Visceral adiposity influences glucose and glycogen metabolism in control and hyperlipidic-fed animals

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Abstract

Introduction: Evidences suggest that fat intake, visceral obesity and intracellular lipids are related to insulin impairment.

Objective: The objective of the present paper was correlate visceral obesity and metabolic alterations in control (CTR) and hyperlipidic cafeteria diet (CFT) fed animals.

Methods: After 6 months of diet treatment, liver and muscle of the male rats were utilized to determined glucose uptake and glycogen metabolism after administration of 0.4 IU/kg insulin in vivo, and correlate the visceral adiposity to these two parameters.

Results: Ample range of physiologic answers to body composition in metabolic profile of the both diets was found. No differences were found in glycemia and triacylglycerol after insulin action in both groups, however CFT group accumulated higher adiposity, mostly visceral fat, and showed lower glycogen content in the liver. We also found an inverse correlation between visceral adiposity and glucose uptake and a decrease of the glycogen synthase active form in the liver. CTR animals demonstrated an inverse correlation between glucose uptake and visceral adiposity in the muscle.

Discussion and conclusion: It was observed a variability of metabolic alterations in animals which can be related to degree of accumulation of abdominal adiposity and ingestion of diet fats. Further studies will be required to clarify the reasons for the observed liver alterations in CFT and muscle alterations in CTR animals.

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Key words: Insulin impairment. Visceral adiposity. Glucose uptake. Glycogen metabolism.

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Abbreviations

CTR: Control.
CFT: Cafeteria.
WAT: White adipose tissue.
BAT: Brown adipose tissue.
GS: Glycogen synthase.
GSI: Active form of the glycogen synthase.
GST: Total form of the glycogen synthase.

Introduction

Obesity is one of the most prevalent and incident diseases worldwide and can lead to insulin resistance, diabetes mellitus type 2 and cardiovascular diseases.1,2

Insulin resistance is characterized by inadequate insulin action to produce a normal response in peripheric tissues.3 Accumulation of lipids and their metabolites, including fatty acyl CoAs, diacylglycerols, and ceramides inside the cells, caused by hyperlipidic diets or by visceral obesity, can damage insulin receptor signal transduction.4,5 As a consequence it increases hepatic gluconeogenesis and causes glycogen depletion by altering glycogen synthase (GS) enzymatic activity in liver and muscle.6,7,8

Rodents are useful experimental models to study the insulin resistance caused by hyperlipidic diets, visceral obesity and genetic factors. Genetically obese mice demonstrating differences in body composition and in insulin action—with or without resistance—after nine months of high-fat diet.9

The cafeteria (CFT) diet can mimic the high energy foods that are ingested by human subjects being considered a robust model of obesity and metabolic syndromes. It was demonstrated previously that CFT diet induced inflammation in liver and white and brown adipose tissues.10

But not only obese subjects can develop an impairment of insulin action. Lean and overweight subjects can accumulate higher levels of intramyocellular lipids and its metabolites and as a consequence presented a decrease of glucose uptake in myocytes.5,11 The aim of the present study was to analyze the correlation of visceral adiposity and glucose and glycogen metabolism in muscle and liver of control (CTR) and cafeteria (CFT) feeding animals after six-months of treatment. In addition, the variability of the body composition and physiologic responses involving glucose and glycogen metabolism to CTR and CFT diet was also considered.

Methods

Chemicals

The analytical degree reagents utilized in the experiments were obtained from Merck SA, Porto Alegre, Brazil. 2-Deoxi-1-13C-glucose (55 mCi/mmol) and D-[U-14C] glucose (3.0 mCi/mmol) were purchased from Amersham.

Animals and treatments

Twenty-one-day-old male Wistar rats supplied by the Instituto de Ciências Básicas da Saúde (Universidade Federal do Rio Grande do Sul - UFRGS, Porto Alegre, Brazil), were housed at 22-24°C and controlled humidity (70-80%) under a 12 h artificial light cycle (8 am to 8 pm). The animals were randomly assigned to two different dietary groups during six months: control (CTR) and high fat – cafeteria diet (CFT).

