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## A Novel Role for Subcutaneous Adipose Tissue in Exercise-Induced Improvements in Glucose Homeostasis



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**Exercise training improves whole-body glucose homeostasis through effects largely attributed to adaptations in skeletal muscle; however, training also affects other tissues, including adipose tissue. To determine whether exercise-induced adaptations to adipose tissue contribute to training-induced improvements in glucose homeostasis, subcutaneous white adipose tissue (scWAT) from exercise-trained or sedentary donor mice was transplanted into the visceral cavity of sedentary recipients. Remarkably, 9 days post-transplantation, mice receiving scWAT from exercise-trained mice had improved glucose tolerance and enhanced insulin sensitivity compared with mice transplanted with scWAT from sedentary or sham-treated mice. Mice transplanted with scWAT from exercise-trained mice had increased insulin-stimulated glucose uptake in tibialis anterior and soleus muscles and brown adipose tissue, suggesting that the transplanted scWAT exerted endocrine effects. Furthermore, the deleterious effects of high-fat feeding on glucose tolerance and insulin sensitivity were completely reversed if high-fat-fed recipient mice were transplanted with scWAT from exercise-trained mice. In additional experiments, voluntary exercise training by wheel running for only 11 days resulted in profound changes in scWAT, including the increased expression of ~1,550 genes involved in numerous cellular functions including metabolism. Exercise training causes adaptations to scWAT that elicit metabolic improvements in other tissues, demonstrating a previously unrecognized role for adipose tissue in the beneficial effects of exercise on systemic glucose homeostasis.**

Regular physical exercise has beneficial effects on overall health, and the role of exercise in the treatment and prevention of metabolic diseases such as obesity and type 2 diabetes is particularly well recognized (1,2). Exercise training improves whole-body glucose homeostasis and increases insulin sensitivity, effects primarily ascribed to adaptations in skeletal muscle, the tissue responsible for the majority of glucose disposal (3). In addition to skeletal muscle, long-term exercise training causes adaptations to multiple tissues throughout the body including adipose tissue. Exercise training decreases adipocyte size, reduces lipid content, increases the number of enzymes involved in mitochondrial biogenesis (4–6), and increases the expression of several metabolic proteins and cytokines in adipose tissue, which are adaptations that presumably function to enhance the supply of free fatty acids to the working muscle (7,8).

Adipose tissue is one of the largest organs in the body, and functions in lipid storage, hormone production, and immune function (9). White adipose tissue (WAT) is located in two major, but distinct, depots: visceral and subcutaneous. In humans, visceral WAT (vWAT) surrounds the internal organs, while subcutaneous WAT (scWAT) is found mainly around the thighs and buttocks. Excessive accumulation of vWAT is associated with insulin resistance and increased risk of type 2 diabetes (10–12), while a predisposition to accumulate scWAT is associated with a more insulin-sensitive phenotype and a lower risk of the development of type 2 diabetes (13,14). It is now known that beige cells (also termed adaptive brown fat cells, recruitable brown fat cells, or brite cells) can be interspersed in the scWAT

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of humans and rodents (15–17). These beige cells express uncoupling protein 1 (UCP1), have a multilocular morphology, and increase in number upon exposure to cold, exercise, or an enriched environment (2,18–20).

In the current study, exercise training of mice resulted in wide-ranging and multifunctional adaptations to scWAT. Based on these findings, we tested the hypothesis that exercise training-induced adaptations to adipose tissue contribute to the improvement in glucose homeostasis that occurs with exercise. Using a mouse model, transplantation of scWAT from exercise-trained mice into sedentary recipient mice resulted in improved whole-body glucose homeostasis, even under conditions of high-fat feeding. Thus, in contrast to the previous concept that the effects of exercise to improve glucose homeostasis results primarily from adaptations to skeletal muscle, these data establish that regular physical activity has marked effects on scWAT that contribute to an advantageous metabolic homeostasis.

## RESEARCH DESIGN AND METHODS

### Mice and Exercise

Male C57BL/6 mice (Charles River Laboratories) were used for all studies. Mice were maintained on either a standard mouse diet (21% kcal from fat) (9F 5020 Laboratory Diet; PharmaServ) or on a high-fat diet (60% kcal from fat) (Research Diets, Inc.). Mice were housed with a standard 12-h light/dark cycle. The Joslin Diabetes Center Institutional Animal Care and Use Committee approved all experiments.

Male mice at 10 weeks of age were divided into two groups. One group of animals was housed individually in wheel cages (Nalgene) where voluntary access to physical activity was available at all times. The total number of wheel cage revolutions was monitored every day and the accumulated running distance was calculated at the end of 11 days ( $70 \pm 8$  km/mouse). Mice trained for 11 days in order to provide a significant training stimulus without a substantial loss of adipose tissue mass, which can occur with longer periods of training. The age-matched control mice were maintained in individual cages and treated identically to the wheel cage-housed mice, except that they did not have access to a running wheel. After 11 days, mice were removed from the wheel cages or static cages, anesthetized, and scWAT and vWAT were immediately removed and either analyzed or used for transplantation.

### Fat Transplantation

Fat transplantation was performed using WAT removed from the subcutaneous (inguinal) and visceral (intra-abdominal perigonadal) areas of trained and sedentary mice. Donor mice were killed by cervical dislocation, and fat pads were removed and kept in saline in a 37°C water bath until transplantation. Recipient mice were anesthetized by intraperitoneal injection of 85–100 mg/kg body wt pentobarbital. For each recipient mouse, 0.85 g scWAT or 1.0 g vWAT was transplanted into the visceral cavity or subcutaneous cavity. For transplantation into the

visceral cavity, the tissue was carefully lodged deep between folds within sliced portions of endogenous perigonadal adipose tissue of the recipient and placed next to the mesenteric adipose tissue just below the liver (21). For transplantation into the subcutaneous cavity, the tissue was placed along both endogenous inguinal adipose tissue pads. Adipose tissue from approximately four trained mice and two sedentary mice was used in order to equalize the amount of transplanted adipose tissue. Sham-treated “recipient” mice underwent the same surgical procedure, but no adipose tissue was transplanted. All recipient mice were sedentary throughout the study (i.e., were housed in static cages). There appeared to be no difference in the rate of recovery after the surgery among the groups based on similar body weights and close monitoring of mice for several days post-transplant (Supplementary Fig. 1B).

### Glucose, Insulin, and Pyruvate Tolerance Tests

For glucose tolerance tests (GTTs), mice were fasted for 11 h (2200–0900 h) with free access to drinking water. A baseline blood sample was collected from the tail of fully conscious mice followed by injection of glucose (2.0 g/kg body wt i.p.); and blood was taken from the tail at 15, 30, 60, and 120 min postinjection. To normalize for differences in basal glucose concentrations, these data are displayed as the area under the curve (AUC) with subtraction of basal glucose concentrations. For insulin tolerance tests (ITTs), mice were fasted for 2 h (1200–1400 h), and baseline blood samples were collected from the tails of fully conscious mice. Insulin (1 unit/kg body wt) (Humulin; Eli Lilly, Indianapolis, IN) was administered by intraperitoneal injection; and blood samples were taken from the tail at 10, 15, 30, 45, and 60 min postinjection. Pyruvate tolerance tests (PTTs) were performed in mice fasted for 11 h (2200–0900 h), and baseline blood samples were collected from the tail of fully conscious mice. Pyruvate (sodium pyruvate, 2 g/kg body wt) was administered by intraperitoneal injection; and blood samples were taken from the tail at 15, 30, 45, 60, and 90 min postinjection. Glucose concentrations were determined from blood using a OneTouch Ultra portable glucometer (LifeScan, Milpitas, CA).

