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EXPERIMENTAL

Fat Grafting Can Induce Browning of White Adipose Tissue

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Background: Fat grafting is commonly used when treating soft-tissue defects. However, much of the basic biology behind fat transfer is still uncovered. Adipocytes can be divided into energy storing white and energy burning brown adipose cells. It is now well known, that also adult humans have metabolically active brown adipose tissue (BAT) within white adipose tissue (WAT). Previously our group showed that transfer of metabolically inactive WAT into a new environment increased the metabolic activity of the fat grafts to resemble the activity in the recipient site and that different WAT depots have variation in the metabolic activity. This led us to speculate, whether the metabolic increase of the graft is a result of "browning" of the transferred WAT toward beige adipose tissue.

Methods: We investigated the metabolic and histological characteristics and BAT marker *Ucp1* gene expression in different types of WAT grafts placed either in subcutaneous or muscle tissue in mice. Metabolic activity of the grafts was investigated by FDG-PET/CT at 4- and 12-week time-points.

Results: The glucose uptake of all transferred fat types was increased when compared with respective control WAT regardless of transfer location. *Ucp1* gene and protein expression was increased in 4 of 15 intramuscularly placed fat graft samples and showed histological resemblance to BAT with multilocular cells.

Conclusions: Grafting of metabolically inactive fat intramuscularly may induce browning of fat grafts toward more active beige adipose tissue. This opens up new research areas in exploiting fat grafting in metabolic diseases. *(Plast Reconstr Surg Glob Open 2018;6:e1804; doi: 10.1097/GOX.0000000000001804; Published online 19 June 2018.)*

INTRODUCTION

Autologous free white adipose tissue (WAT) grafts are commonly used in plastic surgery for reconstruction of soft-tissue defects.[1] Novel experimental therapies include softening and contour of scars and other fibrotic conditions.[2–4] However, the basic biology of fat transfer surgery and the antifibrotic effects are still poorly understood. Many of the effects are thought to be mediated by the immunomodulatory properties of adipose-derived stem cells, which are present in the transferred adipose tissue.[5] They have been shown to reduce proinflammatory cytokine (IL-1, IL-17) levels and increase anti-inflammatory

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protein (IL-10) levels in mice.[6] Only a few experimental reports describe the effects of fat grafting in muscle tissue[7,8] although fat grafting is routinely performed in large volumes for example in pectoralis and latissimus dorsi muscles in breast reconstruction and augmentation.[9,10]

The type of adipose tissue plays a great role in obesity and metabolic disorders such as type 2 diabetes. It is known that there are 2 types of adipose tissue in mammals: thermogenically active brown adipose tissue (BAT) and WAT, which is mainly involved in storing excess energy.[11] Typically BAT contains adipocytes with small multilocular lipid droplets, and numerous mitochondria with uncoupling protein 1 (UCP1), the protein responsible for regulation of thermogenesis and characteristic feature for BAT.[12,13] Previously, BAT was thought to exist only in small mammals and infants, but during the past decade, several studies have demonstrated significant amounts of functional BAT also in adults.[14] Interestingly, also adult humans have inducible brown-like adipocytes called beige (or brite) adipose tissue, which consists of brown-like adipocytes among WAT bringing about similar characteristics in energy metabolism as BAT.[15–17] Recent evidence suggest that beige adipocytes derive from the same mesenchymal stem cells but different precursor cells as WAT[18] and begin to differentiate due to environmental stimuli, such as changes in temperature and nutrition.[19]

We have previously used an experimental model to evaluate the vascularization, survival, and metabolic changes after free fat transfer using ^{18}F-fluorodeoxyglucose (FDG) PET/CT imaging described by Tervala et al.[20] Results showed that transfer of the metabolically inactive (epididymal) fat into a new environment modulates the metabolic activity of the fat grafts to resemble the situation in the recipient site. We also noted metabolic differences in subcutaneous, visceral, and epididymal WAT depots. In this study, we wanted to investigate whether the metabolic increase of the transplant can be a result of "browning" of the transplanted WAT and whether it was donor-site specific. In this study, we examined the metabolic and histological characteristics and BAT marker *Ucp1* expression in different types of WAT grafts placed either subcutaneously or intramuscularly. The purpose of the study was to investigate whether fat grafting can induce browning of WAT, especially when fat is transferred to a metabolically more active area such as muscle tissue, and determine the potential benefits of fat grafting in the surgical treatment of metabolic disorders.

