Adipose tissue redistribution caused by an early consumption of a high sucrose diet in a rat model

Ashley Kate Castellanos Jankiewicz\textsuperscript{1}, Sofía Montserrat Rodríguez Peredo\textsuperscript{1,2}, Guillermo Cardoso Saldaña\textsuperscript{3}, Eulises Díaz Díaz\textsuperscript{1}, María Elizabeth Tejero Barrera\textsuperscript{1}, Laura del Bosque Plata\textsuperscript{1}, Roxana Carbó Zabala\textsuperscript{1}  

\textsuperscript{1}Laboratory of Nutrigenomics and Nutrigenetics, National Institute of Genomic Medicine. Periférico Sur #4809, Col. Arenal Tepepan, Tlalpan, C.P. 14610. \textsuperscript{2}National School of Biological Sciences, National Polytechnic Institute. Prolongación de Carpio y Plan de Ayala S/N, Col. Santo Tomás, Miguel Hidalgo, C.P. 11340. \textsuperscript{3}Department of Reproductive Biology, National Institute of Medical Sciences and Nutrition “Salvador Zubirán”. Vasco de Quiróga #15, Col. Sección XVI, Tlalpan, C.P. 14080. \textsuperscript{4}Department of Cardiovascular Biomedicine, National Institute of Cardiology “Ignacio Chávez”. Juan Bacigalupo #1, Col. Sección XVI, Tlalpan, C.P. 14080. Mexico D.F.

Abstract

\textit{Introduction:} obesity is a major public health problem worldwide. The quantity and site of accumulation of adipose tissue is of great importance for the pathophysiology of this disease.

\textit{Objectives:} the aim of this study was to assess the effect of a high carbohydrate diet on adipose tissue distribution.

\textit{Methods:} male Wistar rats, control (CONT) and high sucrose diet (HSD; 30\% sucrose in their drinking water), were monitored during 24 weeks and total energy and macronutrient intake were estimated by measuring daily average consumption. A bioelectrical impedance procedure was performed at 22 weeks of treatment to assess body compartments and systolic arterial blood pressure was measured. Serum was obtained and retroperitoneal adipose tissue was collected and weighed.

\textit{Results:} HSD ingested less pellets and beverage, consuming less lipids and proteins than CONT, but the same amount of carbohydrates. Retroperitoneal adipose tissue was more abundant in HSD. Both groups were normoglycemic; triglycerides, adiponectin and leptin levels were higher, while total cholesterol and HDL-cholesterol were lower in HSD; insulin, HOMA index and systolic blood pressure had a tendency of being higher in HSD.

\textit{Discussion:} this model presents dyslipidemia and a strong tendency for insulin resistance and hypertension. Even though there was no difference in body compartments between groups, retroperitoneal adipose tissue was significantly increased in HSD. This suggests that a rearrangement of adipose tissue distribution towards the abdominal cavity takes place as a result of the high sucrose diet in early consumption.

RESUMEN

\textit{Introducción:} la obesidad es uno de los mayores problemas de salud pública en todo el mundo. El momento en que se establece, la distribución y cantidad de tejido adiposo son de gran importancia para comprender su fisiopatología.

\textit{Objetivos:} observar la distribución de tejido adiposo en una dieta alta en sacarosa desde una edad temprana en un modelo animal.

\textit{Métodos:} se utilizaron ratas Wistar recién destetadas, animales control (CONT; agua ad libitum) y animales con dieta alta en sacarosa (HSD; 30\% de sacarosa en el agua) durante 24 semanas. Se calcularon las kilocalorías y macronutrientes ingeridos diariamente; se evaluaron por impedancia bioeléctrica los compartimentos corporales, se midió la presión sistólica, se obtuvo el tejido adiposo retroperitoneal y el suero para medir parámetros bioquímicos.

