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Obesity (Silver Spring). Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Obesity (Silver Spring). 2015 January ; 23(1): 105–111. doi:10.1002/oby.20906.

Increase in visceral fat *per se* does not induce insulin resistance in the canine model

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Abstract

Objective—To determine whether a selective increase of visceral adipose tissue content will result in insulin resistance.

Design and Methods—Sympathetic denervation of the omental fat was performed under general inhalant anesthesia by injecting 6-hydroxydopamine in the omental fat of lean mongrel dogs (n=11). In the conscious animal, whole-body insulin sensitivity was assessed by the minimal model (S_I) and the euglycemic hyperinsulinemic clamp (SI_{CLAMP}). Changes in abdominal fat were monitored by magnetic resonance. All assessments were determined before (Wk0) and 2 weeks (Wk2) after denervation. Data are medians (upper and lower interquartile).

Results—Denervation of omental fat resulted in increased percentage (and content) of visceral fat [Wk0: 10.2% (8.5–11.4); Wk2: 12.4% (10.4–13.6); P<0.01]. Abdominal subcutaneous fat remained unchanged. However, we found no changes in S_I [Wk0: 4.7 $(mU/L)^{-1}\bullet min^{-1}$ (3.1–8.8); Wk2: 5.3 $(mU/L)^{-1}\bullet min^{-1}$ (4.5–7.2); P=0.59] or SI_{CLAMP} [Wk0: 42.0 $\times 10^{-4}$ $dL\bullet kg^{-1}\bullet min^{-1}\bullet (mU/L)^{-1}$ (41.0–51.0); Wk2: 40.0 $\times 10^{-4}$ $dL\bullet kg^{-1}\bullet min^{-1}\bullet (mU/L)^{-1}$ (34.0–52.0); P=0.67].

Conclusions—Despite a selective increase in visceral adiposity in dogs, insulin sensitivity*in vivo* does not change, which argues against the concept that accumulation of visceral adipose tissue contributes to insulin resistance.

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Competing interests: the authors have no competing interests.

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Keywords

Insulin sensitivity; Lipolysis; Omental fat; Sympathetic denervation; Visceral adipose tissue

INTRODUCTION

Insulin resistance (low insulin sensitivity) contributes to the pathogenesis of type 2 diabetes (1), yet the pathophysiology of insulin resistance is not fully understood. Abdominal fat accumulation is believed to play an important role in the development of insulin resistance, although the relative contribution of a specific fat depot is less clear. Arguments supporting intra-abdominal fat or abdominal subcutaneous fat as the major contributor of insulin resistance have been extensively discussed (2-7). However, most of the evidence supporting either notion come from epidemiological and cross-sectional studies (8); thus, causality cannot be proved. Visceral adipose tissue is mainly composed of omental and mesenteric fat (8). Omental fat displays high lipolytic activity, resistance to insulin-mediated suppression of lipolysis, and release of pro-inflammatory cytokines (2,8). It is thought that these features, together with the flux of non-esterified fatty acids (NEFA) from this depot via the portal vein to the liver, may contribute to hepatic insulin resistance ("portal hypothesis") (3).

Surgical reduction of visceral fat has been shown to improve insulin sensitivity in rodents (9,10), dogs (11), and humans (12-14). However, surgical removal of omental fat *per se* does not provide additional metabolic benefits in obese individuals with or without type 2 diabetes who underwent gastric bypass surgery (12-15), suggesting that visceral fat mass is not the main determinant in improvement of insulin sensitivity after bariatric surgery. Although numerous studies have explored the effect of removing visceral fat on insulin sensitivity, no study has investigated the effect of selective visceral fat accumulation on insulin sensitivity. Sympathetic denervation of epididymal, retroperitoneal, and inguinal fat tissue has been shown to promote adipocyte hyperplasia in rodents (16,17), offering a tool to induce fat accumulation in the omental fat. Thus, we used this approach in our canine model to selectively increase visceral fat content. We hypothesized that accumulation of visceral adipose tissue impairs insulin sensitivity. To test this hypothesis we assessed insulin sensitivity *in vivo* using widely accepted techniques (euglycemic hyperinsulinemic clamp and the minimal model approach (18)) before and after visceral fat accumulation induced by sympathetic denervation of the omental fat.

METHODS

Animals and housing conditions

Eighteen male mongrel dogs [mean ± SD: 28.3 ± 2.6 kg] were housed in the vivarium at Keck School of Medicine of the University of Southern California and at Cedars-Sinai Medical Center under controlled kennel conditions (12:12-h light-dark cycle). Animals were included in this study following physical examination and a comprehensive blood panel. Dogs were accustomed to laboratory procedures and were used for experiments only if judged to be in good health as determined by visual observation, body temperature, and hematocrit. The experimental protocol was approved by the Institutional Animal Care and

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Use Committee (IACUC) from both the University of Southern California and Cedars-Sinai Medical Center.

