Autologous subcutaneous adipose tissue transplants improve adipose tissue metabolism and reduce insulin resistance and fatty liver in diet-induced obesity rats

Gonzalo Torres-Villalobos1,2,*, Nashla Hamdan-Pérez2,*, Andrea Díaz-Villaseñor3,4, Armando R. Tovar5, Ivan Torre-Villalvazo3, Guillermo Ordaz-Nava3, Sofía Morán-Ramos3, Lilia G. Noriega3, Braulio Martínez-Benítez5, Alejandro López-Garibay2, Samuel Torres-Landa2, Juan C. Ceballos-Cantú2, Claudia Tovar-Palacio6, Elizabeth Figueroa-Juárez6, Marcia Hiriart7, Roberto Medina-Santillán8, Carmen Castillo-Hernández8 & Nimbe Torres3

1 Depto. de Cirugía, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Tlápan, Mexico City, Mexico
2 Depto. de Cirugía Experimental, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Tlápan, Mexico City, Mexico
3 Depto. de Fisiología de la Nutrición, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Tlápan, Mexico City, Mexico
4 Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico
5 Depto. de Patología, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Tlápan, Mexico City, Mexico
6 Depto. de Nefrología y Metabolismo Mineral, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Tlápan, Mexico City, Mexico
7 Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Mexico City, Mexico
8 Departamento de Posgrado e Investigación, Instituto Politécnico Nacional, Escuela Superior de Medicina, Mexico City, Mexico

Keywords
Autologous adipose tissue transplant, diet-induced obesity, insulin resistance, obesity.

Abstract
Long-term dietary and pharmacological treatments for obesity have been questioned, particularly in individuals with severe obesity, so a new approach may involve adipose tissue transplants, particularly autologous transplants. Thus, the aim of this study was to evaluate the metabolic effects of autologous subcutaneous adipose tissue (SAT) transplants into two specific intraabdominal cavity sites (omentum and retroperitoneal) after 90 days. The study was performed using two different diet-induced obesity (DIO) rat models: one using a high-fat diet (HFD) and the other using a high-carbohydrate diet (HCHD). Autologous SAT transplant reduced hypertrophic adipocytes, improved insulin sensitivity, reduced hepatic lipid content, and fasting serum-free fatty acids (FFAs) concentrations in the two DIO models. In addition, the reductions in FFAs and glycerol were accompanied by a greater reduction in lipolysis, assessed via the phosphorylation status of HSL, in the transplanted adipose tissue localized in the omentum compared with that localized in the retroperitoneal compartment. Therefore, the improvement in hepatic lipid content after autologous SAT transplant may be partially attributed to a reduction in lipolysis in the transplanted adipose tissue in the omentum due to the direct drainage of FFAs into the liver. The HCHD resulted in elevated fasting and postprandial serum insulin levels, which were dramatically reduced by the autologous SAT transplant. In conclusion, the specific intraabdominal localization of the autologous SAT transplant improved the carbohydrate and lipid metabolism of adipose tissue in obese rats and selectively corrected the metabolic parameters that are dependent on the type of diet used to generate the DIO model.

*Both authors contributed equally to this work.

© 2016 The Authors. Physiological Reports published by Wiley Periodicals, Inc. on behalf of the American Physiological Society and The Physiological Society. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
Introduction

Obesity is an epidemic disease representing a particular focus of many public health efforts worldwide (Khan et al. 2009; Malik et al. 2013; Ogden et al. 2014). It is a complex metabolic disorder associated with the appearance of insulin resistance and dyslipidemia among other comorbidities; thus, there is an increased risk of developing type 2 diabetes and cardiovascular disease (Perrini et al. 2008).

The distribution of fat in obesity has a direct effect on metabolic abnormalities. It is known that there are several differences between subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (Ibrahim 2010). Some studies have demonstrated that obesity complications are mainly associated with an increase in the amount of VAT (Alvehus et al. 2010).

Different strategies have been used to prevent and treat obesity. However, the success of long-term dietary and pharmacological treatments has been questioned, particularly in individuals with severe obesity (Kakkar and Dahiya 2015). For this reason, new strategies are being explored.