\textit{Resultados:} los animales HSD comieron y bebieron menos, obteniendo menos proteínas y lípidos, sin diferencia en los hidratos de carbono. El tejido adiposo fue más abundante en HSD. Ambos grupos CONT Y HSD fueron normoglucémicos; HSD tuvieron triglicéridos, adiponectina y leptina altas, y el colesterol y las HDL más bajos; la insulina, el HOMA y la presión sistólica tuvieron tendencia a ser mayores en HSD.

\textit{Discusión:} este modelo presenta dislipidemia y una tendencia a tener resistencia a la insulina e hipertensión. A pesar de no haber una diferencia en los compartimentos corporales entre grupos, el tejido adiposo tuvo una localización específica en la espalda y fue más abundante en HSD. En conclusión, la distribución de grasa en el...
chronic high sucrose consumption, which contributes to a higher risk of suffering from metabolic and chronic degenerative diseases.

*(Nutr Hosp.* 2015;31:2546-2553)

**DOIs: 10.3305/nh.2015.31.6.8935**

**Keywords:** Nutrition. Adipose tissue. Bioelectrical impedance. Sucrose. Obesity.

**Abbreviations**

- BIA: bioelectrical impedance analysis
- BMI: body mass index
- CONT: control
- FFM: fat free mass
- GLUT: glucose transporters
- HCO: carbohydrates
- HOMA: Homeostasis Model Assessment
- HDL: high density lipoproteins
- HSD: high sucrose diet
- IL-6: interleukin-6
- L: length
- MMP: matrix metalloproteinase
- TBP: total body fat
- TBS: total body solids
- TBW: total body water
- TNF-α: tumor necrosis factor - α
- VLDL: very low density lipoproteins
- WBR: whole body resistance
- WBXx: whole body reactance

**Introduction**

Nowadays, Western diets have become a major health problem due to highly energetic and rapidly available foods. In the long term, this can reflect in economical issues and social problems, since there is a negative impact on the governmental health care budget and is also cause of productivity loss in the working population. Once considered a problem only in high income countries, overweight and obesity are now dramatically on the rise in both low- and middle-income countries, particularly in urban settings1. The risk of metabolic diseases due to overweight has been strongly linked to adipose tissue distribution, which is dependent of sex, genetic background, disease state, certain drugs and hormones, aging and environmental factors2.

Obese children and adolescents face similar health risks as adults. Whilst symptoms may not become apparent until later in life, in many cases, damage from being overweight as a child has already been done, making it especially important for children to reach a healthy weight as early as possible1.

Overweight and obesity are defined as abnormal or excessive adipose tissue accumulation that present health risks for a number of chronic diseases, including metabolic diseases, cardiovascular diseases and cancer, reducing the life expectancy by up to 8 years1. Adipose tissue can be accumulated in different depots, such as subcutaneous, ectopic, intramuscular, mesenteric and epididymal, but abnormally high deposition of visceral adipose tissue is known to present a higher risk of suffering from metabolic disorders1-3.

Diets rich in simple carbohydrates (HCO), such as sucrose, have been strongly associated with an increased prevalence of obesity, type 2 diabetes and cardiovascular risk factors4, although controversy has risen on whether this type of diet contributes to an obese phenotype1 or simply promotes adipocyte hypertrophy, glucose intolerance, hyperinsulinemia, hyperlipidemia and an increased number of inflammatory cytokines5. Thus, several models have been designed in order to further study this multifactorial condition.

A rat model has been developed by administering a 30% sucrose solution as an unlimited beverage, causing metabolic disruptions by high HCO intake. The characteristics of this particular model are: moderate elevation of blood pressure, hypertriglyceridemia, glucose intolerance, hyperinsulinemia, hyperlipidemia and an increased number of inflammatory cytokines6. Thus, several models have been designed in order to further study this multifactorial condition.

However, a complete characterization of the model has not yet been published in relation to food intake and total body composition.

**Objectives**

The aim of this study was to characterize the effect of a high sucrose diet on blood parameters, body compartments and adipose tissue distribution.

