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Pre-Operative Diet Impacts the Adipose Tissue Response to Surgical Trauma

Binh Nguyen, MD¹, Ming Tao, MD¹, Peng Yu, MD¹, Christine Mauro, MD¹, Michael A. Seidman, MD, PhD², Yaoyu E. Wang, PhD³, James Mitchell, PhD⁴, and C. Keith Ozaki, MD¹

¹Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

²Department of Pathology and Laboratory Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

³Center for Cancer Computational Biology, Dana Farber Cancer Institute, Boston, Massachusetts, USA

⁴Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts, USA

Abstract

Background—Short-term changes in pre-operative nutrition can have profound effects on surgery related outcomes such as ischemia reperfusion injury in pre-clinical models. Dietary interventions that lend protection against stress in animal models (e.g. fasting, dietary restriction [DR]) impact adipose tissue quality/quantity. Adipose tissue holds high surgical relevance due to its anatomic location and high tissue volume, and it is ubiquitously traumatized during surgery. Yet the response of adipose tissue to trauma under clinically relevant circumstances including dietary status remains poorly defined. We hypothesized that pre-operative diet alters the adipose tissue response to surgical trauma.

Methods—A novel mouse model of adipose tissue surgical trauma was employed. Dietary conditions (diet induced obesity [DIO], pre-operative DR) were modulated prior to application of surgical adipose tissue trauma in the context of clinically common scenarios (different ages, simulated bacterial wound contamination). Local/distant adipose tissue phenotypic responses were measured as represented by gene expression of inflammatory, tissue remodeling/growth, and metabolic markers.

Results—Surgical trauma had a profound effect on adipose tissue phenotype at the site of trauma. Milder but significant distal effects on non-traumatized adipose tissue were also observed. DIO exacerbated the inflammatory aspects of this response, and pre-operative DR tended to reverse these changes. Age and LPS-simulated bacterial contamination also impacted the adipose tissue response to trauma, with young adult animals and LPS treatment exacerbating the proinflammatory response.

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Correspondence and reprint requests: C. Keith Ozaki, M.D. 75 Francis Street, Boston, MA 02115 857-307-1920 (phone), 857-307-1922 (fax) CKOzaki@partners.org.

Authorship: Binh Nguyen and Ming Tao contributed equally to this work.

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Conclusions—Surgical trauma dramatically impacts both local and distal adipose tissue biology. Short-term pre-operative DR may offer a strategy to attenuate this response.

Dietary restriction (DR), or reduced food intake without malnutrition, is best known for lifespan extension in a variety of experimental organisms¹, but can also protect against a number of inflammatory injuries. Recent data from animal models clearly indicate that even short-term preoperative dietary interventions, including 2-4 weeks of DR, 6 days of protein deficiency or 3 days of fasting, offer protection against organ injury associated with surgical ischemia reperfusion injury²⁻⁸. Dietary restriction thus holds potential as a clinically relevant strategy to alter the mammalian response to acute stress such as surgical trauma²⁻⁹.

In addition to its role in energetics, mammalian adipose tissue is now recognized as an active participant in homeostasis and immune function via a variety of endocrine and signaling networks¹⁰⁻¹³. While broadly obesity positively correlates with metabolic and cardiovascular disorders, qualitative adipose tissue factors appear to be important determinants of health and disease beyond simple body mass index¹⁴⁻¹⁶. Dietary intake serves as a key determinant of adipose tissue quantity and quality in humans¹⁶. Importantly, the plasticity of adipose tissue in response to food intake makes it a prime potential mechanistic vehicle for dietary effects^{11, 17}.

Due to anatomic proximity and relatively large tissue volume, adipose tissue is ubiquitously traumatized in surgical procedures. Links between adipose tissue biology and clinically relevant surgical outcomes are emerging¹⁸. For instance, exacerbated adipose tissue IL-6 release in obese surgical patients correlates with peri-operative insulin resistance¹⁸. Yet the impact of surgical trauma itself on adipose tissue biology, and how this can be modulated by diet, remains largely unknown.

Leveraging a controlled murine model of typical surgical trauma to adipose tissue, we sought to understand the impact of pre-operative diet on the response to surgery and its modulation by clinically relevant variables including age and bacterial wound contamination. We found that surgical trauma up-regulates markers of inflammation, matrix remodeling and angiogenesis, and that pre-operative diet modulates this response.