A new approach to the treatment of obesity may involve adipose tissue transplants. This is based on some animal studies that have demonstrated that heterologous and autologous adipose tissue transplants can improve several metabolic parameters, especially insulin sensitivity (Konrad et al. 2007; Tran et al. 2008; Foster et al. 2011, 2013; Satoor et al. 2011; Hocking et al. 2015). However, heterologous adipose tissue transplants in humans imply a risk of rejection, the use of immunosuppressants, and an increased risk of infection (Fishman 2007; Seetharam et al. 2010). Therefore, autologous adipose tissue transplant is a much more viable option that has been used for several years for esthetic purposes (Gutowski and Force 2009).

Adipose tissue transplants have been studied mostly in nonobese animals, and although some beneficial metabolic effects have been observed, these models do not represent the metabolic abnormalities that occur in obesity. To the best of our knowledge, few studies involving adipose tissue transplants have been conducted in diet-induced obesity (DIO) models (Foster et al. 2013; Hocking et al. 2015). It has been reported that DIO models using a high-fat diet (HFD) or a high-carbohydrate diet (HCHD) induce different metabolic changes in humans (Thomas et al. 1992) and rodents (Buettner et al. 2007; Chaumontet et al. 2015; Torres-Villalobos et al. 2015). However, whether an adipose tissue transplant can provide the same health benefits in obese animals depending on whether they developed obesity with a HFD or HCHD is unknown.

The inconsistencies between the reported metabolic effects of adipose tissue transplants are possibly related to differences in study duration and the use of obese or lean rodents (Konrad et al. 2007; Tran et al. 2008; Foster et al. 2011, 2013; Satoor et al. 2011; Hocking et al. 2015). In addition, it is important to consider the specific intraabdominal location where the adipose tissue is transplanted. Inside the abdominal cavity, three different fat compartments have been described, mesenteric, omental, and retroperitoneal adipose tissue (Fig. 1A). The venous flow of the intraperitoneal adipose tissue coming from the mesenteric and omental compartments is different than the venous flow of the retroperitoneum. The first two have their venous drainage to the portal vein into the liver, while the retroperitoneal compartment drains into the vena cava and directly to the systemic circulation (Tchernof and Despres 2013). This difference in venous drainage implies that all their products, such as free fatty acids (FFAs) and adipokines, will affect different targets and have been associated with different hypothesis regarding fat accumulation and disease. Therefore, it is important to consider the transplantation site because the metabolic products of the transplanted adipose tissue will reach different tissues (Rytka et al. 2011).

The mechanism by which the transplant improves metabolic parameters has not been well established. According to several studies, adipose tissue transplants can modify the flux of FFAs from adipose tissue to the liver (Foster et al. 2011, 2013; Hocking et al. 2015). It has been demonstrated that an increase in the drainage of FFAs from adipose tissue into the liver is associated with the development of insulin resistance (Ebbert and Jensen 2013). The release of FFAs from adipose tissue is dependent on the rate of lipolysis that is carried out by three different lipases. Specifically, the hormone-sensitive lipase (HSL) and the adipose triglyceride lipase (ATGL) are the enzymes that highly regulate the lipolysis in adipocytes, however, obesity is associated with a decreased expression and activity of HSL, but not ATGL in visceral and subcutaneous adipocytes of obese individuals (Fruhbeck et al. 2014). Thus, HSL may be a key regulator of the flux of FFAs into the circulation after an adipose tissue transplant, although this has not been thoroughly studied.

Therefore, the aim of this study was to evaluate the metabolic effects of an autologous SAT transplant into two specific intraabdominal cavity sites (omentum and retroperitoneal) using two different DIO rat models: one using a HFD and one using a HCHD. In addition, to establish whether the improvements in insulin sensitivity and hepatic lipid accumulation after adipose tissue transplant occur through modifications of adipose tissue lipolysis, the phosphorylation status of HSL in the omental and retroperitoneal adipose tissues was assessed.
Figure 1. Visceral adipose tissue (VAT) compartments and transplant surgery process. (A) mesenteric, omental, and retroperitoneal VAT. Removal of subcutaneous adipose tissue (SAT) from both bilateral inguinal fat pads (B) and cut in small pieces that can pass through a syringe (C). Midventral abdominal incision was performed (D) and the SAT was injected between the two sheets of the omentum through the incision (E) and closed with nonabsorbable suture (F). Incision of the retroperitoneal adipose tissue (G) for the injection of the SAT (H), followed by the nonabsorbable suture (I). A duration of 90 days after the transplant, the site of the surgery did not present necrosis (J and K). The omental (L and M) and the retroperitoneal (N and O) adipose tissue were totally macro- and microscopically viable.
**Materials and Methods**