### Comprehensive Lab Animal Monitoring System and Biochemical Methods

The Comprehensive Lab Animal Monitoring System (Oxymax Opto-M3; Columbus Instruments) was used to measure activity level, food and water intake, volume of O<sub>2</sub> consumption, volume of CO<sub>2</sub> production, and heat production. Total energy expenditure of mice was calculated as described previously (22). To determine the effects of cold exposure on body temperature, mice were fasted overnight, and body temperature was determined using an animal rectal probe thermometer (Physitemp). Basal temperature was determined, and mice were placed at 4°C and body temperature was measured at 15, 30, 60, 90, and 120 min.

Subcutaneous adipose tissue was removed from mice and prepared in paraffin after fixation in 10% phosphate-buffered formalin, and then hematoxylin-eosin (H-E) staining was

performed. mRNA levels of *Ucp1* and *Prdm16* were measured by quantitative RT-PCR using primers shown in Supplementary Table 1. Retro-orbital sinus bleeds were performed after an overnight fast (2200–0900 h), and plasma levels of hormones and metabolites were assessed. Plasma hormone and metabolite levels were measured with mouse ELISA kits for insulin, leptin, triglyceride, cholesterol, total adiponectin, free fatty acid, interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (Crystal Chem Inc. or Alpco Diagnostics); FGF21 (BioVendor); and norepinephrine (IBL-America). Tissue processing and immunoblotting were performed as previously described (22–24). The UCP1 antibody was obtained from a commercial source (AnaSpec, Fremont, CA).

### Hepatic Glucose Production in Isolated Hepatocytes

Hepatic glucose production was measured in primary hepatocytes as previously described (25–28). Briefly, hepatocytes were isolated by liver perfusion using type II collagenase and were grown on collagen-coated plates, and glucose production was assayed. Primary hepatocytes were incubated for 4 h in Krebs-Henseleit-HEPES assay buffer. Cells were incubated with 0.1% BSA containing isotonic or various hypertonic solutions of Krebs-Henseleit-HEPES buffer containing 1 mmol/L sodium pyruvate and 10 mmol/L sodium lactate. Glucose content was determined by a fluorometric enzyme assay (29). Glucose in the culture medium was measured and normalized to total protein levels, and the normalized values were used as an index to estimate glucose production.

### Glucose Uptake In Vivo

Glucose uptake in vivo was measured as previously described (30). Briefly, mice were fasted overnight (2200–0900 h), anesthetized, and injected with either saline or glucose (1.0 mg/kg body wt) in combination with 0.33  $\mu$ Ci [ $^3$ H]2-deoxyglucose administered via the retro-orbital sinus; and blood samples were taken 0, 5, 10, 15, 25, 35, and 45 min later for the determination of glucose and [ $^3$ H] concentrations. After the last blood draw, mice were killed by cervical dislocation and transplanted WAT, brown adipose tissue (BAT), and heart, tibialis anterior, soleus, extensor digitorum longus (EDL), and gastrocnemius muscle tissue were harvested and immediately frozen in liquid nitrogen. Accumulation of [ $^3$ H]2-deoxyglucose-6-P was assessed in tissues using a perchloric acid/BaOH-ZnSO<sub>4</sub> precipitation procedure modified from previous work (31).

### Microarray

Samples for microarray analysis were prepared following the Affymetrix (Santa Clara, CA) GeneChip Expression Analysis Manual. Total RNA was isolated and purified from frozen scWAT from trained or sedentary mice using an RNeasy Mini Kit (Qiagen, Valencia, CA), and was subjected to cDNA and cRNA preparations. From the total RNA, double-stranded cDNA was created using the SuperScript Choice system (Life Technologies, Grand Island, NY). The cRNA was synthesized using MEGAscript T7 Transcription Kit (Life Technologies). Adjusted cRNA was hybridized to Affymetrix U74Av2 arrays. Intensity values

were quantified by MAS version 5.0 software (Affymetrix). All chips were subjected to global scaling to a target intensity of 1,500 to take into account the inherent differences among the chips and their hybridization efficiencies. Hybridization and gene chip expression analysis were performed at the Joslin Diabetes Center Advanced Genomics and Genetics Core Laboratory.

### Oxygen Consumption Rates

Subcutaneous adipose tissue was removed and five pieces (10 mg) of tissue per mouse were used to measure tissue oxygen consumption using an XF<sup>e</sup> Analyzer (Seahorse Bioscience, North Billerica, MA). The tissue was placed on mesh screen circles and “snapped” into a well of an Islet plate (Seahorse Bioscience), and 500  $\mu$ L of low-glucose DMEM was placed in each well. Plates were equilibrated for 1 h without CO<sub>2</sub>, followed by measurement of basal oxygen consumption rates (OCRs) (32). Basal OCR was normalized for total cell protein.

### Fatty Acid Uptake In Vitro

The stromal vascular fraction was digested in DMEM containing 10% FBS, 1% penicillin-streptomycin, and type I collagenase (1 mg/mL). The isolated stromal vascular fraction was cultured in DMEM with 10% FBS. After the cells reached confluence, adipogenic differentiation was initiated by incubation with DMEM media containing 10% FBS, 1% penicillin-streptomycin, insulin (5 mg/mL), T3 (1 nmol/L), dexamethasone (1 mmol/L), isobutylmethylxanthine (0.5 mmol/L), and indomethacin (50 mmol/L). Twelve days after differentiation, fatty acid uptake and oxidation were measured using the conversion of [ $^3$ H] palmitate into CO<sub>2</sub> as previously described (33).