MATERIALS AND METHODS

Mouse Model

Permissions for the animal studies were obtained from the National Animal Experiment Board of Finland. We used 3 different types of WAT as free fat grafts transferred into subcutaneous and muscle tissue, as described in Figure 1. According to the WAT type, 3 groups of graft mice were formed and maintained during the experiment: epididymal (n = 6), visceral (n = 5), and subcutaneous group (n = 5). A control group (n = 5) was used as a reference to determine the basic muscle, BAT, and WAT metabolism. C57BL/6 mice (C57BL/6NCrl, Harlan, The Netherlands) were killed and used as donor mice for harvesting WAT. Athymic nude mice (Hsd:Athymic Nude-Foxn1nu, Harlan, The Netherlands) with inhibited immune system were used as recipients to avoid graft rejection response. All mice were anesthetized with xylazine hydrochloride 5 mg/kg (Rompun 20 mg/mL, Bayer Animal Health, Leverkusen, Germany) and ketamine 70 mg/kg (Ketalar, Hameln Pharmaceuticals, Hameln, Germany). For analgesia, buprenorphine hydrochloride 0.075 mg/kg (Temgesic, RB Pharmaceuticals Limited, Slough, Berkshire, Great Britain) was administered to recipient mice during 2 days postoperatively.

After harvesting, the excised grafts were weighted and subsequently placed under the skin of forehead to subcutaneous space and among muscle tissue in hind leg of the recipient mice (n = 16). Into forehead, the fat was placed as a whole piece, and into leg with a 14 G cannula after mechanical disruption combined with a small amount

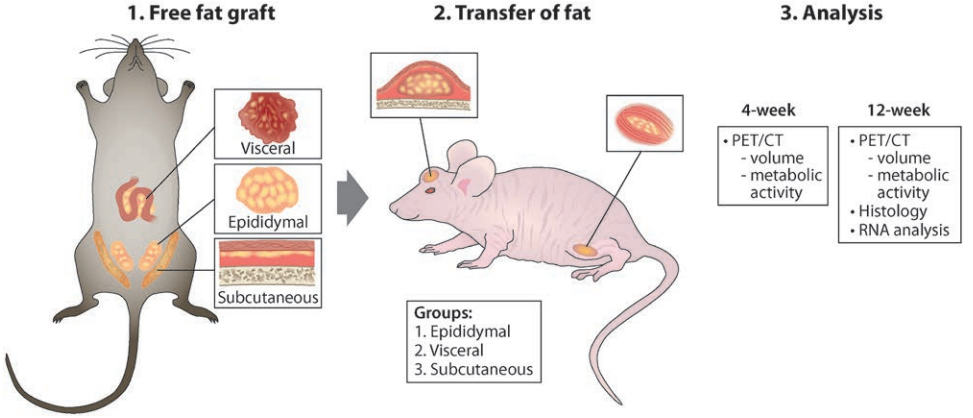

Fig. 1. Study protocol. Three types of WAT (epididymal, visceral, and subcutaneous) were transferred into 2 locations: subcutaneously in forehead and intramuscularly in leg region. Groups according to transferred fat were maintained throughout the follow-up of 12 weeks. Glucose uptake of the grafts was evaluated at 4- and 12-week time points using FDG-PET/CT imaging. At 12 weeks, tissue samples were examined for histological characteristics and BAT marker *Ucp1* gene and protein expression.

of saline. The average volume of grafted fat to forehead was 0.25 ± 0.09 mL and 0.13 ± 0.05 mL to leg, respectively. Wounds were closed using 5-0 Polysorb sutures (Covidien, Dublin, Ireland).

In Vivo FDG-PET/CT Imaging and Data Analysis

Metabolic activity of fat grafts was examined in mice at 4- and 12-week time points by PET/CT (Siemens Medical Solutions USA, Knoxville, Tenn.) using ^{18}F-FDG, a glucose analogue known to accumulate to metabolically active tissues. Metabolism of BAT increases in cold temperature, and to stimulate the activity of BAT and induce thermogenesis, PET/CT scan was performed on 2 consecutive days: first with cold exposure (26–28°C) and the second with warm exposure (36–38°C).[21] FDG was given approximately 5 MBq into the tail vein and the 2-step whole-body PET/CT scan was performed at fasting state (4 h) during isoflurane gas anesthesia on. The cold exposure was accomplished by exposing mice to cold air by circulating propylene glycol through an animal holder using a heating circulator (S-12 Julabo, Seelbach, Germany) with simultaneous temperature monitoring. The warm exposure was carried out in normal room temperature by using a heating pad. At 4-week time point, PET/CT was performed only with warm exposure, and at 12-weeks with both cold and warm scans.