**Animals and diets**

Male Wistar rats (8 weeks-old) were housed in individual cages at controlled temperature (22°C) and humidity with a 12-h light/dark cycle. The rats were fed a HFD or HCHD for 180 days. The HFD was composed of 18.7% of total energy from protein, 45% from fat, and 36.4% from carbohydrates. In addition, these rats consumed water supplemented with 5% sucrose. The HCHD was composed of 28.5% of total energy from protein, 13.5% from fat, and 57.9% from carbohydrates, as well as 20% sucrose in water; both groups were allowed free access to both food and water. The animal protocol was approved by the Animal Care and Research Advisory Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico (CEX-35-10-11-1).

**Experimental design and surgical procedures**

Once the rats developed obesity after consuming the HFD \( n = 25 \) or HCHD \( n = 14 \) for 90 days, they were randomly divided into three subgroups according to different surgical procedures: the sham group (Sh) \( n = 8 \) for HFD and \( n = 5 \) for HCHD) which was the surgical control; the removal group (Rv) \( n = 8 \) for HFD and \( n = 4 \) for HCHD); and the transplant group (Tr) \( n = 9 \) for HFD and \( n = 5 \) for HCHD) (Fig. 2).

For surgery, inhalation of 2% sevoflurane with oxygen at 0.4–0.6 L/min was used to establish anesthesia, and on demand, sevoflurane with oxygen at 0.4–0.6 L/min was used for maintenance, provided by a Kent Scientific ventilator. The Sh group underwent laparotomy and remained under anesthesia for the same amount of time as the other two groups. For the Rv group, SAT was completely removed from both bilateral inguinal fat pads without being transplanted (Fig. 1B). For the Tr group, the SAT that was extracted from the same area in the inguinal fat pad, was weighed and cut in small pieces that could pass through a syringe (Fig. 1B and C) to implant them into two specific intraabdominal compartments: omental and retroperitoneal. A midventral abdominal incision was performed and the SAT (3.15 ± 1.1 g) was injected between the two sheets of the omentum through a small incision and closed with nonabsorbable suture to recognize the transplant site (Table 1 and Fig. 1D–F). Then, the retroperitoneal adipose tissue was localized in both sides, and the SAT was injected (5.8 ± 2.2 g) through an opening in the retroperitoneum. The incision was closed in the same manner as in the omentum (Fig. 1G–I). After the surgery, all animals received prophylactic analgesia.

![Figure 2](image-url)
ples were dissected, weighed, and maintained at
/F0FFAs. Liver and VAT (omental and retroperitoneal) sam-
measure fasting serum glucose, insulin, TGs, glycerol, and
Lakes, NJ) and centrifuged at 1500
ntaining a separating gel and clot activator (BD Franklin
ological assessment.
 weighed. Liver and VAT samples were also fixed for his-
until analysis, whereas SAT was only dissected and
(ª)
ª–
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
ª*
Histological analysis
Liver and VAT (omental and retroperitoneal) samples were fixed in an ice-cold 4% (w/v) formaldehyde in phosphate-buffered saline (PBS) and were embedded in paraffin. Then, sections of 4 μm were stained with hematoxylin–eosin. Images were obtained at 40× and 10× magnification, for liver and adipose tissue samples, respectively.

Western blotting
Proteins were extracted from omental and retroperitoneal adipose tissue and were quantified as described previously (Diaz-Villasenor et al. 2013). Then, 10 μg of total protein was denatured by heating for 5 min in a Laemmlı sample buffer containing β-mercaptoethanol (Bio-Rad, Hercules, CA), separated by SDS-PAGE using 8% polyacrylamide gels, and transferred to PVDF membranes. The blotted membranes were blocked for 1 h at room temperature using 5% nonfat dry milk (Bio-Rad, Hercules CA) and were then incubated overnight at 4°C with the following primary antibodies: HSL #45422 diluted 1:140,000 or UCP-1 #10983 diluted 1:2000 from Abcam (Cambridge, MA), pHSLSer563 #4139 diluted 1:750 from Cell Signaling Technology (Danvers, MA), and Actin #1615 diluted 1:1500 from Santa Cruz Biotechnology (Santa Cruz, CA). After incubation with the secondary antibody, the blots were developed using the enhanced chemiluminescence method with Immobilon Western Chemiluminescent HRP substrate (Millipore, Billerica, MA). Optical densitometric analysis was conducted using ImageJ 1.42p digital imaging processing software (Rasband 1997). The immunoblotting assays were performed with three independent blots, and the values were normalized relative to actin values.