**Materials and methods**

**Animal model**

Male Wistar rats (n=22) were fed during 24 weeks after weaning (starting weight: 70-95g) a standard rodent diet (Laboratory Rodent Diet 5001: protein 28.507%, fat 13.496%, HCO 57.996%, from which sucrose 3.7%, fructose 0.3%, glucose 0.22%, PMI Nutrition International, Brentwood, MO) and water ad libitum. The high sucrose diet (HSD) group (n=11) was...
provided a 30% sucrose solution as their only liquid source. The other group were controls (CONT) (n=11) that received the same diet, with the exception of providing water as their liquid source. All animals were housed in proper facilities in groups of 6 animals per cage under artificial 12 hour light/dark cycles and a mean temperature of 22°C.

Morphological variables and blood pressure measurements

Animals were weighed on an electronic scale (1,500g capacity and accuracy of 1g) every two weeks. Length for body mass index (BMI) calculation was measured from the narium to the base of the tail\(^{12}\). BMI was calculated by dividing the animal’s weight (g) by the square of its length (cm\(^2\)). Systolic arterial blood pressure was measured every 2 months in conscious animals using the tail-cuff method. The cuff was connected to a pneumatic pulse transducer (Narco Bio-systems Inc., Healthdyne Co.) and a programmed electro-sphygmomanometer. Six independent determinations were recorded with a Grass polygraph (model 79, Grass Medical Instruments, Quincy, MA) and the mean was calculated for each animal.

Food intake measurements

In order to estimate total energy intake and macronutrients from the diet, a weighed quantity of food and beverage were added daily at the same time of day (08:00 to 09:00 hours), and remains were collected and measured the day after in order to calculate by difference the average consumption. This was carried out throughout the 24 weeks of treatment. Drinking bottle spouts were rotated among the test and control animals when they were replenished and were monitored for leakage weekly.

Body composition procedure

At 22 weeks of age, the animals were submitted to an 8 hour fasting period. Rats were weighed and anaesthetized with a sodium pentobarbital solution (0.1mL per 100g of body weight, i.p.) in combination with saline solution. Once animals were completely anaesthetized, hair was removed from the dorsal surface and they were placed on a non-conductive surface. Their length (L) was measured from the narium to the pelvic-caudal junction with a non-elastic tape. Afterwards, they were submitted to a bioelectrical impedance procedure (RJL Systems BIA Analyzer, model Quantum X) as described previously\(^{13,14}\) for obtaining body composition estimates using whole body resistance (WBR) and reactance (WBXc).

Four sterile 22G needles bent 1 cm from the tip were used as electrodes and placed as firstly reported\(^{14}\) on the shaved dorsal surface. Three replicate measurements of WBR and WBXc were recorded; this was done twice, after repositioning the animal between measurements. Average of WBR and WBXc measurements were used for analysis. To obtain fat free mass (FFM)\(^{15}\), total body water (TBW)\(^{16}\) and total body solids (TBS)\(^{13}\) of each animal, the following formulas were used:

\[
\text{FFM (g)} = (141.25 \cdot \text{L}^2) / \text{WBR} - 0.02
\]
\[
\text{TBW (g)} = 15.47 + (97.44 \cdot \text{L}^2) / \text{WBR}
\]
\[
\text{TBS (g)} = \text{Body weight} - \text{TBW}
\]

Total body fat (TBF) was obtained by subtracting total weight from the animals during the procedure minus FFM.

Serum measurements

After 24 weeks, both groups were sacrificed and blood samples were collected. Retroperitoneal adipose tissue was collected and weighed after sacrifice. Serum was obtained by blood centrifugation (15,000 rpm during 15 minutes at 4°C) and stored at -20°C until needed.