MATERIAL AND METHODS

Murine Surgical Trauma Model, Local and Distant Adipose Tissue Collection

Male C57BL/6J mice (Jackson Laboratory) were maintained on a 12-hour light-dark cycle for at least one week pre-experiment and throughout the experiments, and received water and chow ad libitum according to the dietary parameters discussed below. Experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee and complied with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, Revised 1996).

Operative procedures (including harvests) were performed aseptically, with general continuous isoflurane inhalant anesthesia (1-2% isoflurane mixed with 1 L/min oxygen), using a Zeiss binocular OPMI-MD Surgical Microscope (Carl Zeiss). A 2 cm by 1 cm “L”-shaped incision was made on the left flank of the animal. After retracting the skin, a 5 mm by 5 mm square of ~2 mm-thick subcutaneous adipose tissue was harvested using sharp dissection and snap frozen in liquid nitrogen (control baseline adipose tissue). A smaller 3 mm by 3 mm square of ~2 mm-thick adipose tissue was harvested for formalin fixation. Simple direct pressure was applied for hemostasis. The following standard surgical manipulations were then applied to the remaining adipose tissue in the surgical field. First, blunt dissection into the fat was performed by spreading and closing a hemostat instrument

ten times. This was followed by cauterizing a 4 mm length along the edge of the adipose tissue using a handheld electrocautery instrument. The skin incision was then closed with 6-0 absorbable suture, and the mouse was allowed to recover.

Twenty-four hours later the mice were again anesthetized. Adipose tissue was first harvested from the right flank of the animal following the same protocol outlined above, representing harvest from a remote adipose tissue site. The left flank operative site was then re-opened and the remaining adipose tissue from the site of the initial surgical manipulations (surgically traumatized adipose tissue) was harvested and snap frozen in liquid nitrogen.

Interventions to Model Clinical Circumstances

Dietary Perturbations—Beginning at 6 weeks of age, animals received either a 10 kcal% fat standard chow diet (normal chow [NC]; D12450B; Research Diets Inc.), a 60 kcal% fat diet (diet induced obesity [DIO]; D12492; Research Diets Inc.), or a high fat diet switched to normal chow (dietary restriction [DR]) 3 weeks before surgical trauma.

Age—Two distinct age group cohorts (11- and 26-week-old mice representing young adult and middle-aged animals, respectively) were investigated to define the impact of age on the adipose tissue response to surgical trauma under various conditions.

Mimicry of Local Wound Infection—LPS (5 µg in 40 µL 40% w/v pluronic gel) was placed in the traumatized surgical field immediately prior to closure.

Tissue Analyses

Total RNA was isolated from fresh frozen adipose tissue (RNeasy Mini Kit; Qiagen), quantified with NanoDrop-1000 (Thermo Scientific) and qualified with Bioanalyzer (2100, total RNA nanochip, Agilent). Quantitative real-time PCR (RT² qPCR Primer Assay, SYBR® Green, Life Technologies) was completed for selected mediators (Supplemental Table 1). The real time PCR assays for the selected genes were performed on a 7500 Sequence Detection System (Applied Biosystems) by using 400 nM of forward and reverse primers, a 10 ng cDNA sample, and RT² qPCR SYBR Green/ ROX Master Mix (Qiagen) in 25 µL reaction volume. Each target gene was simultaneously run with housekeeping genes (Hsp90ab1, Hprt1, and 18SrRNA) on all investigated specimens. The Comparative CT method was used for experimental setup and data analysis. To minimize variations in sample loading and make comparison across multiple experiments, results were expressed as mRNA fold induction normalized to the average expression of housekeeping genes Hsp90ab1 and Hprt1 in individual animals; 18S rRNA expression was not used due to high variation. The normalized values were then standardized to the mean baseline expression of the housekeeping genes across all animals.

Histologic sections of adipose tissue in the vicinity of the surgical site were generated from formalin-fixed paraffin-embedded tissue from mice in all treatment groups. Sections were stained with Masson's trichrome.