The control animals (n = 9) were fed a standard pelleted diet (Nuvital CR-1 6002–NUVILAB, Curitiba, Paraná, Brazil) containing 25.6% of energy as protein, 63.4% of energy as total carbohydrates, and 11% of energy as fat (% of kcal). Water was provided ad libitum.

The CFT group (n = 11) was fed a high-fat diet containing 15% of energy as protein, 60% of energy as total carbohydrates, and 25% of energy as fat (% of kcal). The cafeteria diet was composed of sweet biscuits, bread, french fries, chocolate, fatty ham, banana, liver pate, sausage, fatty cheese, condensed milk, and smoked bacon. All foods of CFT diet and water were offered in natura and ad libitum with the standard diet. Food intake was measured when the animals were four months old and during four weeks. Carbohydrate, lipid, and protein contents of the food constituents were determined by the Instituto de Ciências e Tecnologia de Alimentos at UFRGS.

Special care was taken to limit the number of animals used and their suffering. All animal procedures followed in this study were in accordance with the Principles of Laboratory Animal Care (COBEA-Brazilian College of Animal Experimentation), and the experimental protocol was approved by the UFRGS Animal Care Committee.

Experimental procedure

After six months of treatment, and two days before the experimental procedure, the animals received a canula in a vein through a surgical procedure. One day before the experiment, the animals were submitted to 8 h of fasting and on the day of the experiment the rats were weighed and received 0.4 IU/kg of human insulin. An aliquot of blood was removed from the canula for dosage of fasting glycemia (0 min) and after 16 min of the insulin administration. The blood was centrifuged and the plasma used to measure plasma glucose by an enzymatic glucoseoxidase method (Glucose PAP Liquiform-Kit Labtest Diagnostics). Triacylglycerol was also measured after fasting (0 min) by an enzymatic method according to the manufacturer’s instructions (Trinder Liquiform-Kit Labtest Diagnostics).
Both measurements were performed with the help of the Espectrofotometer Ultraspec 2000 (Pharmacia Biotech) using wave lengths of 505 nm and 520 nm for glucose and triacylglycerol, respectively. The values of glycemia and triacylglycerol were expressed as mg/dL.

Body composition

The animals were weighed and measured (length were measured in cm) after submitted to any treatments or diets. After insulin administration, the animals were sacrificed by ether. White (WAT) and brown adipose tissue (BAT) were dissected from abdomen and perilumbar regions, respectively, and weighed to verify the total adipose tissue. Free-fat weight was obtained by the subtraction of the total weight from WAT and BAT. All weights described are expressed in grams (g).

We also calculated the WAT index obtained by the division of weight of WAT per total weight; the BAT index obtained by the division of weight of BAT per total weight; and the free-fat weight index obtained by the division of weight of free-fat mass per total weight. All the indexes are expressed in percentage (%).

After the experiment, the liver and the gastrocnemic muscle were also dissected and divided in three parts. The first was incubated with 2-Deoxi-D-1-14C-Glucose to assess glucose uptake or with D-[U-14C] glucose to verify 14C-glycogen synthesis. The second was used to verify the total glycogen content, and the third was frozen in nitrogen and utilized to assess glycogen synthase (GS) activity.

In vitro experiments: 2-14C-DeoxiGlucose uptake and 14C-glycogen synthesis

To determine glucose uptake and glycogen synthesis in vitro, liver and muscle were rapidly removed and placed on a Petri dish containing cold incubation buffer and cut in slices.

To measure [2-Deoxi-1-14C-glucose] glucose uptake, slices (about 200 mg/tube) were incubated in 1 mL Krebs Ringer bicarbonate (KRb) buffer, pH 7.4, at 37º C with constant shaking and containing 0.15 mCi 2-Deoxi-1-14C-glucose. The contents of the tubes were gassed with O2:CO2 (95:5, v/v) for 1 min and then closed. The tissues were incubated in a Dubnoff incubator at 37º C for 1 h with constant shaking. After incubation, the tissues were withdrawn, rinsed in cold incubation buffer (three times) and blotted with filter paper. The 2-Deoxi-1-14C-glucose was extracted from the tissue and the radioactivity was measured in LKB-Wallac scintillation counter. Results are expressed in pmol/mg of tissue/ incubation time.