Glucose was measured with a commercial enzymatic kit (DCL- glucose oxidase Diagnostic Chemical Limited de Mexico, Mexico). Insulin was determined with a commercial rat specific radioimmunoassay kit (Linco Research, Inc. Missouri, USA) with 0.1ng/mL sensitivity and intra- and inter-assay coefficients of variation of 5 and 10%, respectively. Homeostasis Model Assessment (HOMA) was used as an index to measure the degree of insulin resistance and was calculated as follows: (insulin (\(\mu UI/mL\)) x glucose (mmol/L) / 22.5)\(^{16}\).

Triglycerides and cholesterol were determined with commercially available procedures (SPINREACT cholesterol-LQ and triglycerides-LQ; Spinreact S.A. Girona, Spain). HDL-cholesterol was measured by enzymatic procedures (Hitachi 902 analyzer; Hitachi LTD, Tokyo, Japan). Accuracy and precision of lipid measurements in our laboratory are under periodic surveillance by the Centers for Disease Control and Prevention (Atlanta, GA, USA).

Leptin and adiponectin were determined using specific ELISA kits for rat from MILLIPORE (EZRL-83K and EZRADP-62K Millipore Corporation, Missouri, USA). Leptin had a specificity of 100% and 0.08 ng/mL sensitivity. The intra and inter-coefficients of variation were 2.13 and 2.95%, respectively. Adiponectin had a specificity of 100% and no reactivity with human or mouse; the sensitivity was 0.4ng/mL. The intra and inter-coefficients of variation were 1.59 and 6.54%, respectively, with 4 duplicates per sample.

Ethics

All animals were lawfully acquired and their manipulation was carried out in accordance with The European Commission Environment (Declaration of Helsinki) EC
Directive 86/609/EEC for animal experiments. The protocol was reviewed and approved by Ethics Committee of the National Institute of Cardiology. Experimental work followed the guidelines from the Norma Oficial Mexicana guide for the use and care of laboratory animals (NOM-062-ZOO-1999) and for the disposal of biological residues (NOM-087-ECOL-1995).

All reagents used were of the highest grade available.

Statistical analysis

Statistical comparisons between experimental groups were conducted using Student’s t-test (Sigma-Plot for Windows version 11.0 Germany), followed by Mann-Whitney post-hoc test. Data were tested for normality with the Shapiro–Wilk test (N-S-W test), and the abnormal variables were transformed to their natural logarithm. Values of $P<0.05$, $P<0.01$ and $P<0.001$ were considered as significant. Data are presented as standard error.

Results

External and macro-parameters

CONT and HSD began the study with no significant differences in body weight or age. At the end of the 24 weeks treatment period, HSD had a different appearance than CONT, since their hair was unevenly distributed along the whole body, and during the extraction of retroperitoneal adipose tissue, they presented more fat pads around and inside the organs, as well as inside the thoracic cavity.

Progressive weight gain during treatment between groups is shown in figure 1A. HSD weighed 49.17 g less than CONT at sacrifice ($P<0.01$). Retroperitoneal adipose tissue was significantly more abundant in HSD ($P<0.001$) (Fig. 1B).

Blood pressure

There was a strong tendency of HSD to present higher systolic blood pressure. However, this parameter did not result in statistically significant differences between groups at the end of treatment (Fig. 1C).

Energy and macronutrient intake

Since the first month of treatment, HSD consumed significantly less pellets ($P<0.001$) and beverage ($P<0.001$) than CONT (Table I), even after adjusting by total weight (data not shown).

Energy consumption (Kcal) followed through the treatment period showed that HSD consumed less Kcal ($P<0.01$) (Table I). In relation to macronutrient ingestion, HSD consumed less protein (g) and lipids (g) than CONT ($P<0.001$). Interestingly, total HCO intake did not change between groups. CONT obtained HCO solely from pellet consumption, while HSD, although they consumed less pellets, compensated HCO consumption by drinking their sucrose rich water (Table I; Fig. 2).
Average weight of animals at the moment of the bioelectrical impedance analysis (BIA) was 519.58 ± 32.5 and 458.75 ± 48.4 g for CONT and HSD, respectively. Results of the procedure did not show significant differences between groups in relation to the measured components FFM, TBW, TBS and TBF after adjusting by total weight (Fig. 3). No significant differences were found in relation to BMI between groups (Fig. 1D).