Statistical Analyses

Each of the twelve total treatment groups had 6 animals per cohort, with individual animals generating their own baseline, ipsilateral surgically traumatized, and contralateral (remote from trauma) adipose tissue specimens. Fold induction from baseline was then determined, and is expressed as mean ± SEM. Clinically meaningful comparisons were then completed for specific scenarios, and gene expression identified as up- or down-regulated if there was a statistical significance of $p < 0.05$. Differential expression was determined using the LIMMA package¹⁹. All p -values reported were corrected for multiple comparisons testing

using Benjamini and Hochberg correction²⁰. We also performed multivariate analyses using a $p < 0.01$. Principle component analysis was performed across all selected genes, and Euclidean distances between all sample pairs were calculated using the first three principle component. All statistical and clustering analyses were performed using R and Bioconductor²¹.

RESULTS

Effect of High Fat Diet (DIO) and Short-Term Pre-Operative Dietary Restriction on Baseline Adipose Tissue Phenotype

Animals were placed on a purified high fat diet containing 60% calories from fat (DIO group) from the age of six weeks until surgery at 26 weeks of age (middle-aged mice). Animals on a purified normal chow diet with 10% calories from fat served as a control (NC group). To simulate a clinically feasible preoperative DR regimen, DIO animals were placed on normal chow three weeks prior to surgery (DR group). Although allowed ad libitum access to control chow, these animals lost weight ($15.7\% \pm 4.5\%$) relative to the DIO group over the 3 week preoperative period. In order to evaluate the effects of age, the same three groups were subjected to surgery at 11 weeks of age (young adult mice), resulting in NC and DIO groups on control and high fat diets, respectively, for 5 weeks, and a DR group on a high fat diet for two weeks and control chow for three weeks prior to surgery. While the 11 week old normal chow and DR groups had a similar weight (28.1 g vs 27.4 g respectively, $P = 0.53$), 26 week old animals that had undergone DR still had substantially higher weights (37.7 g) than NC controls (31.0 g; $P = 0.0006$).

We first surveyed a host of mediators linked to adipose tissue homeostasis and the response to surgical trauma via quantitative RT-PCR (Supplemental Table 1) from adipose tissue samples obtained at baseline as a function of diet and age. Employing a statistical threshold of $p < 0.05$, dietary manipulations significantly impacted adipose tissue gene expression (Table 1). In 26 week old DIO animals (third data column), the pro-inflammatory and anorectic adipokine *Lep* was the most highly upregulated gene observed at baseline. Some additional proinflammatory markers such as *Tnf* and *Icam1* were also significantly upregulated, whereas others were significantly down-regulated (*Il1b*, *Il6*). Expression of the anti-inflammatory cytokine *Il10* and the alternatively activated macrophage marker *Mgl1* were down-regulated by DIO. Similar trends were seen for DIO in the 11 week old animals (Table 1, second data column), although the magnitude of the effects were smaller likely due to the shorter time period on the high fat diet (5 weeks vs. 20 weeks). Interestingly, there were a number of age-dependent changes observed on the control between young animals and middle-aged animals (Table 1, first data column), including a decrease in some proinflammatory markers (*Tnf*, *Icam1*, *Lep*) and an increase in anti-inflammatory markers (*Mgl1*, *Il10*).

Dietary restriction for 3 weeks pushed the adipose tissue phenotype back toward the normal chow baseline for 13/20 significant genes changed by the DIO diet at baseline in 26 week old mice, including *Lep*, *Il1b*, *Il6*, *Mgl1*, *Il10*, and *Mmps* (Table 1). The results were more profound still in young adult mice, in which 12/14 significant changes upon DIO mice were reversed by DR.

Impact of Diet and Surgical Trauma on Adipose Tissue Gene Expression

Each of the groups underwent a defined local trauma to subcutaneous adipose tissue designed to mimic a realistic surgical procedure. One day later, adipose tissue from the local surgical site and a remote site on the opposite flank were harvested. Quantitative RT-PCR revealed induction of all pro- and anti-inflammatory mediators examined except *Vcam1* and

Mg11 (Table 2) when preoperative adipose tissue from each animal was utilized as its own baseline. Adipose tissue derived hormones *Adipoq* and *Lep* were both generally down-regulated by surgical trauma. *Mmp2* was modestly down-regulated in younger animals, while the other matrix remodeling mediators *Tgfb1* and *Ctgf* were widely up-regulated. Full results are detailed in Supplemental Figures 1-4. While more subtle, both age groups did show a modest systemic response evidenced by alterations in gene expression at the remote tissue site. Adipose tissue from 26 week old DIO animals yielded an exaggerated pro-inflammatory response signature (increased *Il1b* and *Il6*, though relatively little impact on *Tnf*) compared to normal chow controls. Short-term DR also significantly attenuated the *Il1b* ($p = 0.002$) and *Il6* ($p = 0.015$) induction in this age cohort (Figure 1). Four other genes were significantly differentially regulated (*Adipoq*, *Mmp9*, *Tgfb1*, *Ctgf*) by DR vs. DIO, all in the direction of expression toward the corresponding normal chow group. Indeed, of all the genes tested, DR resulted in changes from DIO in the direction of the normal chow group in a large portion of the genes tested (Table 2, highlighted data cells). As a control for differences in baseline expression (Table 1), normalization of gene expression after surgical trauma to the corresponding NC group, yielded similar results.