Diet

All animals had access to water *ad libitum*. Dogs were fed a weight-maintaining standard diet that consisted of 415 g of Hill's Prescription Diet (Hill's Pet Nutrition, Topeka, KS) and 825 g of dry chow mixture (Laboratory High Density Canine Diet and Prolab Canine 2000, Richmond, IN). Hill's Prescription Diet's composition was 10% carbohydrate, 9% protein, 8% fat, 0.3% fiber, and 73% moisture. To ensure weight stabilization, dry chow (40% carbohydrate, 26% protein, 14% fat, and 3% fiber) alone was given for a period of 2–3 weeks before the experimental period started. Food was presented from 0900–1200 h daily.

Experimental design

Assessments of body weight, fasting blood biochemistry, insulin sensitivity *in vivo*, β-cell function *in vivo*, and magnetic resonance imaging of abdominal trunk were performed before and 2 weeks after sympathetic denervation of the omental fat.

Chemical sympathetic denervation

We adapted the chemical sympathetic denervation protocol performed in hamsters by Foster and Bartness (19) to induce selective denervation of the omental fat in dogs (n=11). Under general inhalant anesthesia, a median incision in the abdominal wall was performed, the omental fat depot exposed, and 6-hydroxydopamine (6-OHDA, Sigma-Aldrich) 0.6 mg/mL in saline 0.09% containing ascorbic acid 1% (Sigma-Aldrich) was injected multiple times parallel to the vasculature of the greater omentum using a 27-G hypodermic needle. In the control group (n=7), the protocol was identical except that animals received injections of vehicle only (saline 0.09% and ascorbic acid 1%). The total volume of 6-OHDA and vehicle injected was 100.6 ± 8.8 mL (mean ± SEM) and 123.6 ± 16.9 mL, respectively (P=0.26). Approximately, 2.1 ± 0.2 mg of 6-OHDA solution per kg of body weight were injected into the omental fat depot.

Magnetic Resonance Imaging (MRI)

MRI scans of the dog abdominal trunk were performed as previously described (20). Briefly, eleven axial images were obtained, five above, one at, and five below the level of the left renal hilum (middle-point landmark), covering in total an 11-cm axial length. Fat distribution assessment included visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT), and total fat (VAT+SAT). Fat and non-fat contents (volume) were estimated using SliceOmatic (Tomovision, Montreal, Canada). First, regions of interest (VAT, SAT, and non-fat tissue) of each image were determined by manual trace, based on the histogram of image pixel intensity, after which volume was automatically calculated based on field-of-view settings. We could not perform MRI scans in two dogs (one from each group) due to adverse effects of the anesthesia (ketamine, 10 mg/kg and diazepam 0.5 mg/kg). MRI image analyses were performed by V. Ionut, who was blinded to the experimental protocol.

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Adipocyte size

Adipocytes from VAT (visceral adipose tissue), superficial and deep depot, and SAT (subcutaneous adipose tissue) were isolated according to the method performed by Rodbell (21) and Morisset et al (22). Biopsies were taken under general inhalant anesthesia immediately before the injections of 6-OHDA or saline and two weeks after the injections, immediately before euthanasia. We defined superficial VAT as the fat beneath the canine visceral peritoneum that is adjacent to the abdominal wall, and deep VAT as the omental fat or greater omentum. Adipose tissue digestion took place in a shaking water bath for 40 min at 37°C. The suspension was then filtered and the cellular filtrate obtained was washed 3 times with Krebs buffer. Approximately 1 mL of pure fat cell suspension was obtained and re-suspended in 6 mL of Krebs buffer (solution A). Three 10-μL aliquots each of solution A were placed on a glass slide and 300-1,000 cells were photographed at 10X using an inverted microscope. Adipocyte size, denoted as the cell diameter, was measured using Image-Pro Express software (Media Cybernetics, Bethesda, MD) as previously described (23).

Lipolysis *in vitro*

Lipolysis experiments were performed in adipocytes from superficial and deep VAT and from subcutaneous fat. Isolated cell suspensions were incubated for 2 h at 37°C in Krebs-Ringer buffer, with or without β-adrenergic receptor agonist isoproterenol at different concentrations (1×10^{-8}, 6.25×10^{-8}, 1.25×10^{-7}, 2.1×10^{-7}, 2.5×10^{-7}, 5.1×10^{-7}, 5.1×10^{-6}, and 1×10^{-5} mol/L). Lipolysis inhibition in response to insulin at different concentrations (20, 50, 70, 100, 150, and 190 pmol/L) was also determined in adipocytes previously stimulated with isoproterenol at 5×10^{-7} mol/L. Lipolysis was estimated as the rate of glycerol released in the medium, measured in a 100-μL aliquot, using the Glycerol-Free Reagent kit (Sigma-Aldrich Co., St. Louis, MO). Average adipocyte weight and cell number in the suspensions were calculated using lipid weight, average cell volume, and the density of triolein. Lipolysis results were expressed in μmol per 10^6 cells per 2 h. Cell number was counted in three 10-μL aliquots each at 4X using Image-Pro Express.