**Bioelectrical impedance analysis**

Average weight of animals at the moment of the bioelectrical impedance analysis (BIA) was 519.58 ± 32.5 and 458.75 ± 48.4 g for CONT and HSD, respectively. Results of the procedure did not show significant differences between groups in relation to the measured components FFM, TBW, TBS and TBF after adjusting by total weight (Fig. 3). No significant differences were found in relation to BMI between groups (Fig. 1D).

**Serum parameters**

Blood glucose in both groups was normoglycemic. Triglycerides, adiponectin and leptin levels were higher (P<0.01, P<0.001 and P<0.01, respectively), while total cholesterol and HDL-cholesterol were lower (P<0.01 and P<0.001, respectively) in HSD compared to CONT. Serum insulin and HOMA index showed a strong tendency of being higher in HSD (data not significant). Values for each parameter are shown in table II.

**Discussion**

The present study demonstrates that chronic sucrose consumption starting from an early life stage plays a deleterious effect on serum parameters and significantly increases retroperitoneal adipose tissue. Despite having consumed more Kcal in the form of simple HCO (available from sucrose in their beverage (Fig. 2), HSD did not gain more weight than CONT. This diet-induced effect has been previously reported in a similar rat model, where sweetened drinks were administered to animals and no weight difference was observed. Nonetheless, HSD presented significantly more retroperitoneal adipose tissue and a tendency of having more TBF, which could explain the higher serum levels of triglycerides and leptin, as well as the strong tendency of high blood pressure, which is consistent with previous studies.

High sucrose diets do not necessarily induce an obese phenotype, but provoke severe endocrine and metabolic disturbances caused by abdominal adipose tissue accumulation, inducing hepatic synthesis of triglycerides from glucose and transports them to the bloodstream through very low density lipoproteins (VLDL) for storage in the adipose tissue.

Leptin has pleiotropic effects on body weight, energy homeostasis, immune responses, inflammation, and angiogenesis. Circulating levels of leptin correlate with fat mass quantity. It travels from the adipose tissue to the hypothalamus, indicating the state of adipose tissue storage, and it exerts its action by inducing the expression of anorexigenic factors and reducing the hypothalamic production of orexigenic peptides. High levels of leptin have been described in inflammatory states, making leptin resistance a key factor for the development of obesity together with hypertension. Our model presented high levels of leptin in HSD, and are in accordance with the presence of excess retroperitoneal fat.
Adipose tissue redistribution caused by an early consumption of a high sucrose diet in a rat model

<table>
<thead>
<tr>
<th>Table II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum parameters</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
</tr>
<tr>
<td>Insulin (µUI/mL)</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
</tr>
<tr>
<td>HOMA</td>
</tr>
</tbody>
</table>

Values are expressed as means and standard error (±S.E.). HOMA: Homeostasis Model Assessment. **P< 0.01; ***P< 0.001. Insulin and leptin were transformed into the natural logarithm.

Fig. 3.—Body composition of both groups at 22 weeks of age. Values are expressed as percentage adjusted by total weight (g), representing body compartment distribution for CONT and HSD. FFM: fat free mass; TBW: total body water; TBS: total body solids; TBF: total body fat. **P < 0.01.

On the other hand, adiponectin controls systemic glucose and lipid homeostasis in the liver and skeletal muscle and is considered an anti-inflammatory adipokine24,25. Adiponectin levels in plasma and adipose tissue are decreased in obese compared with lean individuals, and its production is inhibited by pro-inflammatory factors, such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6), as well as by hypoxia and oxidative stress26,27. However, it has also been reported that high levels of adiponectin are present during the pathogenesis of rheumatoid arthritis and osteoarthritis as a pro-inflammatory cytokine, inducing the expression of IL-6, IL-8, matrix metalloproteinase (MMP) -3 and MMP-9, via adenosin monophosphate-activated protein kinase, mitogen-activated protein kinases-38, inhibitor of IκB kinase α-β and nuclear factor-κB28, 29. At the time of sacrifice, adiponectin was elevated in our model. We hypothesize that it was exerting an inflammatory effect, due to the pro-inflammatory microenvironment in the adipose tissue of HSD, emulating the behavior of inflammatory states, as in arthritis.