Statistical analysis
The values are expressed as the mean ± SEM. The pre- and postsurgical differences were analyzed by two-way ANOVA test followed by Bonferroni’s multiple comparison test. Differences between the AUCs of presurgery rats fed the HFD or HCHD were evaluated with one-sided unpaired t-tests (using Welch’s correction when the variance between the groups was significantly different) or Mann–Whitney tests (for nonparametric data), as indicated in each figure. Differences between the experimental groups (Rv and Tr) and the control group (Sh) were evaluated using one-way ANOVA followed by Dunn’s multiple comparison test or the Kruskal–Wallis test followed by Dunn’s multiple comparison test for nonparametric data, as indicated in each figure. The normality of the data distribution was evaluated using the Kolmogorov–Smirnov test. In all cases, P < 0.05 was considered significant (GraphPad Prism 5.00, San Diego, CA).

Results
Diet-induced obesity and presurgical metabolic changes after the consumption of a HFD or HCHD
The appearance of metabolic abnormalities differs when obesity is induced using a HFD compared with a HCHD (Thomas et al. 1992; Buettner et al. 2007; Chaumontet et al. 2015; Torres-Villalobos et al. 2015). Therefore, to assess the impact of an autologous fat transplant from the subcutaneous fat pad to the visceral compartments, we developed a diet-induced obesity (DIO) using a HFD or HCHD. We observed that rats fed a HFD gained approximately 313 g after 90 days of dietary treatment, whereas those fed a HCHD gained approximately 355 g during the same period (Table 1).

As a result of 90 days of dietary treatment with both diets and despite the lack of a significant difference in weight gain between the groups, rats fed a HCHD had a significantly higher fasting serum TGs (Fig. 3A and B) and IR index (Fig. 3C and D) than those fed a HFD. The IR index and fasting serum TGs were 34% (P = 0.03) and 102% (P = 0.0001) greater, respectively, in the group fed the HCHD than the group fed the HFD (Fig. 3A–D). Nonetheless, in both groups, the IR index and fasting serum TGs were above the previously reported control values (Eu et al. 2010; Kowalski and Bruce 2014). We observed that the elevation in the IR index was the result of an increase in serum insulin without a significant change in fasting serum glucose in both groups (Fig. 3E–H). In addition, this difference between rats fed a HCHD and rats fed a HFD was evident after the OGTT with insulin measurements because the area under the curve (AUC) for serum insulin was significantly greater in rats fed a HCHD than in rats fed a HFD (Fig. 4A and B).

Body weight change after autologous transplant in obese rats fed a HFD or HCHD
A duration of 90 days after the surgical procedure (Fig. 2), the Sh group fed a HFD gained 83 g, whereas the corresponding Rv and Tr groups gained approximately 49.9 g and 48.6 g, respectively, representing 40.1% and 41.7% significantly less weight gain than the Sh group (Table 1). Interestingly, the Sh group fed a HCHD
gained 116 g, but surprisingly, the Rv and Tr groups gained approximately 4.3 and 7.3 g, respectively, representing 96.2% and 93.7% significantly less weight gain than the Sh group. However, energy consumption and liver weight were similar among the three groups for each diet (Table 1).

At the end of the study, as expected, the amount of SAT in rats fed a HFD was significantly less in the Rv and Tr groups than in the Sh group because it was removed during the surgical procedure (Table 1). However, despite the amount of SAT transplanted to VAT in the Tr group, there was not a significant difference among the groups in the amount of visceral fat (Table 1). Regarding the groups of rats fed a HCHD, the Rv and Tr groups also had significantly less SAT than the Sh group. Nonetheless, there was less

Figure 3. Biochemical parameters before and after surgery. Fasting serum triglycerides (TGs) (A and B), insulin resistance (IR) index (C and D), fasting serum insulin (E and F), and fasting serum glucose, (G and H) were determined in rats fed the high-fat diet (HFD) (A, C, E, and G) and in rats fed the high-carbohydrate diet (HCHD) (B, D, F, and H) a few days prior to surgery (presurgery) and 90 days after surgery (postsurgery) in the sham (Sh), removal (Rv), and transplant (Tr) groups. The data are presented as the mean ± SEM and differences were significant at *p < 0.05 and **p < 0.01 between the rats presurgery and postsurgery evaluated by two-way ANOVA.
subcutaneous fat in the Rv group than in the Tr group. In contrast to the rats fed a HFD, the rats fed a HCHD showed significantly less VAT in the Rv and Tr groups than in the Sh group (Table 1). The ratio of visceral to subcutaneous fat was 5.4 in the Rv group, whereas in the Tr group, this ratio was 1.0. Interestingly, in the Tr group, the amount of VAT was even less than in the Rv group, although the SAT was transplanted to the visceral compartment (Table 1).