In separate cohorts of animals, very low dose lipopolysaccharide (LPS) was applied to the surgical site to mimic low grade bacterial wound contamination. Low dose local LPS further potentiated gene perturbations beyond the surgical trauma itself in 11-week (*Ccl2* and *Icam1* up-regulation; *Cd68* and *Mg11* down-regulation), and 26-week (*Tnf*, *Il1b*, *Ccl2*, *Il6*, *Mmp9*, *Tgfb1*, and *Vcam1* up-regulation; *Mg11* down-regulation) animals. As discussed above, DR brought the expression patterns in response to surgical trauma back toward those seen in the normal chow setting for several key mediators (Table 2).

Global Adipose Tissue Gene Expression Analyses

Figure 2 depicts principle component analyses of the two age groups. While there were subtle differentials in Euclidean distances due to the dietary perturbations, the greatest determinant of position was presence or absence of surgical trauma. LPS incited specific gene expression signatures subtly different from those due to the surgical trauma alone, and these were most apparent in the older animals.

Impact of Diet and Surgical Trauma on Adipose Tissue Histology

In the 26 week old group at baseline, mice fed a high fat diet showed larger adipocytes with thinner interlobar septae. DR partially, but incompletely, restored normal architecture. Following surgical trauma, tissue from mice fed a normal diet exhibited prominent inflammation, edema, and fat necrosis. Surprisingly, these inflammatory changes were apparently significantly mitigated in the context of a DIO high fat diet, and dietary reversal prior to surgery only partially restored the baseline inflammatory phenotype. Exposure to LPS magnified the response to surgery in mice receiving a normal diet, but showed modest relative effect in mice that had been fed high fat with or without DR.

DISCUSSION

Here we used a mammalian model to analyze the effects of surgical trauma on adipose tissue gene expression and histology, and the ability of diet to modulate these phenotypes. In terms of global changes in gene expression, principle component analyses revealed that the surgical trauma itself contributed disproportionately to observed changes in gene expression. Nonetheless, different preoperative diet had significant effects. We found that increased adiposity induced by high fat feeding exacerbated transcriptional changes in adipose tissue subject to surgical trauma. Furthermore, we showed that these changes could be mitigated by three weeks of feeding on a low fat control diet prior to surgery, mimicking a mild

dietary restriction with potential clinical relevance. Note that the dietary perturbation was switching from a high fat diet to a normal chow in mice, and thus differs subtly than many of the traditional definitions of DR. Yet these data provide proof of principle that changes in adipose tissue induced by short-term dietary modifications may contribute to its ability to improve outcomes following surgical trauma. It is also acknowledged that practical issues may limit direct application of these interventions to the surgical patient. However, the findings support further investigation into these mechanisms so that realistic therapeutic strategies can be defined.

The response of organs to trauma such as surgery has undergone intensive interrogation for decades since a variety of clinically significant sequela hold clear links to this host response²². These sequela range from local reactions such as vascular restenosis to systemic multiple organ dysfunction and failure²³⁻²⁵. Humans typically have 20-30% body fat, and adipose tissue depots are directly traumatized in most surgical procedures, yet this organ's response to trauma has not previously been well characterized. This response is clearly relevant for soft tissue augmentation techniques such as fat grafting²⁶. Additionally, there is expanding recognition of adipose tissue-based signaling networks in a variety of clinically relevant pathophysiologies^{10-12, 27-30}.

Early observations linking adipose tissue phenotypes to the response to surgery have recently been made. Even minimal trauma such as catheter insertion has been reported to result in cytokine release from subcutaneous adipose tissue³¹. Gletsu *et al* studied the adipose tissue of normal and obese patients undergoing surgery and concluded that circulating *Il6* concentrations both at baseline and after operation are positively correlated to abdominal adipose tissue volume and are exaggerated in severely obese persons¹⁸.

