuperscript{12}.

In order to respond more adequately to previously established issues, the case-control study with monozygotic twins is an excellent methodological resource\textsuperscript{13,14}. This type of research allowed investigating differences between siblings from a specific discordance, showing little or no hereditary influence\textsuperscript{13}, and suggesting a possible environmental origin of the discordance.

The aim of the present study was to investigate changes in body composition and metabolic profile of adolescent monozygotic twins as a result of discordance, without the influence of physical activity and cardiorespiratory fitness.

METHODOLOGICAL PROCEDURES

Sample

All twins of the same sex aged 11-18 enrolled in the public and private education systems of the city of Rio Claro, SP, were invited to participate in the study. A total of 98 pairs of twins of the same sex were included; however, 13 pairs were not located and 31 refused the invitation. In the end, 54 pairs accepted to participate in the research. To classify zygosit
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The twins were submitted to peripheral blood collection and subsequent DNA analysis. In the evaluated sample, 38 pairs of twins were evaluated as MZ and 16 as DZ. Considering the aims of this study, only results of the 38 MZ pairs were used. The present study was approved by the Ethics Committee under protocol number 5093/2009, Unesp - Rio Claro and participants and / or parents signed the free and informed consent form.

Zygosity assessment

The attribution of monozygosity or dizygosity to twins was performed after the evaluation of the agreement of twins in relation to polymorphic genetic markers (DNA), such as microsatellite loci genes, also known by the acronym STR (Short Tandem Repeat)\textsuperscript{14,15}. For the analysis, approximately 20 µL of blood from each participant was pipetted directly to the QIAcard FIA Spots of QIAGEN with Whatman\textsuperscript{®} FTA technology where later DNA extraction was performed through the Whatman\textsuperscript{®} FTA reagent. DNA analysis of twins occurred using the DNA extraction and amplification technique by polymerase chain reaction (PCR). In all DNA samples, the analysis of 16 autosomal STRs (CSF1PO, D2S1338, D3S1358, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, D5S51, FGA, TH01, TPOX, vWA and the Amelogenin locus) was performed by PCR amplification using the commercial Identifiler kit (AB Applied Biosystems) according to manufacturer’s instructions. Genotyping was performed on ABI 310 Genetic Analyzer apparatus (AB Applied Biosystems), according to manufacturer’s instructions, by determining the size of DNA fragments and comparing with allelic scales provided with commercial kits.

Tests and measurements

Anthropometric measurements of body mass (BM) and height were evaluated and the Body Mass Index (BMI) was calculated. Waist circumference (WC) was measured in duplicate at the midpoint between the last ribs and the iliac crest using an inextensible tape measure (Mabis\textsuperscript{®} / Japan). Triceps and subscapular skinfolds were measured using Harpenden\textsuperscript{®} brand caliper. All these procedures were performed according to international standards\textsuperscript{16}. Fat percentage was determined using equation developed by Slaughter et al.\textsuperscript{17}. Birth weight (BW) was collected from participants’ birth records. When the document was not found, the birth weight reported by parents or guardian was requested. The use of birth weight reported by parents has shown high correlation with measured birth weight\textsuperscript{19}.

Cardiorespiratory fitness and daily physical activity

The test to determine the maximum oxygen uptake (VO\textsubscript{2peak}) was performed at the NAFES / UNESP (Physical Activity, Sport and Health Center). Prior to the test date, subjects received the following guidelines: do not ingest alcoholic beverages or stimulants and avoid vigorous physical activity.
on the previous day. The test was performed on a treadmill model ATL Super® (Inbrasport), without slope, in the morning and afternoon periods at 09:00-11:30 am and 02:00-06:00 pm, respectively, with controlled ambient temperature of 20 to 25º C.