Assessment of insulin sensitivity

The main goal of our study was to assess the effect of selective increase of visceral fat mass on whole-body insulin sensitivity. Insulin sensitivity *in vivo* was assessed by the euglycemic hyperinsulinemic clamp and the minimal model.

Minimal model—Assessment of whole-body insulin sensitivity using the minimal model approach was derived from the frequently sampled intravenous glucose tolerance test, performed as previously described (24). Briefly, basal venous samples were taken at −20, −10, and −1 min, followed by an intravenous bolus of glucose 50% (0.3 g/kg of body weight) at 0 min. At 20 min, an intravenous bolus of rapid-acting insulin (0.03 U/kg porcine insulin; Eli Lilly and Company, Indianapolis, IN, USA) was given. Peripheral venous samples were taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 min.

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Euglycemic hyperinsulinemic clamp (EGC)—Assessment of whole-body insulin sensitivity using EGC was performed in a subset of animals from both treated (denervated) and control animals. However, to explore possible changes on peripheral and/or hepatic insulin sensitivity, we performed glucose-labeled EGC only in a subset of animals that underwent sympathetic denervation. EGC was performed as previously described (25). At –120 min, an intravenous bolus of [3-^{3}H]glucose (25 μCi; DuPont-NEN, Boston, MA) was given followed by a continuous intravenous infusion of [3-^{3}H]glucose (0.25 μCi/min). After tracer equilibration, basal venous samples were taken at –30, –20, –10, and –1 min. At 0 min, somatostatin (1.0 $\mu g \cdot min^{-1} \cdot kg^{-1}$; Bachem California, Torrance, CA) and porcine insulin intravenous infusions (0.75 $mU \cdot kg^{-1} \cdot min^{-1}$; Eli Lilly, Indianapolis, IN) were started and continued for the duration of the clamp. Glucose was clamped at basal concentrations by a variable intravenous infusion of glucose labeled with [3-^{3}H]glucose (2.0 μCi/g). Peripheral venous samples were collected every 10 min from 0 to 180 min.

Calculations of insulin sensitivity and β-cell function

Hepatic glucose production and glucose disappearance were assessed by the euglycemic hyperinsulinemic clamp as previously described (25,26). Derivatives of all time course data were calculated with OOPSEG (27). Basal plasma glucose was defined as the average of four venous samples taken from –30 to −1 min, and steady state was defined as the average of four venous samples taken from 150 to 180 min. whole-body insulin sensitivity (SI_{CLAMP}) was calculated using the equation SI_{CLAMP}= G_{inf}/(I X G), where G_{inf} is the difference of glucose intravenous infusion rate at steady state minus basal,]I is the difference of plasma insulin at steady state minus basal, and G is the steady-state plasma glucose concentration. Peripheral insulin sensitivity (SIp_{CLAMP}) was calculated using the equation SIp_{CLAMP}= R_d/(I X G), where R_d is the difference of glucose dissapearance at steady state minus basal. Hepatic insulin sensitivity or hepatic glucose output ($SIHGO_{CLAMP}$) was calculated using the equation $SIHGO_{CLAMP}$= HGO/(I X G), where HGO is the difference of hepatic glucose output at steady state minus basal.

Insulin sensitivity *in vivo*, derived from the minimal model (S_I), and β-cell function *in vivo*, as determined by acute insulin response to glucose (AIRg), were calculated as previously described (24), using the Minmod Millennium version 6.02, 2004 (MINMOD Inc., Los Angeles, CA).

Assays

Fasting insulin, D-[3-3H]glucose, leptin, and adiponectin were measured in plasma from blood collected in tubes precoated with lithium fluoride and heparin (Brinkmann Instruments, Westbury, NY). In addition, tubes for insulin and glucose also contained 50 μL of EDTA (2% w/vol). Fasting NEFA and glycerol were measured in plasma from blood collected in tubes coated with paraoxon (80 μL paraoxon in 30 mL ether) containing 50 μL of EDTA. After glucose measurement, all plasma samples were stored at –80°C for further analyses. Plasma glucose, insulin, and NEFA were measured as previously described (25). Glycerol (Serum glycerol determination kit, Sigma Chemical, St. Louis, MO) was measured by a colorimetric method. Leptin and adiponectin were measured by ELISA (Linco, Millipore, St. Charles, MO). To determine the content of catecholamines (norepinephrine,

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epinephrine, and dopamine) and the neurotransmitter serotonin, omental fat (~100-200 mg) was biopsied under anesthesia pre- and post-denervation. Tissue specimens were frozen in liquid nitrogen and stored at −80°C until content assay. Omental fat tissue was homogenized in perchloric acid (1:1 or 1:4, w:v) and the mixture was centrifuged for 15 min at 14,000 *g* (4°C). The supernatant was filtered and the filtrate directly measured by HPLC (28). Results were expressed as pmol per g of omental fat.