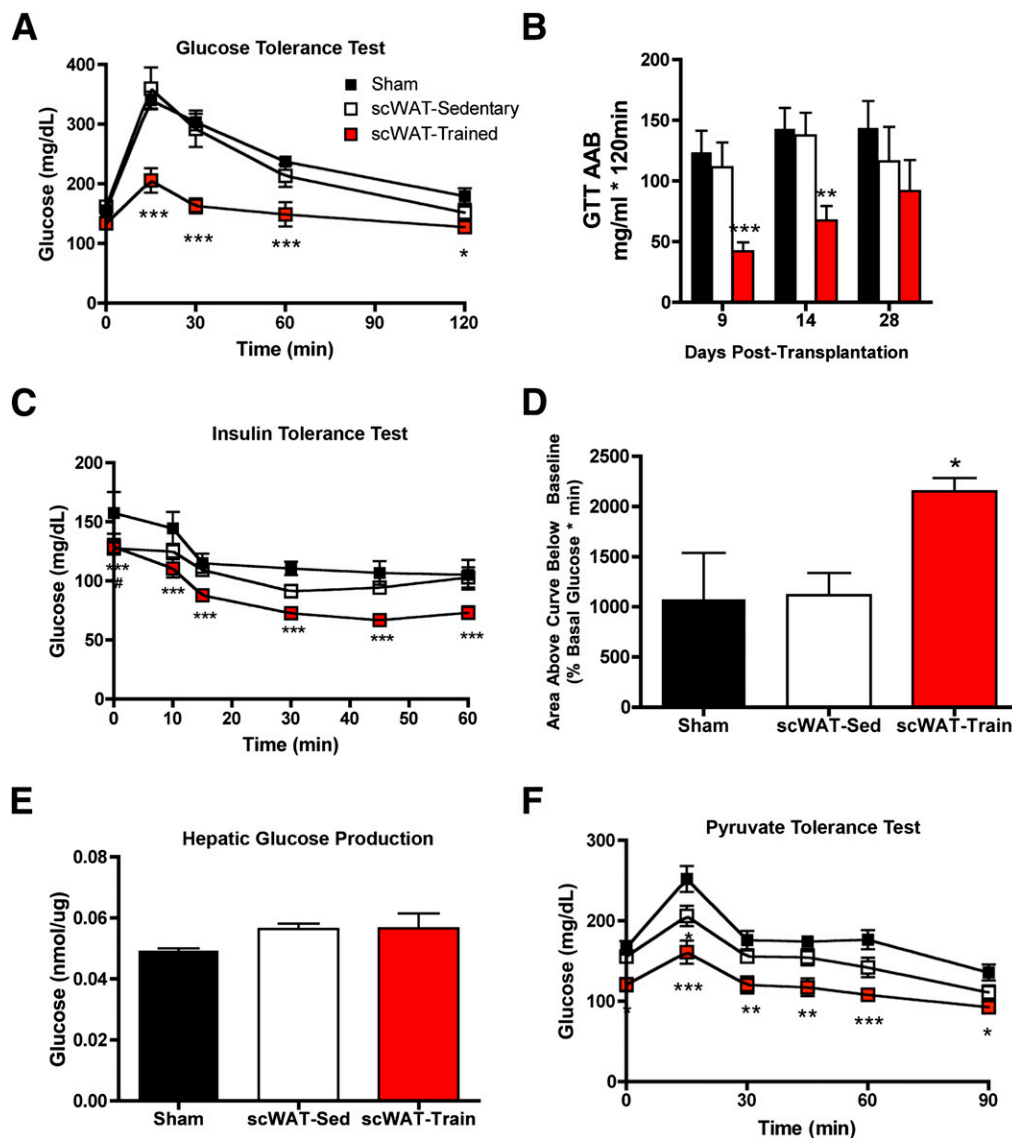
### Statistical Analysis

The data are presented as the mean  $\pm$  SEM. Statistical significance was defined as  $P \leq 0.05$  and determined by Student *t* tests or two-way ANOVA and Bonferroni post hoc analysis. The number of samples used to determine statistical significance is indicated in the figure captions.

## RESULTS

### Transplantation of Subcutaneous Adipose Tissue From Exercise-Trained Mice Improves Glucose Homeostasis

To determine whether adipose tissue from exercise-trained mice exerts metabolic effects on glucose homeostasis, mice were given free access to an exercise wheel or were housed individually in standard cages for 11 days. This protocol resulted in a significant training adaptation, as indicated by a significant increase in HKII protein in the triceps muscles (Supplementary Fig. 1A). scWAT (0.85 g) was removed and transplanted into the visceral cavity of 12-week-old sedentary recipient mice. Nine days post-transplantation, there was a dramatic improvement in glucose tolerance in mice transplanted with scWAT from exercise-trained mice compared with both mice transplanted with scWAT from sedentary mice and sham-treated controls (Fig. 1A). In fact, the calculated glucose



**Figure 1**—Transplantation of scWAT from trained mice improves glucose tolerance and increases whole-body insulin sensitivity. **A** and **B**: Mice were transplanted with 0.85 g scWAT from trained or sedentary mice or were sham operated. For GTTs, mice were injected with 2 g glucose/kg body wt i.p. **A**: GTT at 9 days post-transplantation. **B**: Glucose AAB at 9, 14, and 28 days post-transplantation. Data are the mean  $\pm$  SEM ( $n = 5$ –12/group; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). For ITTs, mice were injected with 1 unit insulin/kg i.p. and measured at 9 days post-transplantation (**C**); the area above the curve below the baseline is shown (**D**). Data are reported as the mean  $\pm$  SEM ( $n = 4$ /group; \* $P < 0.05$ ; \*\*\* $P < 0.001$ ; and # $P < 0.001$  Sedentary scWAT vs. Sham). **E**: Hepatic glucose production was measured in isolated hepatocytes. Data are reported as the mean  $\pm$  SEM ( $n = 6$ –12/group). For PTTs, mice were injected with pyruvate 2 g/kg body wt i.p. Results of PTTs conducted at 9 days post-transplantation (**F**) and represented as the AAB (**G**). Data are the mean  $\pm$  SEM ( $n = 5$ –12/group). **H**: Liver triglycerides at 9 days post-transplantation. Data are the mean  $\pm$  SEM ( $n = 5$ –12/group; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ). **I** and **J**: Mice were transplanted with 0.85 g scWAT from trained or sedentary mice into the subcutaneous cavity or were sham operated. GTT (**I**) and glucose AAB (**J**) are represented as the AAB at 9 days post-transplantation for mice transplanted with 0.85 g scWAT into the subcutaneous cavity. Data are the mean  $\pm$  SEM ( $n = 6$ –8/group). Asterisks represent differences between sham-operated mice and all transplanted groups (\* $P < 0.05$ ; \*\* $P < 0.01$ ). **K**: GTT results at 9 days post-transplantation for mice receiving 0.85 g scWAT from trained and sedentary 12-week-old mice and sedentary 6-week-old mice. Asterisks represent differences between scWAT from exercise-trained (scWAT-Train) mice and all control groups (\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; # $P < 0.05$  compared with mice transplanted with scWAT from sedentary [scWAT-Sed] 6-week-old mice).

AUC above baseline (AAB) for the mice transplanted with scWAT from exercise-trained mice was only 66% of the sham-treated controls (Fig. 1B). However, this effect was of short duration, as the effect was not as pronounced at day 14, and there was only a tendency for improved glucose tolerance 28 days post-transplant.

Transplantation of scWAT from exercise-trained and sedentary mice was not associated with changes in body weight, food intake, or spontaneous activity (Supplementary Fig. 1B–D), while energy expenditure was significantly increased in the fasted state (dark cycle) of mice transplanted with scWAT from exercise-trained mice