Metabolic changes after autologous transplant in obese rats

There was a marked difference in some metabolic parameters in rats fed a HFD or HCHD. In rats fed either a HFD or HCHD, there was a trend to reduce fasting serum glucose; however, this difference did not reach statistical significance (Fig. 3G and H). Nonetheless, fasting serum insulin was significantly reduced after the
transplant with both diets. Rats fed a HFD and HCHD had 54% and 67% less insulin, respectively, than prior to surgery (Fig. 3E and F). As a consequence, there was a significant reduction in the IR index of 59% and 65% in the Tr groups fed a HFD and HCHD, respectively (Fig. 3C and D). Presurgical fasting serum TG concentrations in rats fed a HCHD were greater than those in rats fed a HFD. In rats fed a HFD, fasting serum TG values were similar before and after surgery. However, in rats fed a HCHD, fasting serum TGs tended to reduce after transplant (Fig. 3A and B). Unexpectedly, the Rv group fed a HCHD showed a reduction in fasting serum insulin, IR index, and fasting serum TGs (Fig. 3B, D, and F). This was an effect not observed in the same group fed the HFD (Fig. 3A, C, and E).

**Autologous transplant in obese rats improves insulin sensitivity**

To further determine changes in insulin sensitivity, we performed an OGTT with insulin measurements 90 days after surgery (Fig. 4C–F). The AUC values of serum glucose of the OGTT were not modified among the three groups in both diets (Fig. 4C and D). The AUC of the serum insulin concentrations of the rats fed a HFD tended to decrease in the Tr group compared with the Sh group (Fig. 4E). Nevertheless, in rats fed a HCHD, the AUC was significantly reduced in the Tr group compared to the Sh group (Fig. 4F), indicating that less insulin was required to maintain the same serum glucose concentrations.

**Hepatic lipid reduction after autologous transplant in obese rats**

Hepatic TG concentrations were significantly reduced only in the Tr group of rats fed a HFD or HCHD (Fig. 5A). Rats fed a HFD showed significantly lower hepatic cholesterol concentrations in the Tr group than in the Sh or Rv groups. However, rats fed a HCHD maintained a normal hepatic cholesterol concentration that did not change with the transplant (Fig. 5B). These findings correlated with the histological analysis that revealed that the liver showed fewer lipid deposits only in the case of the autologous transplant (Fig. 5C).

**Autologous transplant reduced FFAs through a decrease in adipose tissue lipolysis**

In the histology of the omental and retroperitoneal adipose tissues, two cell populations of different size can be distinguished in the Tr group (indicated by arrows), in
which the smaller adipocytes corresponded to the transplanted SAT, whereas in the Sh group, only hypertrophic adipocytes were observed. This was more evident in rats fed HCHD than HFD (Fig. 6A).

The mitochondrial uncoupling protein 1 (UCP-1) is present in brown and beige adipocytes, whereas white adipocytes are UCP-1-negative. The function of UCP-1 is to uncouple electron transport from ATP production, which in turn leads to controlled exothermic resolution of the electrochemical gradient and generation of heat (Harms and Seale 2013; Bartelt and Heeren 2014). Thus, since the rats that were transplanted with SAT had less or the same weight of VAT as the Sh or Rv groups (Table 1), the expression of UCP-1 was evaluated in the omental and retroperitoneal adipose tissue as a browning marker. However, UCP-1 levels did not increase in the Tr group in comparison with the Sh and Rv groups (data not shown).

There is evidence that an increase in circulating FFAs causes hepatic and muscle lipid accumulation, leading to insulin resistance and lipotoxicity (Fruhbeck et al. 2014). After the transplant, fasting serum FFAs were significantly reduced compared with the Sh group in both diets (Fig. 6B). Fasting serum glycerol, which was utilized as a lipolysis marker, was also significantly reduced in the Tr group compared with the Sh group (Fig. 6C). To evaluate the mechanism by which lipolysis was decreased with the autologous transplant, the activity of hormone-sensitive lipase (HSL) was measured through its phosphorylation at Ser563 in the omental or retroperitoneal adipose tissue where the SAT was transplanted.