FDG images were reconstructed as described by Tervala et al.[20] Volumes of interest (VOIs) were drawn on the forehead subcutaneous fat graft and leg region intramuscular fat graft by using either radioactivity uptake or the computed tomography template as an anatomical reference. In the leg where grafts were smaller and less visible than that of the forehead, VOIs were drawn to cover the whole leg. VOIs were drawn also on a part of liver, interscapular BAT, and contralateral hind leg (control muscle) as a reference. Standardized uptake value (SUV) was defined as the ratio of FDG radioactivity (Bq) per milliliter (mL) of tissue to the radioactivity in the injected dose corrected by decay and by animal body weight. The SUV of fat grafts were compared with that of liver, and results expressed as fat-to-liver uptake ratios (SUV of fat/SUV of liver).

Ex Vivo Radioactivity Analysis of Tissue Samples

Immediately after the last follow-up scan at 12-week time point, the graft mice were killed for ex vivo tissue sample analysis. Samples were measured for ^{18}F-radioactivity in a gamma counter (Wizard2 3", PerkinElmer, Turku, Finland) and expressed as the percentage of injected dose per gram tissue (% ID/g). Thereafter, samples were fixed in formalin for histologic examination. A separate tissue sample for RNA analysis was snap frozen in liquid nitrogen and stored in -70°C.

Gene Expression Analysis

Tissue samples were homogenized in QIAzol Lysis Reagent (Qiagen), and total RNA was extracted according to the manufacturer's recommendations. RNA was cleaned up from the aqueous phase of the extracts using the RNeasy Micro Kit (Qiagen) according to the manufacturer's recommendations and included an on-column DNase I treatment step. cDNA synthesis was performed using the first Strand cDNA Synthesis Kit for real-time polymerase chain reaction (PCR) (AMV) (Roche). Quantitative real-time PCR was performed on a ViiA7 instrument (Applied Biosystems) using Power SYBR Green PCR Master Mix (Applied Biosystems). The standard curve method with *Rplp0* as the normalizing gene was used to determine relative *Ucp1* gene expression levels. Primer sequences are supplied in Supplementary Table 1 (**see table, Supplementary Digital Content 1**, which displays primer sequences, ***http://links.lww.com/PRSGO/A793***).

Histology and Immunohistochemistry

Tissue samples fixed overnight in formalin were embedded in paraffin. Sections of 4 μm thickness were cut and stained with hematoxylin and eosin for the evaluation and grading (0–5) or percentage (0–100%) of adipose tissue survival, fibrosis, cystic degeneration, presence of multilocular BAT morphology, and degree of inflammation. To confirm the *Ucp1* gene expression results at protein level, UCP1 immunohistochemical staining was performed. Samples were stained with rabbit antimouse anti-UCP1 (dilution 1:1,000, Abcam, United Kingdom). An horseradish peroxidase (HRP)-conjugated goat-antirabbit immunoglobulin G (IgG) polyclonal (ImmunoLogic, Netherlands) was used as the secondary antibody.

Statistical Analysis

All results were analyzed using SPSS (IBM SPSS Statistics IBM SPSS Statistics for Macintosh, version 23.0. Armok, NY: IBM Corp.). Groups were compared using 1-way ANOVA, and pairwise comparisons were made using the Kruskall-Wallis test or Student's *t* test. Statistical significance was set at $P \leq 0.05$.

RESULTS

In Visual Analysis of FDG-PET/CT Imaging, Most Grafts Were Visible and Showed FDG Uptake

At 4-week time point, all subcutaneous grafts at the forehead region were visible and showed FDG uptake, and most grafts (63%) showed increase in volume (mean graft volume 0.32 ± 0.19 mL, median 0.30 mL). Visual FDG uptake in the intramuscular grafts at leg region was seen in 11 of 16 mice (68.8%) evenly distributed in all groups. Intramuscular graft volume was not measurable with PET/CT due to small amount of transferred fat.