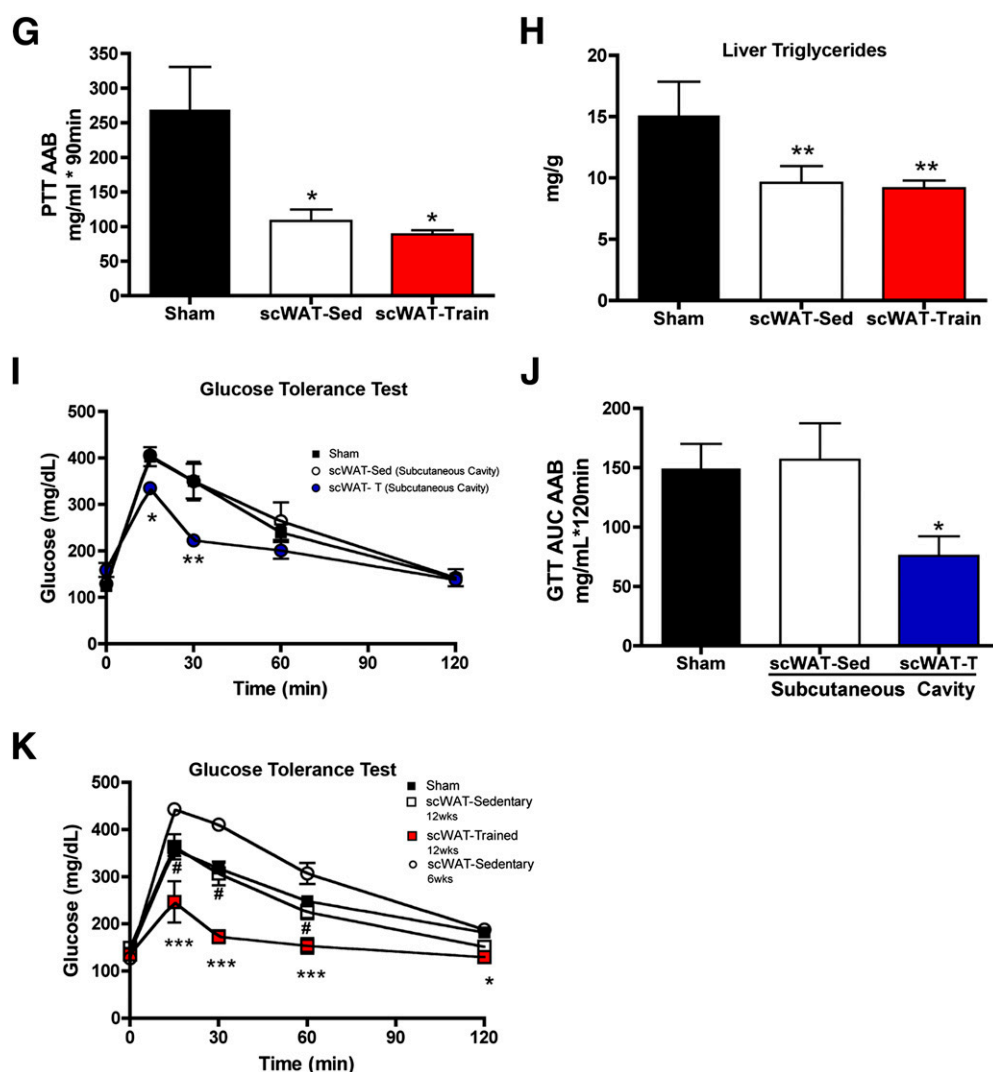


Figure 1—Continued.

(Supplementary Fig. 1E). The respiratory exchange rate was significantly decreased in the fasted state of mice transplanted with scWAT from both sedentary and trained mice (Supplementary Fig. 1F), indicating a preference for fatty acids as fuel. Mice transplanted with scWAT from trained mice exhibited a decrease in fasting blood glucose, insulin, and cholesterol concentrations 9 days post-transplant compared with both sham-treated controls and mice transplanted with scWAT from sedentary mice (Supplementary Table 2). Circulating free fatty acid concentrations were not different among the groups (Supplementary Table 2). An ITT was performed in a separate cohort of mice at 9 days post-transplantation. Mice transplanted with scWAT from trained mice had a greater insulin-induced decrease in glucose concentrations compared with both sham-operated mice and mice transplanted with scWAT from sedentary mice measured by the area subtracted from baseline (Fig. 1C and D), indicating increased peripheral insulin sensitivity.

To investigate a potential role for the liver in the improved glucose tolerance with transplantation, we used the following three approaches: glucose production in isolated hepatocytes, PTTs, and liver triglyceride concentrations. There was no difference in basal rates of glucose production in the isolated hepatocytes among the three groups of mice (Fig. 1E). In contrast, pyruvate tolerance (Fig. 1F), calculated as AAB (Fig. 1G), was improved in mice transplanted with scWAT from both sedentary and exercise-trained mice compared with sham-treated mice. The transplanted mice also had a decrease in liver triglyceride levels compared with sham-treated mice (Fig. 1H). Thus, while transplantation of scWAT improved liver function, since only transplantation of scWAT from trained mice improved glucose tolerance, it is likely that the enhanced glucose tolerance is a result of improved peripheral insulin sensitivity and not of altered liver glucose metabolism.

To determine whether the effects of transplanting scWAT from exercise-trained mice were specific for the location of the transplant, scWAT from trained and sedentary mice was transplanted into the subcutaneous cavity (flank region, directly atop the inguinal adipose tissue). The transplanted scWAT was divided in half, with one half being placed atop each inguinal adipose tissue pad. After 9 days, mice transplanted with scWAT from trained mice in the subcutaneous cavity had a significant improvement in glucose tolerance compared with both sham-treated mice and mice receiving sedentary scWAT (22% decrease in GTT AUC;  $P < 0.01$ ) (Fig. 1I and J). Thus, transplantation of scWAT from trained mice into both the visceral and subcutaneous cavities improves glucose tolerance.

Exercise training decreases adipocyte size (18,34), and thus a potential mechanism for the improved glucose homeostasis with transplantation of scWAT from trained mice could stem from the recipient mice receiving an increased number of adipocytes. To test this hypothesis, 12-week-old recipient mice were transplanted with scWAT from young, 6-week-old sedentary mice with a cell size similar to that of the trained mice. Surprisingly, transplantation of scWAT obtained from the young sedentary mice into the visceral cavity of 12-week-old mice resulted in an impaired glucose tolerance compared with all other groups of mice (Fig. 1K). The mechanism for this effect is unclear, but these findings suggest that exercise training results in adaptations to adipose tissue that confer beneficial effects on systemic glucose homeostasis independent of adipocyte size.

To determine whether the effects of transplanting vWAT from sedentary and trained mice affected glucose homeostasis, similar experiments were performed, except that 1.0 g vWAT from trained and sedentary mice was removed and transplanted into the visceral cavity of 12-week-old sedentary recipient mice. In contrast to what was observed with transplantation of scWAT, there was no difference in glucose tolerance in mice receiving vWAT from trained or sedentary mice compared with sham-treated controls 9, 14, or 28 days post-transplantation (Supplementary Fig. 2A and B). There was no difference in body weight among the groups (Supplementary Fig. 2C). Thus, the transplantation of vWAT from trained mice does not improve glucose homeostasis in recipient mice, and the remainder of experiments focused on scWAT.

#### **Transplantation of Subcutaneous Adipose Tissue Increases Glucose Uptake in Skeletal Muscle and BAT**

To determine the tissues responsible for the improvement in glucose tolerance 9 days post-transplantation in mice receiving scWAT from trained mice, *in vivo* glucose disposal was measured in a separate cohort of mice. Mice were injected with [ $^3$ H]-2-deoxyglucose in saline (basal) or a 20% glucose solution (glucose) that results in a physiological insulin release (29). Glucose and insulin concentrations were measured in the mice injected with

glucose, revealing significantly lower glucose and insulin concentrations in the mice transplanted with scWAT from trained mice (Fig. 2A and B). Forty-five minutes following saline or glucose injection, multiple tissue samples were removed and glucose uptake was determined. There was no difference in basal glucose uptake among groups in any tissue. In response to glucose stimulation, glucose uptake in the tibialis anterior (57% oxidative fibers) (Fig. 2C) and soleus (95% oxidative fibers) (Fig. 2D) muscles was significantly increased in mice transplanted with scWAT from trained mice. There was no difference in glucose uptake in the more glycolytic muscles, gastrocnemius (40% oxidative fibers) and EDL (30% oxidative fibers) (Fig. 2E and F) (35,36). In intrascapular BAT, the increase in glucose-stimulated glucose uptake was most pronounced in mice transplanted with scWAT from trained mice (Fig. 2G). There was no significant effect of transplantation on glucose uptake in the heart (Fig. 2H). Thus, transplantation of scWAT from trained mice into the visceral cavity of sedentary mice results in increased insulin-stimulated glucose uptake into multiple tissues, specifically in more oxidative skeletal muscles and BAT.