Sucrose rich diets do not necessarily promote obesity per se, but lead to adipose hypertrophy, causing major metabolic disturbances, such as glucose intolerance, hyperinsulinemia, hyperlipidemia and an increase in inflammation markers8. Furthermore, high sucrose diets induce hepatic synthesis of triglycerides from HCO, which are then transported to the blood through the VLDL and stored as adipose tissue30. In our model, this is shown by the elevated storage of retroperitoneal adiposity and hypertriglyceridemia in HSD. In a study by Ruiz-Ramírez et al.31, elevated lipid levels in the liver by development of non-alcoholic fatty liver disease was confirmed in the same model.

There are many hypotheses that link these variables with inflammation. It is well known that pro-inflammatory cytokines produced by adipose tissue secrete TNF-α and IL-6, which reduce the expression of glucose transporters (GLUTs), such as GLUT4, that can result in lowering glucose uptake by the muscle, and can take part in the compensatory hyperinsulinemic state22, which is also reflected in the trend of elevated HOMA index seen in our model.

By performing BIA, no significant differences were observed between groups in body compartments after adjusting by total weight. However, HSD had significantly more retroperitoneal adipose tissue than CONT. This suggests that adiposity distribution varied widely between groups. This fact provides novel information on how a sucrose rich diet can lead to the rearrangement of fat depots towards the abdomen, contributing to the appearance of abdominal obesity and greatly increasing the risk of suffering from metabolic diseases as discussed by several authors33. A limitation of this study was that BIA was only performed once in the animals, and the evolution of this redistribution could not be determined.

Abdominal adipose tissue storage has become a key factor in developing several metabolic risk factors. Such is this tendency that some authors have proposed that excessive fat depot would be one of the main features in insulin resistance34. Others propose that the cause of insulin resistance is the excess storage of visceral adipose tissue. In our model, both excess of abdominal fat depots and insulin resistance were present in HSD when compared to CONT.

BIA allowed the comparison of body compartments in both groups, where the most interesting finding was that both groups had the same TBF after adjusting by total body weight, although HSD had significantly higher amounts of retroperitoneal fat.

These findings confirm that high sucrose diets play an important role in the development of dyslipidemia, and suggest that in this animal model, a clear rearrangement of adiposity takes place after a 24 week sucrose dietary intervention, rearranging most of the fat as abdominal adipose tissue and contribu-
ning to a higher risk of metabolic disturbances, such as cardiovascular diseases, dyslipemias and type 2 diabetes. Future research should focus on elucidating the molecular mechanisms by which this rearrangement takes place.

Conclusions
A chronic sucrose rich diet induces high serum lipid and insulin levels, together with hypertension and excess abdominal adiposity. Studying macronutrient intake in animal models is relevant for unraveling the molecular and physiological disturbances of metabolic diseases. Adipose tissue rearrangement towards the abdominal cavity in HSD could be the cause of all metabolic disturbances, since abdominal obesity presents higher risk than when accumulated in other depots. Future work could be aimed on studying the mechanisms that contribute to the fat redistribution towards the abdominal cavity to understand the etiopathogenesis of different metabolic diseases.

Acknowledgements
The authors acknowledge financial support to A.K.C.J., who received a scholarship from the Coordinating Committee of National Institutes of Health and High Specialty Hospitals (PROBEI) and S.M.R.P., who received a PhD, scholarship from the National Council for Science and Technology (CONACYT #230762).

References