A premise of our approach builds on the clinical observation that adipose tissue quality rather than quantity in part determines its impact on human health. Key surgical outcomes have not consistently correlated with simple obesity³², and there is increasing recognition of metabolically healthy¹⁴ and unhealthy adipose tissue^{33, 34}. By testing two age groups and cohorts of animals that consumed a high fat "Western" diet with or without mild, short-term DR in comparison to a low-fat control diet, we were able to interrogate the response of varying baseline adipose tissue phenotypes in a homogenous genetic background. At baseline, the proinflammatory phenotype that we observed associated with the DIO mouse model correlated with previous cell sorting studies of these animals³⁵. These animals were generally "primed" for a hyper-acute response to the surgical trauma.

We explored the effect of short-term DR, an emerging strategy to attenuate the hyper-acute response to ischemia reperfusion injury associated with surgical trauma^{3, 5, 36}. Although defined as reduced food intake with adequate nutrition, experimental DR regimens vary widely in terms of dietary composition, temporal aspects of food consumption, duration of restriction, and percentage calorie or nutrient restriction. Most DR experiments are performed on lean, young adult animals; restriction of total food intake in the range of 30-40% is typically calculated from the amount eaten by ad libitum fed control animals³⁷. It is much more challenging to define caloric content and dietary composition of elective surgery candidates, who are often obese individuals on relatively high calorie/high fat diets. It is also not clear if calculating the percent restriction in this context should be based on current dietary intake or that normalized to a corresponding lean individual. Thinking ahead toward clinical translation, we chose a mild restriction consisting simply of a return to a low-fat diet for the period of three weeks in a DIO model. Importantly, this non-traditional pre-operative dietary restriction showed modest reversibility of the DIO induced changes, particularly in the young adult group. Histological assessment of adipose tissue also indicated the ability of short-term DR to partially restore the normal architecture. However,

more research is needed to define temporal aspects of DR and particular dietary components (e.g. total calories and weight change, change in fat content) that are important for this effect, and the biologic mediators.

Interestingly, increased age was generally associated with an attenuated inflammatory gene response to surgical trauma (less *Il1b*, *Ccl2*, *Il6*). This is paradoxical in the sense that aging is typically associated with an overall increase in inflammatory processes. The answer to this apparent paradox may lie in the difference between chronic, steady-state inflammation, which increases with age, and the ability of the innate immune system to mount an acute inflammatory response, which declines with age. The importance of the latter is emphasized by the heightened susceptibility of younger patients to morbidity/mortality associated with acute inflammatory reactions such as those associated with septic shock. Here, DR attenuated preoperative and postoperative DIO-induced changes in gene expression in both age groups, although DR effects at baseline were stronger in the young adult group.

The effect of DR on adipose tissue histology following surgical trauma was similarly paradoxical. Despite evidence of increased inflammation in the adipose tissue of the DIO model after surgical trauma at the level of gene expression, DIO appeared to protect adipose tissue from infiltration of leukocytes, edema and necrosis following surgical trauma on a histological level, an apparently protective effect that was mitigated by DR. One possible explanation for this is that the infiltrating leukocytes are qualitatively different between control and DR groups, promoting inflammation in the former while helping to suppress it in the latter. In this context, leukocytes such as macrophages may serve the beneficial function in traumatized tissue of clearing out cellular debris and thus preventing further activation of the immune system. Another possibility is that increased adiposity can be beneficial in some cases. Consistent with this possibility, increased adiposity is associated with better outcome in some surgical scenarios such as carotid endarterectomy³⁸. Future studies will be required to resolve these paradoxical findings.

We also modeled the impact of a low grade bacterial wound contamination. For complex surgical procedures, low grade wound bacterial contamination rates may be as high as 80%³⁹. Even beyond the dramatic gene perturbations observed with trauma, LPS administration was associated with even further inflammatory mediator up-regulation (in both normal chow and DIO animals). LPS did have a clear down-regulatory effect on *Mgl1*.