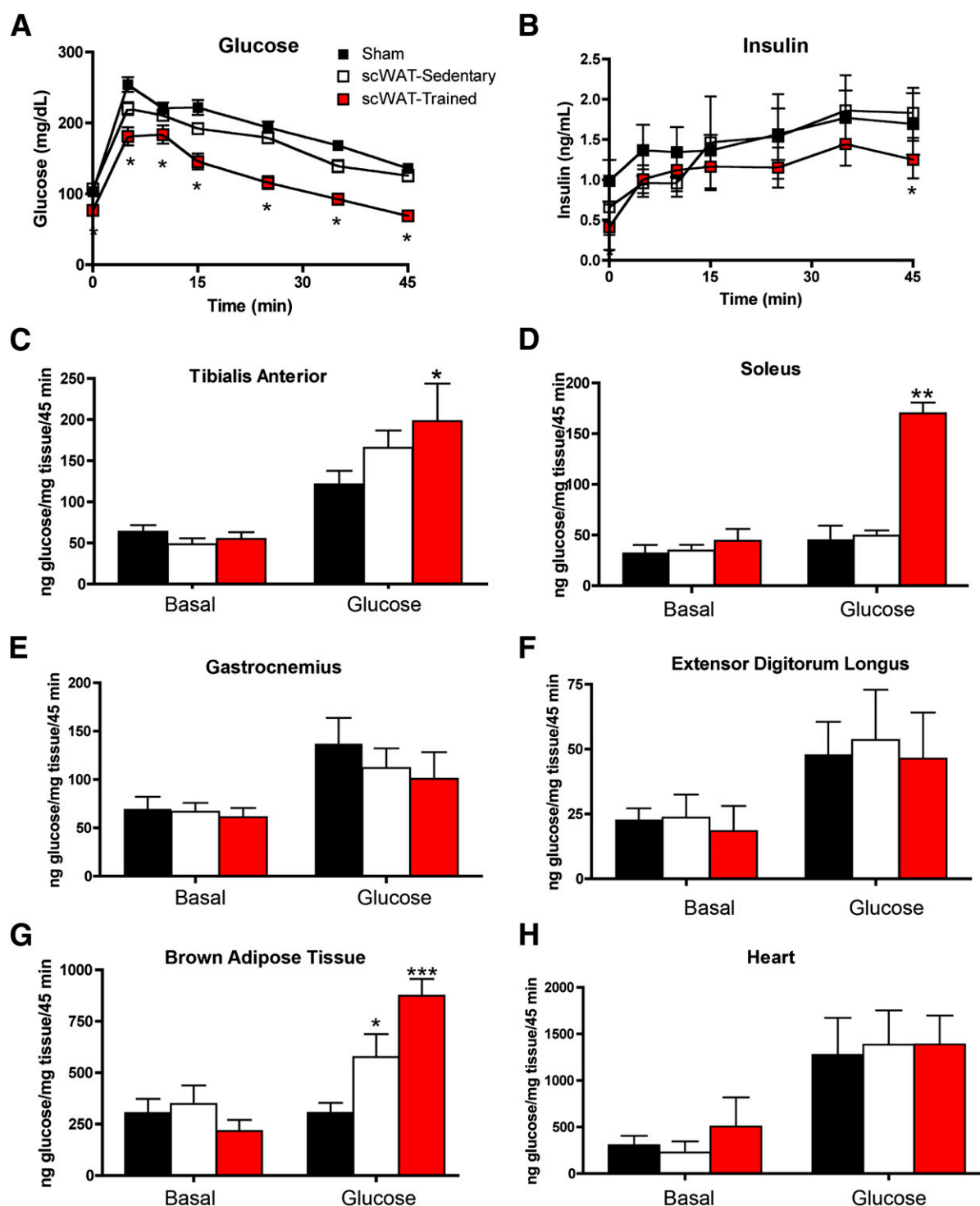
#### **Transplantation of Subcutaneous Adipose Tissue From Trained Mice Ameliorates the Effects of a High-Fat Diet**

We tested the hypothesis that the transplantation of scWAT from trained mice would improve glucose homeostasis under conditions of metabolic stress. Mice were fed a high-fat diet for 6 weeks and were sham operated or transplanted with scWAT from sedentary or trained mice and continued on the high-fat diet until studied at 9 days post-transplant. Mice transplanted with scWAT from either trained or sedentary mice had an improved glucose homeostasis compared with sham-treated high-fat-fed mice (Fig. 3A and B). However, the effect was greater in the mice transplanted with scWAT from trained mice, as demonstrated by significantly lower basal glucose concentrations, significantly lower circulating glucose concentrations 30 min after glucose injection, and a significantly improved overall glucose excursion curve compared with mice transplanted with scWAT from sedentary mice.

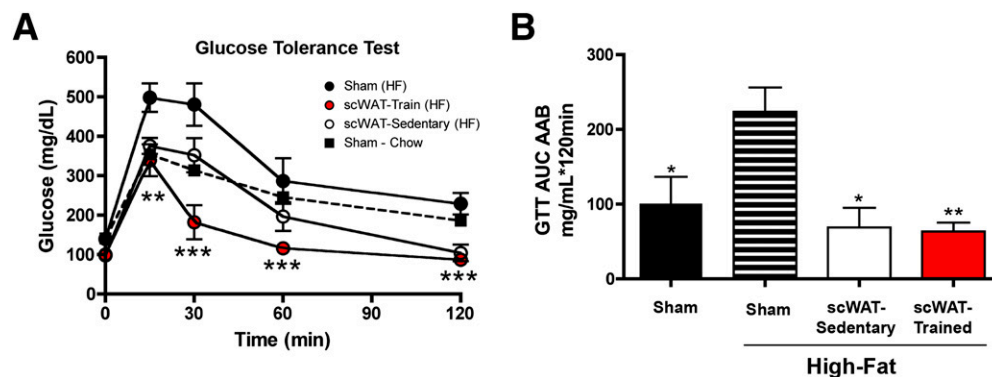
#### **Exercise Training Alters Gene Expression of Subcutaneous Adipose Tissue**

Given the dramatic improvement in whole-body glucose homeostasis with the transplantation of scWAT from trained mice, we hypothesized that exercise training causes marked adaptations to the scWAT. Therefore, we determined the gene expression profile of scWAT obtained from a cohort of mice that were housed in wheel cages for 11 days and compared it with those from sedentary control mice. Using the criteria of  $P < 0.05$  and  $Q < 0.25$ , exercise training had a profound effect on the expression profile of the scWAT with 1,549 genes significantly increased by training, and 1,156 genes significantly downregulated by training (microarray data will be submitted to the Gene Expression Omnibus Database





**Figure 2**—Transplantation of scWAT increases glucose uptake into skeletal muscle and BAT. *A–H*: Mice were transplanted with 0.85 g scWAT from sedentary or trained mice or were sham operated and were studied 9 days post-transplantation. Mice were fasted overnight and anesthetized, and [ $^3$ H]2-deoxyglucose/g body wt was administered via retro-orbital injection in the presence of saline (Basal) or 1 mg/kg body wt glucose (Glucose). Blood glucose (*A*) and insulin (*B*) levels were measured, and glucose uptake was measured in tibialis anterior (*C*), soleus (*D*), gastrocnemius (*E*), EDL (*F*), BAT (*G*), and heart (*H*) muscles. Data are reported as the mean  $\pm$  SEM. Asterisks indicate statistical significance compared with sham-operated mice ( $n = 6$ /group; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).