At 12-week time point, the FDG uptake remained visible in 10 of 16 subcutaneous grafts during follow-up. Poorest survival of grafts was in the visceral group, where only 1 graft was visible. Graft volumes showed great variability (mean 0.53 ± 0.61 mL, median 0.24 mL). Eight intramuscular grafts showed visual FDG uptake with even distribution between groups (Fig. 2).

FDG-PET/CT Quantitative Analysis Showed High Uptake in Both Graft Regions and BAT But Low Uptake in Control WATs

The FDG uptake in VOI was expressed as fat-to-liver ratio shown in Table 1. PET/CT scans at both 4-week and

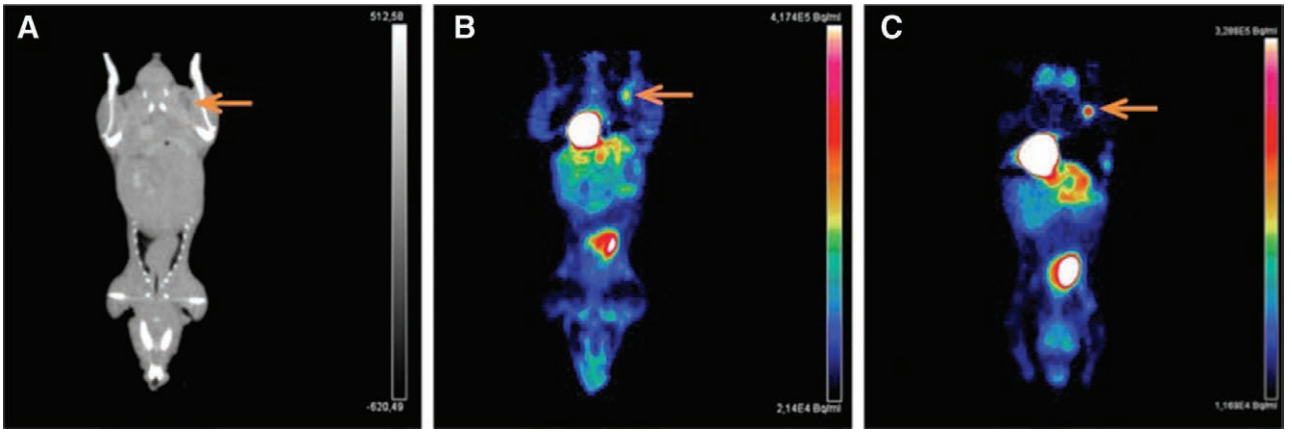

Fig. 2. CT image showing a fat graft in the left hind leg (A) and corresponding PET images with FDG uptake at 4-week (B) and 12-week (C) time points. Arrow indicates the location of the intramuscular graft. CT, computed tomography.

Table 1. Quantitative Results of FDG-PET/CT Imaging (Expressed as Fat-to-liver Ratio) by Groups at 4- and 12-week Time Points from Subcutaneous (scGraft) and Intramuscular (imGraft) Grafts, Control Muscle Tissue (cMuscle) from the Contralateral Leg, and Brown Fat (BAT)

			12-wk Time Point	
Transferred fat	**Location**	**4-wk Time Point**	**Cold**	**Warm**
	scGraft	1.6±0.4	1.6±0.4	2.8±0.4
Epididymal fat (n = 6)	imGraft	0.7±0.2	0.6±0.2	0.9±0.3
	cMuscle	0.5±0.1	0.4±0.4	0.6±0.2
	BAT	2.2±0.3	1.2±0.2	2.3±0.5
	scGraft	3.1±0.6	1.7	2.3
Visceral fat (n = 5)	imGraft	1.4±0.2	0.9±0.7	1.3±1.1
	cMuscle	0.6±0.2	0.5±0.2	0.6±0.2
	BAT	2.5±0.3	2.1±1.0	2.7±0.6
	scGraft	2.5±1.2	1.6±0.3	2.6±0.5
Subcutaneous fat (n = 5)	imGraft	1.0±0.3	0.8±0.5	1.2±0.7
	cMuscle	0.7±0.1	0.5±0.2	0.7±0.2
	BAT	2.3±0.5	1.7±0.4	2.8±0.8
Control (n = 5)	cMuscle		0.5±0.2	0.8±0.2
	BAT		2.2±1.6	2.6±0.8

At 12-week time point, the scan was performed on 2 consecutive days: first with cold exposure to activate brown fat, second with warm exposure. At 4 weeks, imaging was done only after warm exposure.