Statistical analyses

Data frequency distribution was not normal. Thus, data were presented as medians (lower and upper quartiles), unless otherwise indicated. To compare *in vivo* results pre- and post-denervation, Wilcoxon matched pairs test was used. For repeated-measure experiments, Friedman ANOVA was used followed by Wilcoxon matched pairs test if the P value was significant ($P<0.05$). Mann-Whitney U test was used to compare values between groups. Mixed-model analysis was used to determine the effect of denervation on lipolysis, while controlling for time between lipolysis assessments, and accounting for the overall response to serial isoproterenol concentrations. Likewise, mixed-model analysis was used to determine the effect of denervation on adipocyte size and adipocyte number, while controlling for time between adipocyte morphometry assessments. All statistical analyses were performed in Statistica 7.0 (StatSoft, Inc., Tulsa, OK) or Stata/SE 10.0 for Windows (StataCorp LP, College Station, TX).

RESULTS

Overall, visceral injections of 6-OHDA or saline did not result in significant changes in vital signs (blood pressure, heart rate, and peripheral oxygen saturation). Confirming sympathetic denervation of the visceral fat depot, injection with 6-OHDA of the omental fat caused a significant decrease in norepinephrine content [median (interquartile range): 1.3 pmol/g (0.7 to 6.5) versus 0.4 pmol/g (0.2 to 1.5); P=0.012], but not epinephrine content [0.2 pmol/g (0.1 to 1.1) versus 0.6 pmol/g (0.0 to 1.1); P=0.92]. In contrast, control dogs receiving injections of vehicle (saline and ascorbic acid) showed only a nonsignificant trend for a decrease in norepinephrine content [9.6 pmol/g (8.3 to 10.3) versus 1.8 pmol/g (1.4 to 3.6); P=0.109]. The content of dopamine was not affected by denervation [0.2 pmol/g (0.1 to 0.3) and 0.2 pmol/g (0.1 to 0.2); P=0.401; pre- and post-denervation, respectively]. Likewise, serotonin content did not change post. denervation [0.1 pmol/g (0.1 to 0.2) and 0.1 pmol/g (0.1 to 0.7); P=0.208; pre-and post-denervation, respectively].

Sympathetic denervation was used to increase visceral fat mass without changing subcutaneous fat, as evidenced by MRI (Figure 1). After denervation, we did observe a sizable increase in VAT content of 25.2% (6.9–49.6) (P<0.01) compared with pre. denervation (Table 1). The relative percentage of VAT of total abdominal tissue was also increased [10.2% (8.5–11.4) versus 12.4% (10.4–13.6); P<0.01]. As expected, sympathetic denervation of the omental fat did not significantly alter SAT content (P=0.114) or relative SAT percentage (P=0.169). Thus, we were able to cause an increase in visceral fat mass only. Proving that the increase in VAT was due to sympathetic denervation, there was no change in either fat depot with the vehicle alone [VAT (P=0.917) or SAT content

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(P=0.753)]. We only found significant differences between groups in VAT content at week 2 (P=0.017).

Basal lipolysis did not significantly change after denervation: superficial VAT 0.34 (0.27-0.43) and 0.57 (0.48-0.81) μmol per 10^6 cells/2 h (P=0.365); deep VAT 0.27 (0.23-0.46) and 0.44 (0.36-0.60) μmol per 10^6 cells/2 h (P=0.304); SAT 0.07 (0.01-0.112) and 0.22 (0.16.0.31) μmol per 10^6 cells/2 h (P=0.484); pre- and post-denervation, respectively. Overall, isoproterenol-stimulated lipolysis did not significantly changed after denervation (Figure 2). Likewise, we found no greater effect of denervation as compared with control on isoproterenol-stimulated lipolysis (superficial VAT: P=0.814; deep VAT: P=0.918; SAT: P=0.160) or lipolysis inhibition in response to insulin in superficial VAT (P=0.533) or deep VAT (P=0.521). Moreover, sympathetic denervation of the omental fat slightly increased adipocyte diameter in omental fat (deep VAT), from 69.7 (68.3-72.9) μm to 70.5 (65.2-74.7) μm (P<0.001). In contrast, denervation decreased adipocyte size from superficial VAT [from 74.7 (67.0-78.8) μm to 71.0 (68.4-76.9) μm (P=0.011)] and SAT [from 62.4 (57.0-73.0) μm to 61.5 (59.2-71.5) μm (P<0.001)]. We found no effect of denervation on adipocyte number (superficial VAT: P=0.755; deep VAT: P=0.244; SAT: P=0.720) as compared with control group.