**Figure 3**—Transplantation of scWAT from exercise-trained mice improves glucose tolerance in mice on a high-fat diet. In GTTs, mice were injected with 2 g glucose/kg body wt i.p. **A:** GTT results at 9 days post-transplantation in mice fed a high-fat diet. The dashed line indicates a representative group of chow-fed, sham-operated mice 9 days post-transplantation. **B:** Glucose AUC represented as the AAB at 9 days post-transplantation. Data are reported as the mean  $\pm$  SEM ( $n = 5$ /group). Asterisks represent differences between sham-treated high-fat-fed mice and all transplanted groups (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

upon publication). Gene Set Enrichment Analysis ( $P < 0.05$  and  $Q < 0.25$ ) determined that exercise training resulted in significant increases in the number of adipose tissue genes involved in metabolism, mitochondrial biogenesis, oxidative stress and signaling, membrane transport, cell stress, proteolysis, apoptosis, and replication. A representative heat map displaying the top 50 genes upregulated (Fig. 4A) or downregulated (Fig. 4B) by training illustrates the notable effects of exercise on scWAT. Thus, exercise training results in extensive adaptations to scWAT.

#### Exercise Training Alters Metabolic Characteristics of Subcutaneous Adipose Tissue in Mice

One of the most striking findings of the microarray experiments was the marked upregulation of beige adipocyte marker genes and regulators of mitochondrial biogenesis, including significant increases in *Ucp1*, *Prdm16*, *Cidea*, *Elovl3*, *Pgc1a*, *Cox8b*, *Dio2*, *Otopetrin*, *Tbx1*, *Tfam*, *CoxIV*, *Cox7a1*, *Elovl6*, and citrate synthase (Supplementary Table 3) (37). To confirm several of these findings, scWAT from exercise-trained and sedentary mice was analyzed by quantitative PCR and was compared with BAT from sedentary mice. The expression of *Prdm16* mRNA in sedentary scWAT was approximately half that in intrascapular BAT, which is similar to previous reports (38). Remarkably, the expression of *Prdm16* in scWAT from exercise-trained mice was equivalent to the expression in BAT (Fig. 4C). Exercise training also dramatically increased *Ucp1* mRNA by  $\sim 30$ -fold in scWAT, to a level that was nearly 50% of that observed in endogenous BAT (Fig. 4D). Consistent with microarray and quantitative PCR data, UCP1 immunofluorescence was increased in the trained adipose tissue (Fig. 4E). In comparison with the pronounced effects of training on *Ucp1* in scWAT, training did not increase *Ucp1* mRNA expression in vWAT (Supplementary Fig. 2D). This is interesting in light of the lack of effect of transplanting vWAT from trained mice on glucose tolerance.

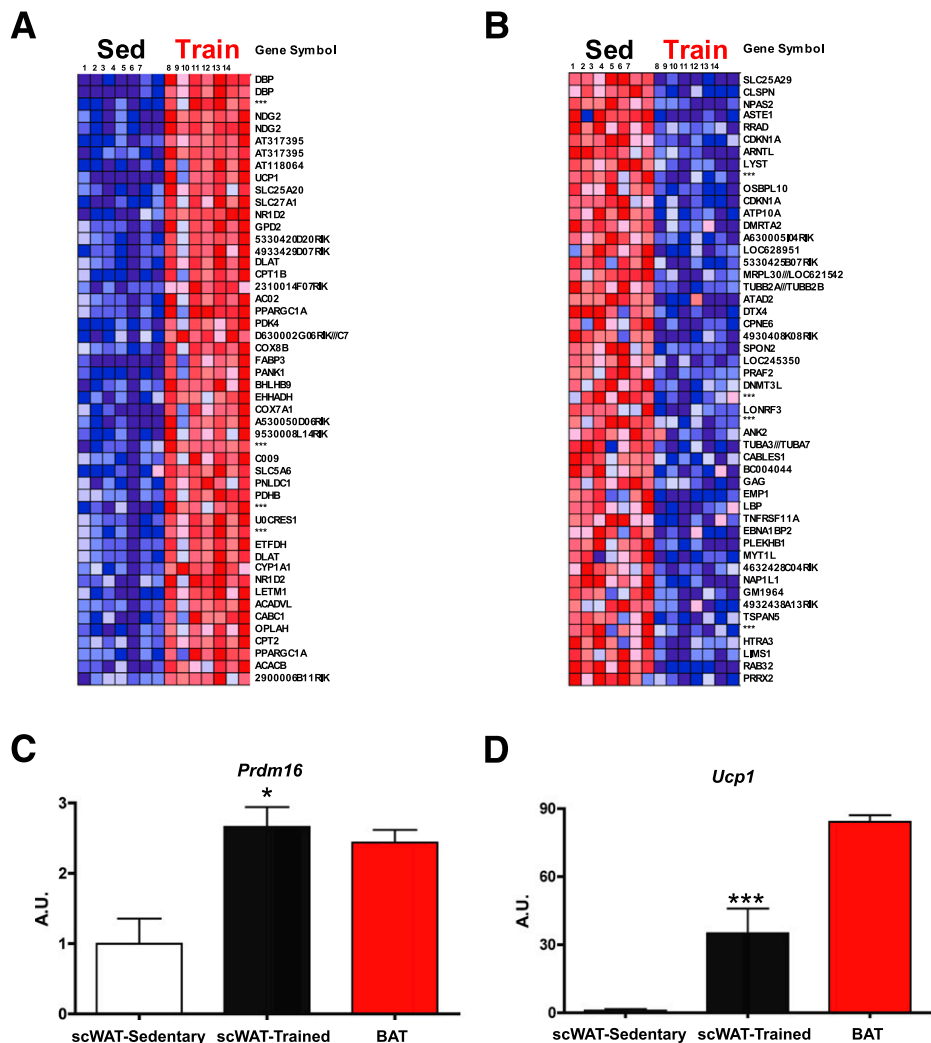
Basal OCRs were significantly increased in the scWAT from exercise-trained mice (Fig. 4F), which is consistent with an increase in mitochondrial uncoupling and a beige fat-like phenotype (39). Additional histological analysis of the scWAT from trained mice revealed characteristics of beige adipose tissue, including the presence of several multilocular cells in the scWAT from the trained mice that were not present in the scWAT from sedentary mice (Fig. 4G) and an increased number of blood vessels present in the scWAT from trained mice (Fig. 4G). The increase in the number of blood vessels is consistent with our microarray data, indicating an increase in the markers of vascularization (e.g., *Vegfa*, *Pdgf*, *Angptl2*; Supplementary Table 3).

Our recent study (40) has shown that the beneficial effects of BAT transplantation on metabolism could involve the release of circulating factors such as IL-6, norepinephrine, and FGF21. To determine whether transplantation of scWAT from trained mice increases circulating concentrations of these factors, we measured serum concentrations of IL-6, norepinephrine, and FGF21 in sham-operated mice or mice transplanted with scWAT. Interestingly, mice transplanted with scWAT from trained mice had a significant increase in circulating concentrations of FGF21 compared with sham-operated mice (Supplementary Table 3), raising the possibility that FGF21 could contribute to the improved metabolism in these mice. IL-6 and norepinephrine concentrations were not different among sham-operated mice or mice transplanted with scWAT from trained or sedentary mice 9 days post-transplantation.