Hepatic and muscle glycogen content in vivo

Hepatic and muscle tissues were frozen at -20º C until ready for analysis. Glycogen was extracted essentially according to Van Handel, and determined as glucose after acid hydrolysis, using rabbit glycogen as standard. Glucose content after hydrolysis was determined by enzymatic glucoseoxidase method (Glucose PAP Liquiform-Kit Labtest) and measured in espectrofotometer (Espectrofotometer Ultraspec 2000-Pharmacia Biotech) using 505 nm wave length. Values were expressed as g% of glycogen.

Glycogen synthase (GS) activity assay

To verify GS activity, liver and muscle were quickly dissected, frozen on liquid nitrogen, and kept at -70º C until ready for analysis. GS activity was measured according to Nuttall and Gannon, but following the modifications described previously. Protein concentration was determined by the method described by Bradford. Enzyme activity was calculated in nmoles of UDP-glucose-U-14C incorporated into glycogen/ milligram of protein/minute.

Statistical analyzes

Data from the experimental procedures were used to compare the effects of insulin in the serum glucose of the CTR versus CFT diets by two-way ANOVA followed by Tukey’s least significance test. Student’s t-test was used to verify statistical differences between glycemia at fasting (0 min) and 16 min after insulin administration and to compare the glycemia between CTR and CFT groups for each time (0 or 16 min).

Differences in food intake along the four weeks were analyzed by one-way ANOVA and next by Tukey’s least significance test. The other experimental conditions between the two groups were compared using
Results

Food intake

Food intake of the CFT rats did not show statistical difference during the 4 weeks of measurement (data not shown; p = 0.679). However, the food ingestion of CFT animals had higher fat content (46.41%) and lower protein and carbohydrate content (14.04 and 39.45%, respectively) than the diet provided to the CTR animals.

Body composition and serum parameters

Body composition and indexes demonstrated an increase in total fat mass content with visceral (WAT) and perilumbar (BAT) fat accumulation in animals fed with CFT diet (Table I).

<table>
<thead>
<tr>
<th>Body composition</th>
<th>CTR group [n]</th>
<th>CFT group [n]</th>
<th>P significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (cm)</td>
<td>252.22 ± 14.6</td>
<td>259.36 ± 19.43</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>Total weight (g)</td>
<td>425.06 ± 42.99[9]</td>
<td>534.06 ± 44.93[11]</td>
<td>p ≤ 0.001</td>
</tr>
<tr>
<td>Total fat weight (g)</td>
<td>11.13 ± 2.73[9]</td>
<td>39.44 ± 9.64[11]</td>
<td>p ≤ 0.001</td>
</tr>
<tr>
<td>BAT index (%)</td>
<td>0.07 ± 0.02[9]</td>
<td>0.11 ± 0.03[11]</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>WAT index (%)</td>
<td>2.49 ± 0.65[9]</td>
<td>7.15 ± 1.69[11]</td>
<td>p ≤ 0.001</td>
</tr>
<tr>
<td>Free-fat mass index (%)</td>
<td>97.44 ± 0.63[9]</td>
<td>92.74 ± 1.70[11]</td>
<td>p ≤ 0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum parameters</th>
<th>CTR [n]</th>
<th>CFT [n]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glycemia (0 min)</td>
<td>89.71 ± 12.01[8]</td>
<td>77.92 ± 28.82[9]</td>
</tr>
<tr>
<td>After 16 min of insulin administration</td>
<td>44.85 ± 5.76[5]</td>
<td>42.27 ± 10.32[7]</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>39.93 ± 12.0[7]</td>
<td>42.89 ± 17.52[8]</td>
</tr>
</tbody>
</table>

The profile of glycemia and triacylglycerol observed in the serum was not different between CTR and CFT groups (Table I).