The abundance of total HSL protein, as well as pHSL at Ser563, was greater in rats fed a HFD than in rats fed a HCHD in both compartments (omentum and retroperitoneal) (Fig. 6D). The proportion of pHSL at Ser563 in relation to total HSL content was significantly reduced in the Tr group compared with the Sh and Rv groups with both diets in both compartments. Finally, this effect was much more evident in the adipose tissue transplanted to the omental compartment than the adipose tissue transplanted to the retroperitoneal compartment in both diets (Fig. 6D and E).

**Discussion**

Autologous SAT transplant improved the metabolic parameters associated with the DIO model. A duration of 90 days after SAT transplant into the omental and retroperitoneal intraabdominal compartments in the HFD- or HCHD-induced obesity models, insulin sensitivity was improved, hepatic lipid content was reduced, and FFAs concentrations were also decreased.

It is interesting to remark on the significant reduction in the amount of VAT in the Tr group compared with the Sh group in rats fed a HCHD, taking into account that in the Tr group, the SAT was removed and then transplanted into the VAT. However, this effect was not observed in rats fed a HFD, suggesting that energy substrates play an important role in this finding. In fact, we have previously reported that adipose tissue metabolism depends on diet composition (Frigolo et al. 2011; Tovar et al. 2011; Diaz-Villasenor et al. 2013). Therefore, this reduction in VAT after the transplant may explain the significant improvement in insulin sensitivity, and consequently, the remarkable reduction in body weight in rats fed the HCHD. This reduction in the mass of the VAT and the metabolic improvement observed with the transplant was not associated with VAT browning, nevertheless is probably associated with the shift in the size of the adipocytes, since small adipocytes are more insulin sensitive and functional than hypertrophic adipocytes (Laforest et al. 2015). However, future studies will be required to understand the mechanism by which VAT was decreased in rats fed a HCHD after the transplant.

The mechanism by which the transplant improves metabolic parameters is still unknown. Although there is no consensus regarding the site where the transplant should be located, we believe that not only the type of adipose tissue that is transplanted (SAT or VAT) but also the intraabdominal localization of the transplant plays a major role in the metabolic effects.

It has been reported in several articles that serum FFAs are reduced after adipose tissue transplant (Gavrilova et al. 2000; Foster et al. 2011, 2013; Hocking et al. 2015). However, approximately one-third of the FFAs released from the adipose tissue to the circulation are reesterified, so a more accurate marker of lipolysis is the measurement of serum glycerol (Forest et al. 2003). Here, we demonstrate that both serum FFAs and glycerol decreased after the transplant in both DIO models, indicating that less lipolysis occurs in these rats, which possibly explains the observed reduction in hepatic lipid content. In fact, the venous drainage of the omentum goes directly to the portal system that ultimately reaches the liver, whereas the venous drainage of the retroperitoneum goes to the systemic circulation through the vena cava (Rytka et al. 2011; Tchernof and Despres 2013).