Most important, despite rapid accumulation of VAT after sympathetic denervation, there were no measureable changes in overall metabolic function. We did not observe significant alterations of fasting plasma levels of NEFA, adiponectin, or leptin (Table 2), changes in whole body insulin sensitivity or β-cell function measured with the minimal model. Furthermore, hepatic, peripheral, and whole-body insulin sensitivity derived from the euglycemic hyperinsulinemic clamp did not change after denervation (Table 2). No differences in metabolic features were found between groups.

DISCUSSION

Epidemiological evidence has supported the concept that visceral fat *per se* is detrimental to metabolic function, contributing to insulin resistance. In fact, reduction of visceral fat improved insulin sensitivity in experimental animals (9-11), while the data in humans is equivocal, particularly when performed concomitant with bariatric surgery (12-14). One problem is that it is difficult to change visceral fat content separate from subcutaneous fat, and overall fat distribution. In the present study, we used sympathetic denervation of the omental fat as a method to change visceral fat alone, independent of other fat depots. We tested the hypothesis that an increase in visceral fat mass impairs insulin sensitivity. Despite a greater than 25% increase in VAT content, and no change in subcutaneous fat deposition, we found no significant changes in overall metabolic function, including insulin sensitivity assessed by both the minimal model and the euglycemic hyperinsulinemic clamp. Therefore these studies in the canine model do not support the concept that accumulation of visceral fat content *per se* is an important causal factor in overall insulin resistance.

Fasting plasma leptin and adiponectin also remained unchanged. We expected no changes in leptin but a decrease in adiponectin since adiponectin is inversely correlated with VAT (29), and leptin is directly correlated with SAT (30). We also found no changes in plasma NEFA

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concentrations. No changes in NEFA has also been reported in humans who underwent omentectomy (13). Together, these findings support earlier observations that VAT may not be the major source of circulating NEFA (31). However, we did not measure nocturnal plasma NEFA levels, a potential contributor to the pathogenesis of insulin resistance (32), nor did we measure portal NEFA levels, which has been shown to contribute to hepatic insulin resistance (33).

Adipocytes from mesenteric fat as compared with those isolated from the omental and subcutaneous fat depots have higher rates of lipolysis *in vitro* (34). Thus, it is possible that the mesenteric component of VAT could play a more important role than the omental fat on the pathogenesis of insulin resistance. Assuming that VAT accumulation induced by sympathetic denervation of the omental fat was mainly or entirely due to omental fat increase, this could explain the lack of effect on insulin sensitivity. However, we were not able to quantify the fat content of each subcompartment within VAT. Therefore, this possibility remains speculative and deserves further investigation.

Local sympathetic denervation *per se* may suppress lipolysis in the targeted tissue (35), therefore inducing selective adiposity. Surprisingly, an increase in adipocyte number (hyperplasia) but not adipocyte size (hypertrophy) is typically noted (16,36). In contrast, in the present study, we did not find hyperplasia of the adipocytes from the omental fat. We found a slight increase in adipocyte diameter in deep VAT from dogs that underwent denervation as compared with control; however, this latter finding does not explain the ~25% increase in VAT as detected by MRI. Thus, the mechanism by which sympathetic denervation of the omental fat induced accumulation of VAT remains elusive.

Our findings have limitations that should be taken in account. The present study was performed in a small number of dogs. Despite that chemical sympathetic denervation with 6-OHDA, a catecholaminergic-specific toxin (19), is a widely accepted technique to selectively induce fat accumulation, in our canine model this procedure involved multiple needle punctures into the fat tissue, so the possibility of an effect of the puncture *per se* to cause unselective denervation not only of noradrenergic and dopaminergic neurons but also other types of neurons, cannot be ruled out. Moreover, the selective increase of canine VAT after sympathetic denervation of omental fat may not resemble visceral fat gain secondary to increased calorie intake. In fact, it can be argued that the increase in VAT in dogs that underwent sympathetic denervation of the omental fat is due to an *in vivo* inhibition of lipolysis, resulting in unaltered or reduced exposure of the liver to NEFA and cytokines released from the VAT. Thus, our findings argue against, but do not totally refute, the “portal hypothesis”. Another limitation of the study is the short period of observation after denervation, purposely intended to avoid the confounding effect of possible reinnervation of adrenergic nerves (37,38). It remains unknown if insulin resistance may have developed in the long term under the same protocol conditions, with further VAT accumulation. Finally, since we did not obtain serial random fat biopsies across the intra-abdominal fat depot to perform the adipocyte experiments *in vitro*, the fact that this study found no changes in adipocyte hyperplasia or hypertrophy does not invalidate this possibility.