After guidelines and explanations regarding the accomplishment and interruption of the test, a period of 5 to 10 minutes of familiarization at different speeds (4 to 7 km/h) was given to subjects, following one minute at rest on the treadmill in orthostatic position. The test started with speed of 4 km / h and a progressive increase of the workload of 1 km / h every minute. Verbal encouragement was used in an attempt to achieve maximum physical effort. The criteria used to interrupt the test were voluntary exhaustion, respiratory exchange rate (QR) greater than 1.15, perceived exertion score of 20 (Borg scale from 6 to 20) and predicted maximum heart rate (220-age). At rest and during the test, the minute volume (VE), the oxygen uptake (VO$_2$) and the carbon dioxide production (VCO$_2$) were continuously recorded by the analysis of pulmonary gas exchange (MedGraphics VO2000® metabolic analyzer - Aerosport Inc.). VO$_{2peak}$ was collected breath by breath and the value adopted for data analysis was recorded as the average oxygen consumption in the 30 seconds that preceded the test interruption.

**Habitual physical activity**

For the determination of habitual physical activity (HPA), the activities (steps) performed during three days were recorded, of which two of these, week days (Monday to Friday) and one day of the weekend (Saturday or Sunday). The pedometer model used was Yamax Digi-Walker SW 701. Participants were told to use the pedometer all day long, and to take it off only for bathing or swimming. At the end of the day, they recorded the value of steps taken. From the weighted average of the three days, habitual PA was calculated according to the following equation: $PA = \frac{[(\text{Average steps week days} \times 5) + (\text{Steps day of the weekend} \times 2)]}{7}$.

**Biological samples**

The twins, accompanied by their parents / guardians, were instructed and attended the Hemodiag clinical analysis laboratory in Rio Claro in the condition of nocturnal fasting (10 to 12 hours). Blood collection was performed by puncture of the antecubital vein using the vacuum collection system (Vacutainer™ Becton Dickinson Company, Plymouth, UK), in 4.0 mL tubes with anticoagulant (fluoride associated to EDTA 1 mg / mL blood and EDTA 1 mg / mL) and 3.5 mL heparin tubes. Plasma was used to determine the lipid concentration, HDL, LDL, Total Cholesterol and triglycerides, as well as fasting glucose and insulin. The estimation of insulin resistance and beta cell function was performed using the Homa index (Homa-IR and Homa-β, respectively), which is known as the glycemic homeostatic model (homeostasis model assessment)\(^{20}\). The Homa-IR and the Homa-β indexes were calculated using the formula: Homa-IR =
(fasting insulin (µU / mL) x fasting glucose (mmol / L)) / 22.5; Homa-β = 20x (fasting insulin (µU / mL) / (fasting glycemia (mmol / L)) - 3.5.

To determine the fasting concentrations of glucose, insulin and lipids (HDL-C, TG, TC), specific kits were used. LDL-C cholesterol was estimated using Friedewald's formula: LDL-C = TC-HDL - (TG / 5)\(^2\).

**Statistical analysis**

Twins were grouped into higher and lower Homa-IR index, and were separated according to the disagreement or agreement for the index cutoff point (Homa-IR = 2.5). Groups were compared using the Wilcoxon paired test.

In a second moment, the differences observed by the disagreement in the Homa-IR index 2.5 in the variables were described in absolute frequency. Thus, twins were separated according to the presence of a greater or lower body fat percentage, and the number of adolescents with an index higher than the cutoff point to characterize IR was counted.

**RESULTS**

Overall, the twins had mean age of 14.5 years, ranging from 11 to 18 years. The pairs of male twins presented median 13.0, ranging from 11 to 17 years, whereas the pairs of female twins presented median 15.0, ranging from 11 and 18 years.

In the body composition results, a significant difference (p<0.05) was observed for BW, body mass, BMI, waist circumference, skinfold sum and body fat percentage, favoring twins of the insulin resistance group among those discordant for Homa-IR (Table 1).