An important step in lipolysis activation in response to catecholaminergic stimulation comprises the translocation of HSL from a cytosolic compartment to the surface of the lipid droplet, through the protein kinase A-mediated phosphorylation of HSL at Ser563, Ser659, and Ser660 (Fruhbeck et al. 2014). Accordingly, the decrease in the active state of HSL, evaluated through the phosphorylation at Ser563, was significantly more evident in the...
Figure 6. Adipose tissue morphology and lipolytic functionality. Representative images of the omental and retroperitoneal adipose tissue samples stained with hematoxylin and eosin from rats fed the HFD or the HCHD 90 days after surgery in the sham (Sh) and transplant (Tr) groups (A). Fasting serum FFAs (B) and glycerol concentrations (C) in rats fed the HFD and the HCHD 90 days after surgery in the sham (Sh), removal (Rv), and transplant (Tr) groups. Representative blots of pHSL Ser563, total HSL, and actin in the omental and retroperitoneal adipose tissue of rats fed the HFD and the HCHD 90 days after surgery in the sham (Sh), removal (Rv), and transplant (Tr) groups (D), and the ratio of pHSL Ser563 to HSL was evaluated via optical density analyses of the blots normalized to actin (E). The data are presented as the mean ± SEM, and symbols denote significant differences between the removal (Rv) or transplant (Tr) groups versus the Sham group (Sh) at *P < 0.05, **P < 0.01 and ***P < 0.001. Significant differences for free fatty acids (FFAs) in rats fed the HFD and glycerol in both diets were evaluated using the Kruskal–Wallis test followed by the Dunn posttest, whereas FFAs in the rats fed the HCHD and the ratio of the phosphorylation of HSL were determined by ANOVA followed by the Dunnett posttest.
In rats fed either the HCHD or the HFD, the transplant of SAT generated metabolic improvements in both groups. The mechanism that we propose is that in the omental and retroperitoneal adipose tissue transplanted with SAT, the size of the adipocytes became less hypertrophic, thus improving adipose tissue insulin sensitivity, resulting in greater insulin-mediated suppression of lipolysis, since insulin antagonizes catecholamine-induced lipolysis. Thus, fasting lipolysis was reduced due to a decrease in the active state of HSL in VAT, and in consequence, there was a decrease in circulating FFAs and glycerol. A decreased flux of FFAs to circulation impacted on less lipid accumulation in liver and probably in muscle, consequently improving systemic insulin sensitivity evaluated through the IR index and resulting in lower serum insulin levels. As a consequence, several metabolic variables were improved compared with the Sh obese rats. However, more studies are needed to understand the molecular mechanism by which the transplant can modulate the activation of HSL.

In conclusion, our study clearly showed that an autologous SAT transplant may be considered an important strategy to improve the homeostasis of carbohydrate and lipid metabolism during obesity. It is necessary to take into account that the location of the SAT transplant into the visceral compartments of adipose tissue is an important issue to generate beneficial health effects. Further studies are needed to demonstrate that the use of autologous SAT transplant can be utilized in humans with obesity.

Conflict of Interest
None declared.
2013. Differential modulation of the functionality of white adipose tissue of obese Zucker (fa/fa) rats by the type of protein and the amount and type of fat. J. Nutr. Biochem. 24:1798–1809.

Ebbert, J. O., and M. D. Jensen. 2013. Fat depots, free fatty acids, and dyslipidemia. Nutrients 5:498–508.

Eu, C. H., W. Y. Lim, S. H. Ton, and K. Bin Abdul Kadir. 2010. Glycyrrhizic acid improved lipoprotein lipase expression, insulin sensitivity, serum lipid and lipid deposition in high-fat diet-induced obese rats. Lipids Health Dis. 9:81.

Fishman, J. A. 2007. Infection in solid-organ transplant recipients. N. Engl. J. Med. 357:2601–2614.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497–509.

Forest, C., J. Tordjman, M. Glorian, E. Duplus, G. Chauvet, J. Quette, et al. 2003. Fatty acid recycling in adipocytes: a role for glyceroneogenesis and phosphoenolpyruvate carboxikinase. Biochem. Soc. Trans. 31:1125–1129.

Foster, M. T., H. Shi, S. Sofic, R. Kohli, R. J. Seeley, and S. C. Woods. 2011. Transplantation of non-visceral fat to the visceral cavity improves glucose tolerance in mice: investigation of hepatic lipids and insulin sensitivity. Diabetologia 54:2890–2899.

Foster, M. T., S. Sofic, J. Caldwell, R. Kohli, A. D. De Kloet, and R. J. Seeley. 2013. Subcutaneous adipose tissue transplantation in diet-induced obese mice attenuates metabolic deregulation while removal exacerbates it. Physiol. Rep. 1:e00015.

Frigolet, M. E., N. Torres, L. Uribe-Figueroa, C. Rangel, G. Jimenez-Sanchez, and A. R. Tovar. 2011. White adipose tissue genome wide-expression profiling and adipocyte metabolic functions after soy protein consumption in rats. J. Nutr. Biochem. 22:118–129.

Frubheber, G., L. Mendez-Gimenez, J. A. Fernandez-Formoso, S. Fernandez, and A. Rodriguez. 2014. Regulation of adipocyte lipolysis. Nutr. Res. Rev. 27:63–93.

Gavrilova, O., B. Marcus-Samuels, D. Graham, J. K. Kim, G. I. Shulman, A. L. Castle, et al. 2000. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J. Clin. Invest. 105:271–278.

Gutowski, K. A., and A. F. G. T. Force. 2009. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. Plast. Reconstr. Surg. 124:272–280.