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In conclusion, our results suggest that a significant increase in canine VAT content *per se* does not alter insulin sensitivity. This study argues against a direct effect of VAT on insulin sensitivity.

ACKNOWLEDGEMENTS

We are very grateful to Edgardo Paredes for animal care and handling, Laura G. Smith for performing the MRI scanning, and Rita Thomas for performing biochemical assays. We also want to thank Dr. John Young, Dr. Erin M. Goodwin, and the Comparative Medicine staff for their assistance in animal care. We are also very grateful to Dr. Igor Rebrin and coworkers from the University of Southern California, Los Angeles, CA, for helping us with the assay of tissue catecholamine content.

This study was funded by the American Diabetes Association (ADA-7-08-MI-0) and the National Institutes of Health (NIH EK 029867).

AVBC and RNB conceived the experimental design. AVBC and OOW performed data analysis. AVBC, OOW, and RNB performed data interpretation; AVBC, OOW, MSI, MK, CMK, LSS, EWS, ELK, HCR, HM, QW carried out the experiments; AVBC, OOW, VI, RNB contributed with the writing of the manuscript. OOW and DS performed statistical analysis; VI, analyzed MRI data; AVBC, MK, IAB, RLP, JLB, performed data collection; VI, SPK and MA provided intellectual input. All authors read and approved the final version of the manuscript. AVBC had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

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What is already known about this subject?

- Numerous studies have explored the effect of removing visceral fat on insulin sensitivity; however, no study has investigated the effect of selective visceral fat accumulation on insulin sensitivity *in vivo*.

What does this study add?

- A selective increase in visceral adipose tissue content in dogs does not affect insulin sensitivity *in vivo*.
- The findings from this animal study do not support the concept that accumulation of visceral fat content *per se* is an important causal factor in overall insulin resistance.

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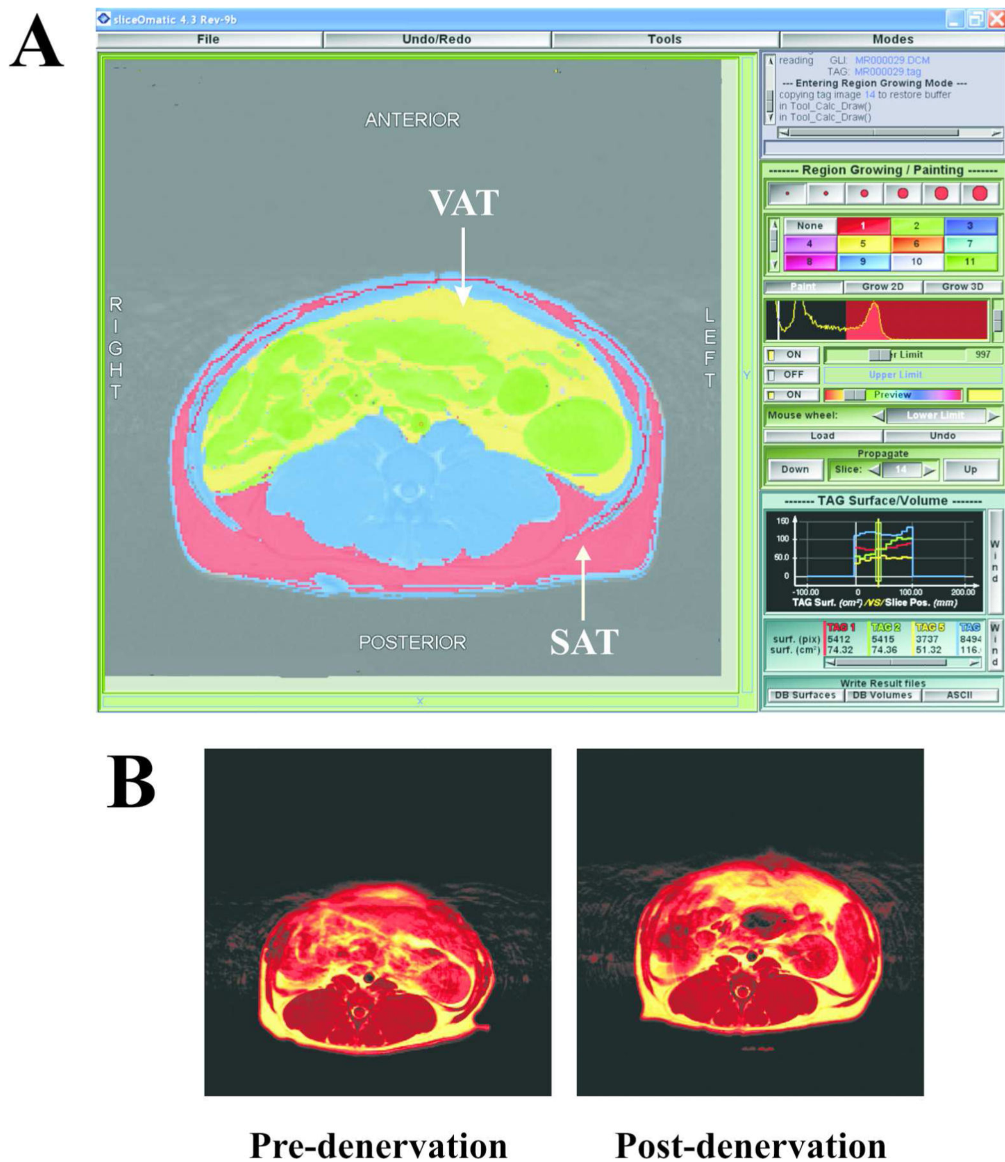