**Table 1.** Body composition and anthropometric characteristics of concordant and discordant monozygotic (MZ) for IR

<table>
<thead>
<tr>
<th></th>
<th>MZ concordant for IR (n=28 pairs)</th>
<th>MZ discordant for IR (n=10 pairs)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Twins &gt; HOMA-IR</td>
<td>Twins &lt; HOMA-IR</td>
</tr>
<tr>
<td></td>
<td>Median P75-P25</td>
<td>Median P75-P25</td>
</tr>
<tr>
<td>Birth Weight (g)</td>
<td>2390.0 600.0</td>
<td>2210 490.0</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>47.5 18.9</td>
<td>48.6 15.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.6 12.4</td>
<td>157.8 12.4</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>18.9 3.1</td>
<td>18.8 3.2</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>65.0 9.0</td>
<td>66.3 9.5</td>
</tr>
<tr>
<td>Sum (mm)</td>
<td>20.5 13.0</td>
<td>19.9 9.7</td>
</tr>
<tr>
<td>%BF (%)</td>
<td>18.8 10.1</td>
<td>17.4 8.1</td>
</tr>
</tbody>
</table>

MZ: Monozygotes; BMI: body mass index (BM/m\(^2\)); WC: waist circumference; SUM: sum of skinfolds; %BF: body fat percentage; HOMA: homeostasis model assessment; IR: Insulin Resistance. P75-P25: Interquartile Interval.

For the metabolic results (Table 2), a significant difference was observed for HDL, lower for the Insulin resistance group. Only fasting insulin and the HOMA-IR index presented differences for concordants and discordants, with higher values for the insulin resistance group among discordants.
This result was accompanied by the absence of significant differences for physical activity and cardiorespiratory fitness.

When counting adolescents with index higher than the cutoff point (HOMA-IR = 2.5), it was observed that the majority (10 adolescents) had higher fat percentage compared to their siblings (figure 1).

Table 2. Daily physical activity (step / day), cardiorespiratory fitness (VO2), glycemia and fasting insulin of monozygotic twins (MZ) concordant and discordant for IR

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>G &gt; HOMA-IR</td>
<td>G &lt; HOMA-IR</td>
</tr>
<tr>
<td>Median</td>
<td>P75-P25</td>
<td>Median</td>
</tr>
<tr>
<td>VO2peak (ml.kg.min⁻¹)</td>
<td>42.5</td>
<td>14.5</td>
</tr>
<tr>
<td>VO2peak (L.min⁻¹)</td>
<td>1.8</td>
<td>0.8</td>
</tr>
<tr>
<td>DPA (steps/day)</td>
<td>11487.0</td>
<td>5724.8</td>
</tr>
<tr>
<td>GLI j (mg.dL⁻¹)</td>
<td>85.5</td>
<td>11.0</td>
</tr>
<tr>
<td>GLI 1h (mg.dL⁻¹)</td>
<td>93.0</td>
<td>22.0</td>
</tr>
<tr>
<td>INS fasting (uU/ml)</td>
<td>7.2</td>
<td>3.4</td>
</tr>
<tr>
<td>INS 2h (uU/ml)</td>
<td>37.7</td>
<td>3.0</td>
</tr>
<tr>
<td>HOMA-IR (UA)</td>
<td>1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>HOMA-β (UA)</td>
<td>109.5</td>
<td>70.8</td>
</tr>
<tr>
<td>TC (mg.dL⁻¹)</td>
<td>152.5</td>
<td>34.0</td>
</tr>
<tr>
<td>HDL (mg.dL⁻¹)</td>
<td>43.5</td>
<td>13.0</td>
</tr>
<tr>
<td>LDL (mg.dL⁻¹)</td>
<td>96.2</td>
<td>39.2</td>
</tr>
<tr>
<td>TG (mg.dL⁻¹)</td>
<td>69.0</td>
<td>44.0</td>
</tr>
</tbody>
</table>


This result was accompanied by the absence of significant differences for physical activity and cardiorespiratory fitness.

When counting adolescents with index higher than the cutoff point (HOMA-IR = 2.5), it was observed that the majority (10 adolescents) had higher fat percentage compared to their siblings (figure 1).

Figure 1. Body fat percentage and HOMA-IR among monozygotic (MZ) twins concordant and discordant for IR

DISCUSSION

The results confirmed some hypotheses raised in literature over the years \(^{7,10,11}\). At the same time, it was observed that the development of compensatory body fat may not be the basis of the metabolic problem, since siblings who presented higher %BF also presented higher BW and greater insulin sensitivity, contrary to some hypotheses in this subject such as the theory of fetal disease development \(^{11}\).
In the present study, it was observed that twins with HOMA-IR index greater than 2.5 presented higher BM, BMI, WC, BF% and body fat values (sum of skinfolds) compared to their corresponding pairs with HOMA-IR index lower than 2.5. In addition, the results are controlled, with little or no influence of heredity, as well as similarities in physical activity level and cardiorespiratory fitness among pairs. However, when analyzing the complex initiated at BW up to the development of IR in adolescence, it is evident that the path may be a reflection of mechanisms still unknown.