Glucose and glycogen metabolism: correlation to visceral adiposity

No difference in 2-14C-DeoxyGlucose uptake and 14C-glycogen synthesis in vitro was observed between the CTR and CFT groups (fig. 1). However, simple linear regression demonstrated an inverse correlation between 2-14C-DeoxyGlucose uptake and total fat mass weight (r² = -0.79, p = 0.03), and between 2-14C-DeoxyGlucose uptake and WAT weight (r² = -0.80, p = 0.033) in the liver from CFT group, but not in muscle (r² = -0.43, p = 0.32 and r² = -0.43, p = 0.326, respectively). Corroborating these results, glycogen content of liver from CFT rats was decreased after insulin administration (p = 0.021) (Table II).

Table II

<table>
<thead>
<tr>
<th>Muscle and liver glycogen concentration after insulin administration to control (CTR) and cafeteria-diet-fed (CFT) animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR [n]</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Muscle</td>
</tr>
<tr>
<td>Liver</td>
</tr>
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</table>

*Statistical difference from the control (p = 0.021). Results are expressed as g%. The value for each n is represented in each line.
For the CTR group we found an inverse correlation between 2-\(^{14}\)C-DeoxiGlucose uptake, and total fat mass weight ($r^2 = -0.89$, $p = 0.017$), and 2-\(^{14}\)C-DeoxiGlucose uptake and WAT weight ($r^2 = -0.89$, $p = 0.018$) in muscle. This data indicates that after six months of treatment, rats with higher visceral fat weight have impaired muscle glucose uptake, as demonstrated by insulin administration. Results for the CTR group liver revealed no statistical significance when same correlations were analyzed (data not shown).

No correlation between \(^{14}\)C-glycogen synthesis \textit{in vitro} and total fat mass weight or WAT weight was observed (data not shown). The correlation between \(^{14}\)C-glycogen synthesis or 2-\(^{14}\)C-DeoxiGlucose uptake and BAT was also tested but no difference was revealed in liver and muscle (data not shown).

\textit{Glycogen synthase (GS) activity}

The measured GS activity did not demonstrate any difference between CTR and CFT (figure 2). However, the active form of the enzyme GS (GSI) was lower than that of the total form (GST) in the liver of CFT and in CTR even after insulin administration (fig. 2).

\textit{Multivariate cluster analyzes}

Multivariate Cluster analyzes were performed to determine the range of physiologic response regarding the body composition profile shown by the animals from both diet groups (fig. 3). The CTR group showed heterogenic responses to the diet as demonstrated by body composition analyzes. The response to CFT diet also varied among the animals, although the observed response was more severe than that found for the CTR group. These finds can be related to the heterogenic metabolic profiles described in the present paper.

\textit{Discussion}

Our study attempted to determine the effect of visceral obesity in the parameters of glucose and glycogen metabolism in muscle and liver of six-months old rats. We found that some parameters such as glycemia and tryacylglicerol were not significantly different between the CTR and CFT groups. In part, the no significant responses can be explicated by heterogenic responses observed from clustered data of body composition. When data were distributed correlating glucose uptake \textit{in vitro} and adiposity (mostly visceral
adiposity) differences were observed, demonstrating variations in the animal responses inside the same group. In addition, GSI activity was decreased in the liver of CTR and CFT animals, but not in muscle, demonstrating alterations in glycogen metabolism related to visceral obesity.

In the CFT diet, the caloric intake was high-fat, low-carbohydrate, and low-protein when compared to CTR animals, demonstrating a preference for fat-intake by the rats that corroborated to previous studies. It was also observed that CFT-fed rats gained total fat mass, mostly visceral, when compared to CTR animals. Visceral adiposity is related to intrahepatic triacylglycerol and other lipids contents and can be responsible for many of the metabolic abnormalities associated with abdominal obesity and insulin resistance. It was not observed any negative influence of CFT diet in the growing process of the animals. Multivariate cluster analyzes of body composition data demonstrated a heterogenic response to the diet in both the CTR and CFT groups, however it was more pronounced in the CFT group. The hyperlipidic diet allowed animals to develop a wide range of weight gain and, as a consequence, heterogenic metabolic profiles. The CFT animals that accumulated more visceral adiposity demonstrated less glucose uptake in liver.