Figure 1.

MRI scan of the canine trunk fat. A) Example of semi-automated fat segmentation using SliceOmatic in a well established canine model of high-fat diet. The yellow area denotes visceral adipose tissue (VAT); the red area, subcutaneous adipose tissue (SAT). B) Representative images of MRI scan of a dog that underwent sympathetic denervation of the omental fat. For clarity purposes, images in B) were edited with the table color option fire-2 using Scion Image for Windows (NIH, USA). Yellow indicates total fat tissue.

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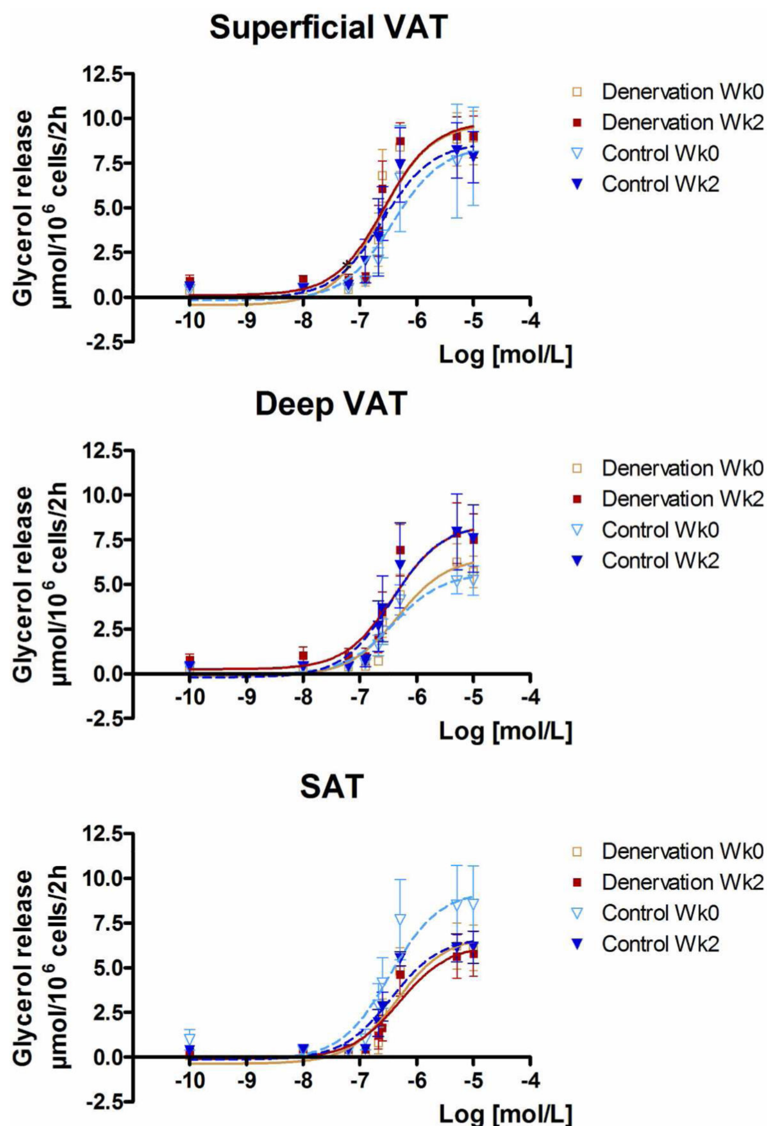

Figure 2.

Effect of sympathetic denervation of the canine omental fat on lipolysis *in vitro*. Plots are concentration-response curves to isoproterenol, pre- (Denervation Wk0) and post-denervation (Denervation Wk2) with injections of 6-OHDA in the omental fat (n=8) and pre- (Control Wk0) and post-injection (Control Wk2) of saline (n=4). See Methods for further details. Lipolysis was determined as the rate of glycerol release. Plots and bars are mean and SEM, respectively. SAT, subcutaneous adipose tissue (n=7); VAT, visceral adipose tissue.