It is known that low BW has been correlated to a variety of metabolic diseases. In a systematic review published in 2008, it was observed that at every 1 kg increased in birth weight reduced the development of DM2 in adolescence and adult life by 20%. Moreover, even after adjustment for socioeconomic status, BMI, age and sex, and exclusion of macrosomic participants, the results remained significant. Other systematic reviews found similar results for metabolic syndrome, obesity and cardiovascular diseases. The results of reviews point to an association between low BW and metabolic diseases; however, many studies that were included in the systematic reviews did not take into account some criteria, which were considered in the present study. The majority of systematic reviews used epidemiological studies, especially cohort studies, which presents difficulties in explaining the difference between environmental and genetic causes, which is better investigated in studies with twins. Thus, factors such as body adiposity and physical activity practice need to be explored, preferably isolated from hereditary effects, since the time interval between birth and the onset of diseases is rarely short.

Understanding the role of adipose tissue in triggering IR for subjects born with low BW was addressed in a review. The observed results suggest that individuals with growth restriction in the gestational phase may exhibit accelerated (compensatory) but unbalanced growth in early childhood, which is associated with increase of adipose tissue. In addition, individuals born with lower birth weight present alterations in the distribution of muscle fibers, which may reduce GLUT4 expression in muscle and adipose tissue, an important insulin receptor.

The role of adipose tissue in the development of IR still needs to be elucidated; however, some results already indicate the pathways to be followed. Animals with growth restriction in the gestational phase and subsequent compensatory growth present an increase in adipocyte size, up-regulation in the expression of genes that control the glucose flow towards lipogenesis, increase in the expression of genes involved in lipogenesis and angiogenesis, and inflammatory process.

In the present study, the results were different from this process, since greater %BF was observed for the sibling with higher BW. Among the possibilities to explain this fact, the metabolic pathways can have an intervening effect in this network of events. At molecular level, insulin resistance has strong association with the expression of genes related to peripheral receptors. In this sense, PPARγ (Peroxisome Proliferator-Activated Recep-
tor Gamma) is a marker of increased insulin sensitivity. Elevated serum levels of PPARγ mRNA expression are associated with adipogenesis and glycemic homeostasis control. Thus, it is likely that modulations in adipose tissue may be associated with increased PPARγ expression in insulin-resistant subjects. This fact would explain the higher insulin sensitivity index values (HOMA-β) in IR young subjects with greater %BF however, it is not possible to discuss its connection with higher BW.

Another interesting result of the present study is the observation of lower HDL concentration in the group of insulin-resistant twins and with higher BW and %BF compared to their corresponding non insulin-resistant pairs with lower BW and BF%. This fact is contrary to findings observed in literature. At each reduction of 1 kg/m² in BMI of the BW, an increase of 0.051-mmol/L of cholesterol was observed, except for HDL. Thus, an association between low BW and dyslipidemia has been suggested.

Finally, the understanding of these results must be accompanied by the understanding of the study limitations. Initially, the analytical and cross-sectional observational characteristic is highlighted. However, as a strong point of the study, the methodology controlled by the comparison of monozygotic twins stands out, which allows the isolation of observations, with little or no influence of heredity.

**CONCLUSION**

Insulin-resistant young subjects had higher values in anthropometry and body composition, as well as glycemic and insulin indexes and HDL. These events may have been triggered at distal level by birth weight and due to metabolic alterations possibly originated in the gestational phase. Thus, under conditions of hereditary and genomic similarity, the difference between twins was conditioned by environmental and behavioral factors.

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CORRESPONDING AUTHOR
Maria Raquel de Oliveira Bueno
E-mail:netzer87@hotmail.com