#### Transplanted scWAT From Trained Mice Maintains Improved Metabolic Characteristics

Based on the molecular adaptations to scWAT from trained mice described above and the effects of transplanting scWAT from trained mice on glucose homeostasis, we next determined whether the trained phenotype of the





**Figure 4**—Exercise training increases the “browning” of scWAT. **A–G**: Mice were housed in wheel cages for 11 days of exercise training, and scWAT was analyzed. Representative heat map of the 50 genes most significantly increased (**A**) and decreased (**B**) by 11 days of exercise training. Expressions of *Prdm16* (**C**) and *Ucp1* (**D**) mRNA of scWAT from trained mice were increased compared with scWAT from sedentary mice, and *Prdm16* expression was increased to the expression level of BAT; immunofluorescence of UCP1 revealed an increased expression in scWAT from trained mice (**E**); and the OCR was significantly increased in scWAT from trained mice (**F**) compared with scWAT from sedentary mice ( $n = 7/\text{group}$ ;  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ). **G**: H-E staining revealed the presence of multilocular droplets in the scWAT from trained mice (black arrows indicate the presence of multilocular droplets; open arrows indicate blood vessels). A.U., arbitrary units; Sed, sedentary mice; Train, trained mice.

scWAT persisted throughout the transplantation period. When the transplanted scWAT was removed after 9 days, multilocular cells were still present in tissue transplanted from trained mice (Fig. 5A) and actively took up glucose at similar rates (Fig. 5B). The mice transplanted with scWAT from both sedentary and trained mice had higher basal body temperatures when compared with those of sham-operated mice. Exposure to a temperature of 4°C decreased body temperature in all groups, although mice transplanted with scWAT from trained mice maintained body temperature more effectively than mice receiving scWAT from sedentary or sham-treated mice (Fig. 5C). The higher temperature in the transplanted mice is likely due to thermogenesis and is not likely to be a result

of fever, as there was no difference among groups in circulating concentrations of the inflammatory or fever markers tumor necrosis factor- $\alpha$  and IL-6 (Supplementary Table 2). These findings demonstrate that the transplanted tissue remains active and maintains its multilocular characteristics, including increased thermogenic capacity (41), with no detrimental effects on the recipient mice.

**Glucose and Fatty Acid Uptake in scWAT**

To investigate the hypothesis that the transplanted scWAT acted as a metabolic “sink” to take up glucose and fatty acids, we measured glucose uptake in vivo in the transplanted scWAT and fatty acid uptake in vitro. In

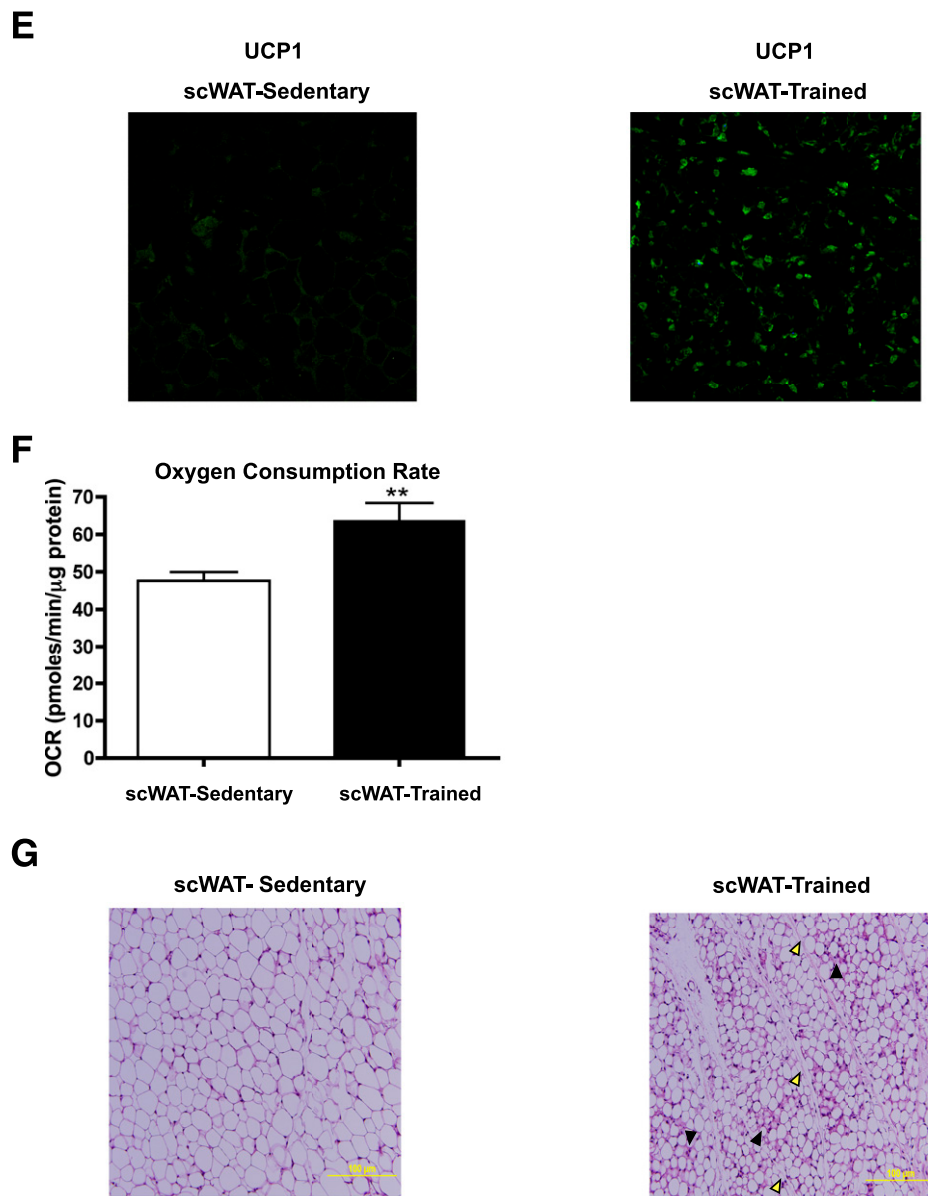


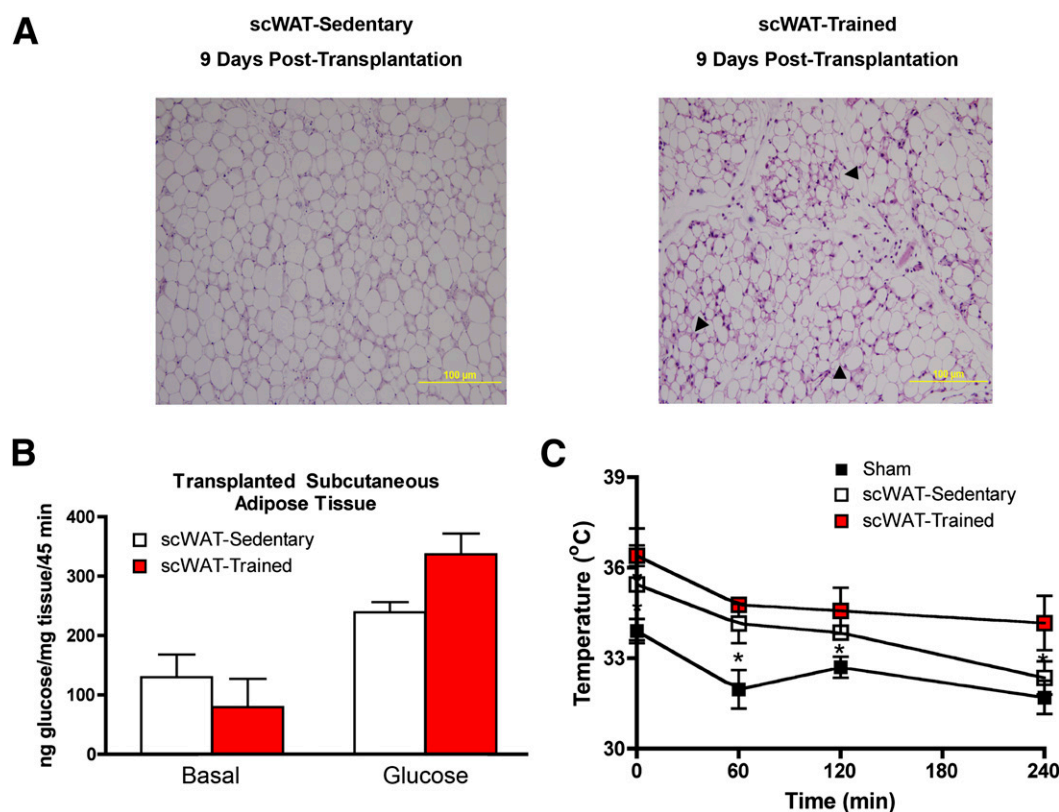
Figure 4—Continued.