Harms, M., and P. Seale. 2013. Brown and beige fat: development, function and therapeutic potential. Nat. Med. 19:1252–1263.

Hocking, S. L., R. L. Stewart, A. E. Brandon, E. Suryana, E. Stuart, E. M. Baldwin, et al. 2015. Subcutaneous fat transplantation alleviates diet-induced glucose intolerance and inflammation in mice. Diabetologia 58:1587–1600.

Ibrahim, M. M. 2010. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes. Rev. 11:11–18.

Kahn, S. E. 2003. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 46:3–19.

Kakkar, A. K., and N. Dahiya. 2015. Drug treatment of obesity: current status and future prospects. Eur. J. Intern. Med. 26:89–94.

Khan, L. K., K. Sobush, D. Keener, K. Goodman, A. Lowry, J. Kakietek, et al. ; Centers for Disease Control and Prevention. 2009. Recommended community strategies and measurements to prevent obesity in the United States. MMWR Recomm. Rep. 58:1–26.

Konrad, D., A. Rudich, and E. J. Schoenle. 2007. Improved glucose tolerance in mice receiving intraperitoneal transplantation of normal fat tissue. Diabetologia 50:833–839.

Kowalski, G. M., and C. R. Bruce. 2014. The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. Am. J. Physiol. Endocrinol. Metab. 307:E859–E871.

Laforest, S., J. Labrecque, A. Michaud, K. Cianflone, and A. Tchernof. 2015. Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction. Crit. Rev. Clin. Lab. Sci. 52:301–313.

Malik, V. S., W. C. Willett, and F. B. Hu. 2013. Global obesity: trends, risk factors and policy implications. Nat. Rev. Endocrinol. 9:13–27.

Ogden, C. L., M. D. Carroll, B. K. Kit, and K. M. Flegal. 2014. Prevalence of childhood and adult obesity in the United States, 2011–2012. JAMA 311:806–814.

Patel, P., and N. Abate. 2013. Body fat distribution and insulin resistance. Nutrients 5:2019–2027.

Perrini, S., A. Leonardi, L. Laviola, and F. Giorgino. 2008. Biological specificity of visceral adipose tissue and therapeutic intervention. Arch. Physiol. Biochem. 114:277–286.

Rasband, W. 1997. ImageJ Software, Java 1.6.0_33, version 1.44n9 ed.: National Institutes of Health. Available at http://imagej.nih.gov/ij/index.html. (accessed 30 July 2016).

Rosen, E. D., and B. M. Spiegelman. 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444:847–853.

Rytka, J. M., S. Wueest, E. J. Schoenle, and D. Konrad. 2011. The portal theory supported by venous drainage-selective fat transplantation. Diabetes 60:56–63.

Satoor, S. N., A. S. Puranik, S. Kumar, M. D. Williams, M. Ghale, A. Rahalkar, et al. 2011. Location, location, location: beneficial effects of autologous fat transplantation. Sci. Rep. 1:81.

Seetharam, A., V. Tiriveedhi, and T. Mohanakumar. 2010. Alloimmunity and autoimmunity in chronic rejection. Curr. Opin. Organ Transplant. 15:531–536.
Tchernof, A., and J. P. Despres. 2013. Pathophysiology of human visceral obesity: an update. Physiol. Rev. 93:359–404.

Thomas, C. D., J. C. Peters, G. W. Reed, N. N. Abumrad, M. Sun, and J. O. Hill. 1992. Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. Am. J. Clin. Nutr. 55:934–942.

Torres-Villalobos, G., N. Hamdan-Perez, A. R. Tovar, G. Ordaz-Nava, B. Martinez-Benitez, I. Torre-Villalvazo, et al. 2015. Combined high-fat diet and sustained high sucrose consumption promotes NAFLD in a murine model. Ann. Hepatol. 14:540–546.

Tovar, A. R., A. Diaz-Villasenor, N. Cruz-Salazar, G. Ordaz, O. Granados, B. Palacios-Gonzalez, et al. 2011. Dietary type and amount of fat modulate lipid metabolism gene expression in liver and in adipose tissue in high-fat diet-fed rats. Arch. Med. Res. 42:540–553.

Tran, T. T., Y. Yamamoto, S. Gesta, and C. R. Kahn. 2008. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 7:410–420.

Wong, R. H., and H. S. Sul. 2010. Insulin signaling in fatty acid and fat synthesis: a transcriptional perspective. Curr. Opin. Pharmacol. 10:684–691.