12-week time points showed increased FDG uptake subcutaneous grafts without significant difference between the groups ($P = 0.069$). In intramuscular grafts, FDG uptake was greater in all graft groups compared with control leg ($P = 0.019$). There was no statistical difference between fat-to-liver ratios in different time points at 4 and 12 weeks ($P = 0.421$). Overall, FDG uptake in all regions was higher in warm temperature scans ($P < 0.001$; Fig. 3A). Ex vivo tissue samples were also measured for ^{18}F-FDG uptake (Fig. 3B) showing high uptake in both graft regions and BAT but low uptake in control WATs.

Gene Expression Analysis Showed Elevated *Ucp1* Expression in 4 out of 15 Intramuscular Grafts

Fifteen intramuscular and 13 subcutaneous graft samples were analyzed in addition to control BAT and WAT samples (Fig. 4). The highest *Ucp1/Rplp0* ratio of all fat tissue samples was used as a reference, and results are expressed as percentage (%) of the reference value. The *Ucp1* expression was relatively low in all subcutaneous grafts (mean 0.0015% ± 0.0022%), whereas the *Ucp1* level in intramuscular grafts was elevated in all groups (mean 2.51% ± 7.65%), but the comparison did not reach statistical significance ($P = 0.25$). The mean *Ucp1* expression in the control samples was 0.0015% ± 0.00077% in epididymal fat, 0.017% ± 0.025% in visceral fat, and 0.12% ± 0.10% in subcutaneous fat. Average BAT *Ucp1* level was 63% ± 12%. In individual analysis, 4 intramuscular fat grafts showed significantly elevated expression of *Ucp1* (mean, 9.4% ± 14%) compared with control WAT and presented later in this section.

Histological Analysis Revealed Brown Adipose Tissue-like, UCP1-positive Multilocular Fat in 4 of 15 Intramuscular Grafts

The histological analysis did not make any difference between the types of WAT used in grafting, and therefore comparison was performed mainly between the graft locations. The average percentage of survived fat was 23% ± 28%, and there seemed to be a trend toward a greater adipose tissue percentage in the intramuscular grafts ($P = 0.12$). No significant statistical difference was observed with respect to the degree of inflammation and fibrosis between graft locations ($P = 0.92$ and $P = 0.28$, respectively), but cystic de-