Serum glycemia and triacylglycerol was not different between CFT-fed and CTR-fed animals. Other
researchers have reported similar results of plasma lipids, although their studies reported an increase in other lipids, i.e., cholesterol and nonesterified fatty acids. The serum profile can be also related to the heterogenic physiologic responses of animals of both diet treatments as observed in cluster analyzes.

Data dispersion indicated a great and strong variability in the responses by the CFT group. It was not possible to observe a direct effect of CFT diet on plasma glucose, 2-14C-DeoxiGlucose uptake, and 14C-glycogen synthesis after insulin administration when compared to the CTR group. However, when the results of 2-14C-DeoxiGlucose uptake were correlated to the total fat and visceral adiposity, they revealed that this progressive accumulation negatively altered glucose uptake in the liver. This strong effect was demonstrated by the decreased glycogen levels found in the livers of CFT-fed animals. Decrease of glycogen levels in the liver are related to lipid accumulation inside the cells that decrease of insulin action. Consistent with this notion, our results showed that glycogen levels in the liver decrease following insulin administration.

The visceral adiposity may be related to the great fat ingestion by the CFT groups and the first tissue that metabolizes the lipids is the liver. Liver is a key player in the control of whole body energy metabolism because of its ability to regulate glucose and fatty acid metabolism and to act as a systemic lipid buffer during periods of high lipid influx (24; 25) and higher waist circumference is correlated to low levels of HDL-c (26). The CFT showed clear alterations of insulin signaling that can be related to accumulation of lipids and metabolites as fatty acyl CoAs, diacylglycerols, and ceramides caused by hyperlipidic diets and visceral obesity (4).

We also observed a significant decrease in GSI activity in the liver but not in muscle of the CFT group. Previous studies reported that glycogen depletion and altered GS enzymatic activity are characteristic features of insulin impairment in insulin-metabolic dependent tissues. In the CTR group an inverse correlation was observed between 2-14C-DeoxiGlucose uptake in the muscle and body adiposity, mostly visceral fat. This was observed when the animal accumulated total fat mass and WAT weight, although the values of both weights were significantly lower compared to CFT-fed animals.

The CTR group showed some variability responses to control diet, as cluster data can demonstrate. Previous reports revealed that lean and overweight subjects can present an inverse correlation between glucose uptake and intramyocellular lipid content related to impairment of insulin action. In the CTR group we also observed an interesting decrease of GSI activity in the liver, in the presence of insulin administration however no negative impact in glycogen content was observed.

**Conclusion**

The accumulation of intracellular lipids and the impairment of insulin action are possible answers for the observed differences in the metabolic state of the CTR and CFT animals. In addition, multivariate cluster demonstrated the variable range of physiologic responses to the diets as observed by the body composition profiles of both experimental groups. In this study we reveal that in the case of the CFT group primary tissue for lipid metabolism and accumulation seems to be the liver. Because the CTR group was fed with a low-fat diet, the animals may respond accumulating lipids in their muscle however the reasons for that need to be clarify. Our results indicate a clear correlation of visceral adiposity and impaired glucose uptake that is influenced by the diet, the degree of fat accumulates in the tissues and the degree of overweight and/or obesity. Further studies are required to unveil the endocrine and molecular mechanisms which account for the differences found for the two groups of animals studied herein.

**Competing interests**

The authors declare that they have no competing interests and did not receive any funding.

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**Author’s contributions**

Danielle Kaiser de Souza wrote the present paper and did all the experiments. Fabiana A. de Souza, and Signor Peres Konrad contributed significantly to all the experimental procedures. Luciano Stürmer de Fraga helped us in the GS activity measurement.

Adriane Belló-Klein and Roselis Silveira Martins da Silva provided materials and intellectual support for the experiments. Luiz Carlos R. Kucharski was the supervising professor of the present paper.

**References**