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Table 1

Effect of sympathetic denervation of the omental fat on body weight and abdominal fat distribution.

	Sympathetic denervation (n=10)		P value	Control (n=6)		P value
	Before	**After**		**Before**	**After**	
Body weight (kg)	27.8 (26.2–29.1)	27.2 (26.2–28.9)	0.563	29 (27.7–29.5)	28.6 (27.4–29.5)	0.463
%VAT of abdominal tissue	10.2 (8.5–11.4)	12.4 (10.4–13.6)	<0.01	11.2 (6.6–11.3)	10.9 (6.7–14.7)	0.60
%SAT of abdominal tissue	9.8 (7.0–14.5)	8.0 (7.6–10.3)	0.169	9.7 (6.2–15.5)	9.9 (7.1–13.1)	0.753
% Total fat of abdominal tissue	18.9 (15.7–25.9)	19.7 (17.3–24.8)	0.093	21.0 (17.2–25.3)	24.0 (15.4–26.9)	0.60
VAT content (cm^3)	259.4 (230.0–325.9)	328.0 (289.5–398.3)	<0.01	219.0 (182.0–228.0)	221.1 (151.0–266.0)*	0.917
SAT content (cm^3)	248.3 (228.0–345.0)	228.5 (210.6–318.4)	0.114	187.0 (128.4–335.0)	189.7 (151.0–309.2)	0.753
Total fat content (cm^3)	507.0 (490.8–575.0)	551.9 (500.1–716.5)	0.074	406.0 (356.0–657.0)	447.5 (343.6–711.3)	0.753

Data are medians (interquartile range).

VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue. Total fat represents VAT and SAT together.

Fat distribution was assessed by magnetic resonance imaging.

* $P<0.05$ vs. after sympathetic denervation.

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Table 2

Effect of sympathetic denervation of the omental fat on metabolic features.

	Sympathetic denervation (n=11)		P value	Control (n=7)		P value
	Before	**After**		**Before**	**After**	
NEFA (Limol/L)[*]	599.8 (533.1–870.0)	801.2 (512.6–924.4)	0.29	725.1 (486.1–846.2)	494.0 (411.6–980.0)	0.87
Leptin (ng/mL)[*]	0.42 (0.23–0.46)[†]	0.41 (0.24–0.44)[†]	0.69	1.2 (0.4–1.3)[‡]	0.9 (0.3–1.3)[‡]	0.109
Adiponectin (mg/L)[*]	19.6 (11.5–27.7)[§]	18.8 (11.6–23.4)[§]	0.31	12.0 (10.2–19.9)[‡]	14.2 (13.9–21.6)[‡]	0.109
S_I [(mU/L)$^{-1}$·min^{-1}]	4.7 (3.1–8.8)	5.3 (4.5–7.2)	0.59	4.5 (3.1–6.9)	4.9 (3.4–6.5)	0.61
AIRg (mU·L^{-1}·min)	543.5 (360.4–687.1)	509.0 (437.2–687.1)	1.00	536.5 (423.9–624.9)	520.5 (382.7–610.7)	0.40
SI_{CLAMP} (×10^{-4} dL·kg^{-1}·min^{-1}·(mU/L)$^{-1}$	42.0 (41.0–51.0)[§]	40.0 (34.0–52.0)[§]	0.67	65.0 (28.1–67.0)[‡]	67.0 (38.0–77.0)[‡]	0.67
SIp_{CLAMP} (×10^{-4} dL·kg^{-1}·min^{-1}·(mU/L)$^{-1}$	40.0 (37.0–45.0)[†]	39.6 (32.0–54.0)[†]	0.35	NA	NA	--
$SIHGO_{CLAMP}$ (×10^{-4} dL·kg^{-1}·min^{-1}·(mU/L)$^{-1}$	5.7 (5.3–9.0)[†]	6.6 (5.7–8.9)[†]	0.69	NA	NA	--

Data are medians (interquartile range).

AIRg, acute insulin response to glucose assessed by the minimal model approach; NEFA, non-esterified fatty acids; S_I , insulin sensitivity assessed by the minimal model approach; SI_{CLAMP}, whole-body insulin sensitivity assessed by the euglycemic hyperinsulinemic clamp; $SIHGO_{CLAMP}$, hepatic insulin sensitivity (hepatic glucose output) assessed by the euglycemic hyperinsulinemic clamp; SIp_{CLAMP}, peripheral insulin sensitivity assessed by the euglycemic hyperinsulinemic clamp.

NA, data not available.

[*] Fasting values.

[†] n=5

[‡] n=3

[§] n=7