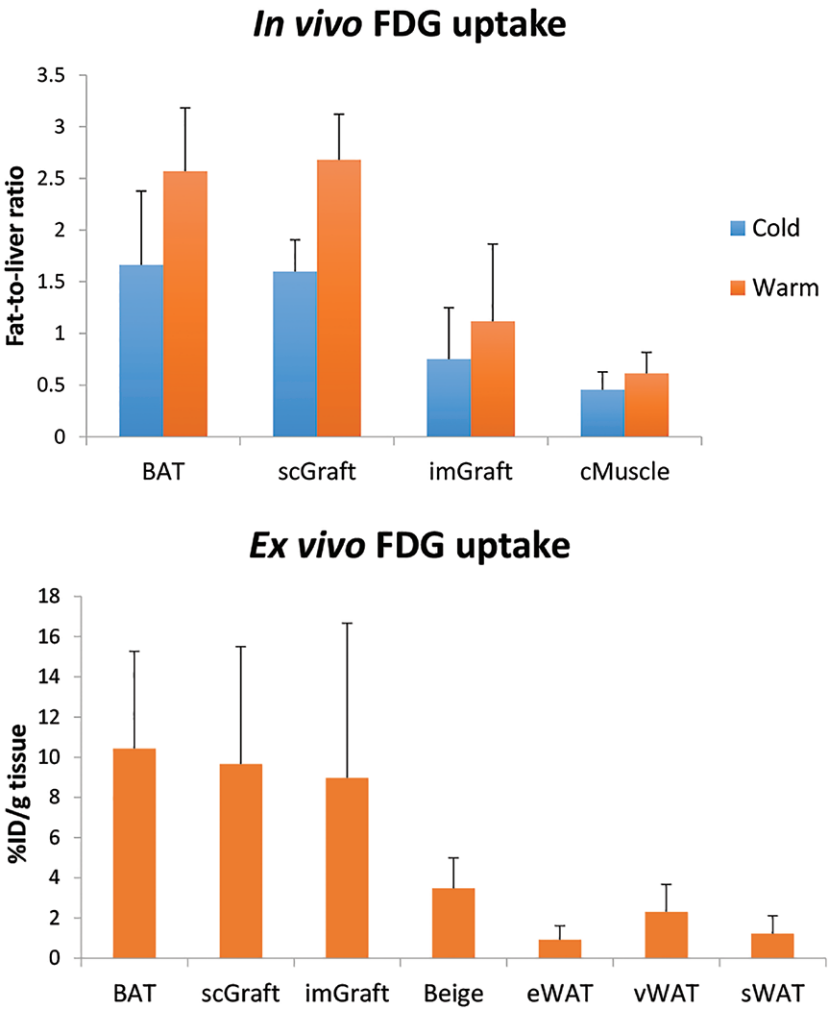

Fig. 3. A, In vivo FDG-PET/CT scans at 12-week time point were performed on 2 consecutive days: first with cold exposure and the second with warm exposure. Figure shows mean fat-to-liver ratios in BAT, subcutaneous (scGraft) and intramuscular (imGraft) grafts, and control muscle tissue (cMuscle). B Ex vivo absolute ^{18}F-FDG uptakes in BAT, subcutaneous (scGraft) and intramuscular (imGraft) grafts, grafts with beige adipose tissue (Beige), and control white adipose tissues epididymal (eWAT), visceral (vWAT), and subcutaneous (sWAT) fat expressed as the percentage of injected dose per gram tissue (%ID/g tissue).

generation was significantly lower in intramuscular grafts ($P < 0.001$). BAT-like multilocular fat was seen only in intramuscular grafts (mean, 11% ± 21%), but also in control WAT in some degree (7% ± 14%; Fig. 5). However, UCP1 staining was negative and *Ucp1* gene expression low regarding these control samples. When examining samples individually, multilocular fat was found in 4 intramuscular grafts (2 in the epididymal group, 1 in the visceral group, and 1 in the subcutaneous group), and these samples showed also positive UCP1 staining and elevated *Ucp1* gene and protein expression as a difference to control samples. Detailed results of histological analysis by groups are shown in Table 2.

In Individual Sample Analysis, 4 of 15 Intramuscular Fat Grafts Showed Browning Toward Beige Fat

According to the results, the same 4 intramuscular fat grafts were characterized by multilocular fat droplets and increased *Ucp1* gene and protein expression, both typical for BAT, indicating metabolic and morphologic change toward beige adipose tissue. As seen in Figure 5, with hematoxylin and eosin (HE) staining beige fat was characterized by both multilocular BAT-like fat and large lipid droplets similar WAT, but other grafts showed mostly original WAT-like fat with large oil cysts. To confirm the presence of beige fat, we examined the samples also with UCP1 staining, which showed positively stained tissue samples. Also some WAT samples showed multilocular fat but UCP1 staining remained negative. The amount of the multilocular fat varied in the beige samples (60%, 50%, 10%, and 40%). The beige fat was well preserved in the target region (fat cells 76±8 % versus 15±18 % in other fat grafts, $P < 0.001$), and there was less inflammation (grading 0.25±0.5 versus 2.96±1.30, $P < 0.001$), fibrosis (0.5±0.6 versus 3.1±1.2, $P < 0.001$) and cystic degenera-

tion (0 in beige grafts, others 2.0±1.7, $P = 0.03$). Glucose uptake in beige grafts was low: the mean ex vivo FDG uptake was 3.5±1.5 %ID/g in the beige grafts and 11.1±6.7 %ID/g in others ($P = 0.140$; Fig. 3B). Mean *Ucp1* expression was 9.4% ± 14 % for beige grafts and 0.002% ± 0.004% for other grafts, respectively, which makes the *Ucp1* expression in beige grafts 4,700-fold greater compared with the other grafts ($P < 0.001$) and from 430 to 1,383-fold greater than in the respective control fat (Fig. 4).

DISCUSSION

In this article, we aimed to investigate the metabolic adaptation of WAT grafts in subcutaneous and muscle tissue, as the metabolic and morphologic changes in fat grafts are still poorly understood despite the fact that lipotransfer is commonly used in plastic surgery. Our results show a novel phenomenon: browning of intramuscularly placed fat grafts. This was demonstrated with HE and UCP1-stained histology samples showing multilocular lipid droplets and an increase in *Ucp1* gene and protein expression. These grafts were also of good quality with limited inflammation, fibrosis, and cystic degeneration.