addition, if the transplanted adipose tissue were to function as a “metabolic sink,” there may be lower circulating lipid and glucose concentrations, resulting in decreased lipid levels in endogenous tissues such as skeletal muscle and liver. As noted above, there was no difference in circulating free fatty acid levels among groups (Supplementary Table 2), and liver triglyceride levels were significantly reduced in mice transplanted with scWAT from both trained or sedentary mice compared with sham-operated mice (Fig. 1H). Triglyceride concentrations in tibialis anterior muscles (Supplementary Fig. 3A) were not different among sham-operated mice or mice transplanted with scWAT from trained or sedentary mice. There were also no differences in glucose uptake *in vivo* in transplanted scWAT from sedentary or trained mice

(Fig. 5B). There was no difference in fatty acid uptake in cultured adipocytes isolated from scWAT from sedentary or trained mice (Supplementary Fig. 3B). Training adaptations were still observed after 12 days in culture, confirmed by increased UCP1 protein expression in cultured adipocytes isolated from trained mice (Supplementary Fig. 3C). These data suggest that the improved glucose tolerance with transplantation of scWAT from trained mice is not due to these cells functioning as a sink for the disposal of glucose or lipids.

## DISCUSSION

Exercise is a primary treatment for obesity and type 2 diabetes, functioning to decrease adiposity, improve glucose homeostasis, and reduce insulin resistance. While the



**Figure 5**—Characteristics of mice transplanted with scWAT. **A**: H-E stains of transplanted scWAT from sedentary (scWAT-Sed) and trained (scWAT-Train) mice 9 days post-transplantation. Arrows indicate the presence of multilocular droplets. **B**: Glucose uptake into transplanted scWAT from mice transplanted with 0.85 g scWAT from sedentary or trained mice ( $n = 6$ /group). **C**: At 9 days post-transplantation, awake mice were exposed to a temperature of 4°C, and body temperature was measured. Data are reported as the mean  $\pm$  SEM ( $n = 6$ /group; \* $P < 0.05$  compared with sham-treated controls).

benefits of exercise on metabolic homeostasis have largely been attributed to adaptations in skeletal muscle, in the current study we establish that physical activity results in profound adaptations to scWAT. We found that scWAT from exercise-trained mice, when transplanted into the visceral or subcutaneous cavity of recipient mice, improves glucose tolerance and insulin sensitivity compared with sham-treated mice or mice receiving scWAT from sedentary animals. These findings suggest a novel paradigm whereby scWAT adapts to exercise training in a manner that exerts beneficial metabolic effects systemically and on other tissues in the body.

A remarkable finding from the current study was the dramatic improvement in glucose homeostasis observed after only 9 days of transplantation of scWAT from trained mice. The effects on glucose tolerance must involve the transplanted tissue exerting endocrine effects on other tissues, since the similar and very low rates of glucose uptake in transplanted tissue from both the sedentary and trained mice cannot account for the increased glucose disposal. Moreover, the transplantation of scWAT from trained mice resulted in increased glucose uptake in the endogenous BAT and oxidative skeletal

muscles of recipient mice. The rapid nature of the effects on glucose tolerance, which occurred as early as 9 days post-transplantation, also supports an endocrine effect. Thus, these short-term beneficial effects of transplanting scWAT from trained mice on glucose homeostasis are likely mediated by an acute factor, with altered expression as a result of exercise.

Skeletal muscles actively contract during exercise and are highly plastic, while adipose tissue has been thought to primarily function to supply fuel for the working muscle. Therefore, we were surprised that the microarray data revealed that a vast number of genes involved with many cellular functions were significantly upregulated or down-regulated in scWAT from trained mice. In fact, although a direct comparison cannot be made, the number of genes upregulated by exercise training in scWAT is substantially greater than what has been reported to be increased in skeletal muscle with exercise training (42–44). Exercise training had significant effects on adipose tissue genes involved in metabolism, mitochondrial biogenesis, oxidant stress and signaling, membrane transport, cell stress, proteolysis, apoptosis, and replication. This degree of plasticity in adipose tissue, along with the dramatic beneficial metabolic effects of transplantation of trained scWAT, suggests

that scWAT plays a prominent role in whole-body adaptations to exercise training.

In the current study, multiple approaches were used to demonstrate that exercise training increases the beige phenotype of the scWAT. We found that the scWAT from the trained mice have the following: 1) the induction of numerous beige adipocyte marker genes (e.g., *Prdm16*, *Ucp1*, *Pgc1α*, *Elovl3*, *Cidea*); 2) increased UCP1 staining; 3) a multilocular appearance; and 4) increased oxygen consumption. These changes occurred after only 11 days of training, a significantly shorter length of time than other studies have reported. Four weeks of exposure to an enriched environment, which included the presence of a running wheel, resulted in the emergence of beige adipocytes in white adipocytes marked by an increase in *Ucp1*, *Prdm16*, and other markers of BAT (20), while another recent study (2) showed that wheel running for 3 weeks significantly increased *Ucp1* mRNA levels. Our other findings, demonstrating that exercise training in mice results in significant increases in *Vegfa* mRNA, suggest that the expression of this protein is likely critical for the training phenotype and are consistent with recent studies showing that transgenic overexpression of vascular endothelial growth factor results in a browning phenotype of WAT (45,46).

Transplantation of scWAT from sedentary mice into the visceral cavity of mice has been previously reported (21) to improve glucose tolerance by 10%. However, these effects did not occur until 12 weeks post-transplantation (21). Our current data are consistent with this study, as there was no effect of transplanting scWAT from sedentary mice on recipient mice at early time points post-transplant. Interestingly, when the recipient mice were stressed with a high-fat diet, transplanting scWAT from both sedentary and exercise-trained mice improved glucose tolerance, but the effect was more pronounced with transplanting scWAT from trained mice. These data demonstrate that the transplantation of scWAT negates the detrimental effects of a high-fat diet on glucose tolerance after only 9 days. Importantly, because the mice receiving scWAT from trained mice have an even greater improvement in glucose tolerance, it is possible that the beneficial effects of transplanting scWAT from trained mice are amplified under conditions of metabolic stress, perhaps providing a potential therapeutic tool.

In summary, we find that exercise training results in profound changes to scWAT, altering a vast array of genes involved in a multitude of cellular functions. The striking effects of transplanting scWAT from trained mice to improve glucose homeostasis suggest that scWAT from trained mice exerts endocrine-type actions that play a previously unrecognized role in the beneficial effects of exercise training on metabolic health. While the concept of contracting skeletal muscle tissue releasing “myokines” has been proposed for many years (47), the current data demonstrate that exercise training causes adaptations to

scWAT that result in tissue-to-tissue communication, contributing to improved metabolic homeostasis.

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**Author Contributions.** K.I.S. designed the research, performed the experiments, analyzed the data, and wrote the paper. R.J.W.M., M.-Y.L., H.T., K.S., K.M.H., K.R.M., and K.H. performed the experiments. K.L.T. and M.F.H. performed the experiments and analyzed the data. Y.-H.T. analyzed the data. L.J.G. designed the research, analyzed the data, and wrote the paper. L.J.G. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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