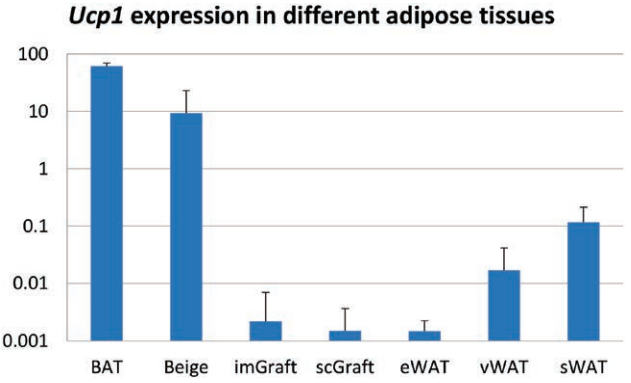

Fig. 4. Mean *Ucp1* expression (expressed as percentage of the reference value) in BAT, intramuscular grafts with beige fat (Beige), other intramuscular grafts (imGraft), subcutaneous grafts (scGraft), and in epididymal (eWAT), visceral (vWAT), and subcutaneous (sWAT) WATs. *$P < 0.001$ between beige and other grafts.

This finding presents an interesting outcome of a common procedure in the field of plastic surgery. Obesity and the large amount of inactive fat are major factors in metabolic disorders. By changing the metabolism of fat into a more active form, fat grafting could have beneficial effects also on glucose tolerance and body weight. To this point, many experimental studies have shown that fat has effects on glucose balance, but different depots of WAT seem to have different metabolic characteristics. Previous reports have shown that the fatty acid metabolism is lower in epididymal than in subcutaneous WAT.[22] It has also been reported that transfer of epididymal or inguinal WAT into the visceral cavity improves glucose tolerance,[23] decreases the total amount of fat and weight and insulin resistance in mice.[24] Especially the transfer of subcutaneous fat into the visceral cavity reduces the amount of several proinflammatory cytokines (TNF alfa, IL-17, IL-12) in plasma.[25]

A limited degree of inflammation may be 1 factor allowing browning to initiate. A recent study has shown that inflammation of WAT is associated with diminished generation of *Ucp1* expressing beige adipocytes[26] giving a possible explanation why browning occurred only in limited amount of samples in our study. Unlike previous studies have shown,[27,28] also interscapular BAT showed increased glucose (FDG) uptake during warm exposure than in cold exposure. However, recent reports demonstrate that FDG uptake can be increased in BAT of UCP1-deficient mice suggesting also UCP1 independent mechanisms for the increased uptake.[29]

The amount of BAT precursors in fat grafts also varies, and therefore some grafts may be more prone to browning than others. According to previous studies, the metabolic adaptation of white adipose cells to beige cells through direct transformation is regulated by many factors.[16,30,31] Beige adipocytes, at least in the subcutaneous depot, have been shown to arise from a precursor population rather than from preexisting adipocytes.[32] The beige adipocyte precursor population is different from the white precursor population,[18] and beige adipocytes have been shown to be most abundant in the inguinal WAT, which is a major subcutaneous depot in rodents.[33]

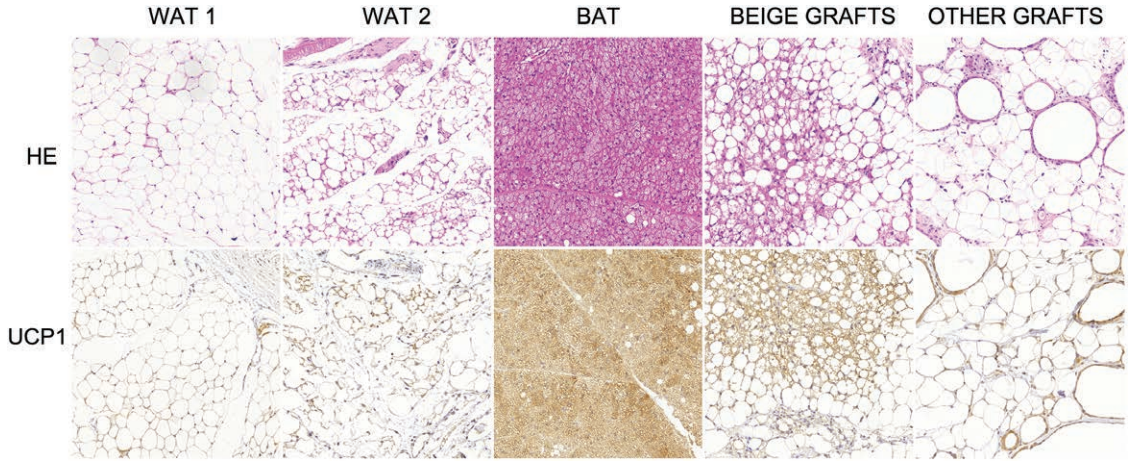

Fig. 5. HE (above) and UCP1 (below) staining (20×) of normal WAT1, multilocular WAT2, BAT, beige grafts, and other fat grafts.

Table 2. Table Shows Histological Analysis of Tissue Samples Specified by the Transferred White Fat (Epididymal, Visceral, or Subcutaneous) and the Graft Type (Subcutaneous or Intramuscular)

Fat Graft	Fat Cells (%)	Multilocular Fat (%)	Inflammation	Cystic Degeneration	Fibrosis
Epididymal	27±31	10±22	2.5±1.6	2.3±1.8	2.9±1.5
Visceral	16±27	1±3	2.6±1.5	0.8±1.4	2.6±1.6
Subcutaneous	24±24	6±15	3.0±1.9	1.6±1.6	2.9±1.4
All	23±28	6±17	2.6±1.5	1.7±1.7	2.8±1.5
Subcutaneous fat grafts					
Fat graft	Fat cells (%)	Multilocular fat (%)	Inflammation	Cystic degeneration	Fibrosis
Epididymal	12±8	0	2.8±1.0	3.8±0.8	3.3±0.5
Visceral	9±11	0	2.5±2.1	1.3±1.9	2.8±1.9
Subcutaneous	24±19	0	2.8±1.5	2.3±1.7	3.3±1.0
All	14±13	0	2.7±1.4	2.6±1.7	3.1±1.1
Intramuscular fat grafts					
Fat graft	Fat cells (%)	Multilocular fat (%)	Inflammation	Cystic degeneration	Fibrosis
Epididymal	43±38	18±29	2.2±2.0	0.7±0.8	2.5±2.1
Visceral	22±36	2±5	2.6±1.1	0.4±0.9	2.4±1.5
Subcutaneous	25±31	11±20	3.3±2.4	1.0±1.4	2.5±1.7
All	31±34	11±21	2.6±1.8	0.7±1.0	2.5±1.7
Control WAT					
Control fat	Fat cells (%)	Multilocular fat (%)	Inflammation	Cystic degeneration	Fibrosis
Epididymal	92±5	1±1	0.8±0.4	0.6±0.5	0.2±0.4
Visceral	89±7	11±22	0.8±0.4	0.2±0.4	0.6±0.5
Subcutaneous	78±12	9±12	0.4±0.5	0.2±0.4	0.8±0.8
All	86±10	7±14	0.7±0.5	0.3±0.5	0.5±0.6

HE-stained samples were examined to determine the amount of survived fat and multilocular BAT-like fat (as percentage), and evaluated for degree of inflammation, cystic degeneration, and fibrosis (on a scale 0–5).

An autocrine/paracrine mechanism between muscle and adipose cells may also be the cause behind browning. Myocytes secrete irisin, and its concentrations increase in mice and humans by exercise training and it has been shown to stimulate the browning of WAT through specific actions on the beige preadipocyte population.[34] An advantage in muscle tissue is also the abundant vascular bed improving the survival of fat graft.

As a conclusion, this study suggests a novel finding: fat grafting can induce browning of the graft. Subcutaneous and intramuscular grafting might have different outcomes as browning of fat was only seen in grafts placed in muscle tissue. It is known that many factors can initiate browning, and external manipulation (liposuction and lipotransfer) may be 1 of these factors. Also transferring WAT to metabolically more active muscle tissue may bring about metabolic adaptation in the fat. Therefore, fat transfer may have beneficial effects on body metabolism by increasing the amount of metabolically active tissue. Clinical research is still needed to investigate the metabolic changes in autologous fat grafting in humans, but the potential browning and metabolic activation of WAT opens up new research areas in exploiting fat grafting in metabolic diseases.

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