Due to its plasticity and its ability to modulate inflammatory status by secretion of pro- and anti-inflammatory adipokines including *lep* and *adipoq*, adipose tissue stands as a prime interventional target^{11, 40, 41}. Diet-induced changes in quantitative and qualitative aspects of adipose tissue phenotype occur rapidly in the context of reduced calorie intake, for example during fasting or DR. Although primarily thought of as a depot for energy storage, adipose tissue changes similar to DR can also occur in the absence of reduced calorie intake, for example in the context of a protein or essential amino acid deficient diet². Furthermore, adipose tissue phenotypes can be modulated by pharmaceutical compounds in the absence of dietary interventions. Ikeoka *et al* infused an intravenous lipid-heparin compound to increase nonesterified fatty acids in humans, leading to adipose tissue cytokine production⁴². The multitude of emerging pharmacologic compounds that impact adipose tissue biology might have a role in altering the mammalian response to trauma⁴¹.

Despite rigorous adherence to standardized animal acquisition, care, anesthetic, and tissue harvest, four of the baseline samples for the LPS normal chow cohort were outliers when examined via principle component analysis (Figure 2). These results are included for transparency regarding the potential for variation in our approach, a reality likely to be even more relevant in a genetically and environmentally heterogeneous human patient population.

Despite this subtle baseline phenotypic differential, however, the response to surgical trauma and LPS administration was similar to the other LPS treated animals.

Other limitations to the data presented are acknowledged. The relatively minor trauma inflicted in this mouse model may not well represent the variety of human surgical interventions since many procedures expose adipose tissue for several hours. Only an early time point was examined, and over time there may be important differential adaptations to the trauma among various conditions both in terms of gene expression and histology. However, we designed the experiments to assay short-term RNA dynamics at a time point when the biologic and clinical response to trauma tends to be high. Specific cellular mediators and confirmatory protein quantifications are not offered, but the intention was to broadly yet accurately portray the impact of clinically relevant conditions on peri-operative adipose tissue phenotypic signatures. We employed LPS rather than live bacteria to better control conditions for modeling wound contamination at the time of surgery; organisms such as Gram positive cocci would likely incite other host mediators. Finally, the influence of numerous clinically relevant scenarios such as diabetes is not considered, but description of this investigative approach and the fundamental dynamics herein should accelerate such work in a murine platform. As is frequently the case with mouse models, reconciliation of rodent nutrition and lifespan with the human condition is challenging, but this mouse model at least offers a tool to dissect common mammalian biologic mechanisms.

As clinical consequences are increasingly linked to adipose tissue driven signaling, the current results point to potential approaches to alter the outcomes of elective surgical procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1. Selected adipose tissue gene expression patterns in 26-week-old mice under 3 different dietary conditions and subjected to surgical trauma. Fold induction is presented relative to the baseline value of the corresponding individual animals in that group.

Fig 2.

Principle component analysis of the assayed genes for the young and old age groups. Red = normal chow, Blue = diet induced obesity, Green = dietary reversal; spheres represent animals baseline (Day 0) and after surgical trauma (Day 1); squares represent animals receiving LPS in addition to the surgical trauma. While the dietary perturbations and LPS modestly impacted global gene expression, the surgical trauma itself dominated, in both young and old mice.

TABLE 1

Fold Induction by Age and Dietary Manipulations for Baseline Adipose Tissue

Gene	NC 26-week vs. NC 11- week	DIO 11-week vs. NC 11- week	DIO 26-week vs. NC 26- week	DR 11-week vs. DIO 11- week	DR 26-week vs. DIO 26- week
<u>Proinflammatory</u>					
<i>Tnf</i>	-1.9		2.4	-1.4	1.5
<i>Il1b</i>	1.9		-3.9	1.9	3.3
<i>Ccl2</i>		2.1		-1.9	
<i>Il6</i>	1.8		-3.3		2.1
<i>Icam1</i>	-5.6	-1.1	4.5		
<i>Vcam1</i>				1.3	
<i>Cd68</i>	1.4		-1.4		1.6
<i>Mgl1</i>	1.7	-1.4	-2.9	1.6	1.9
<u>Anti-Inflammatory</u>					
<i>Il10</i>	1.4	1.5	-2.4	1.6	1.6
<u>Adipose Derived Hormones</u>					
<i>Adipoq</i>	-3.9		3.1		
<i>Lep</i>	-11.4	3.5	50.0	-4.7	-4.1
<u>Matrix Remodeling</u>					
<i>Mmp2</i>	1.5	-1.5	-2.7	1.7	2.0
<i>Mmp9</i>		-4.3	-10.3	2.8	6.0
<i>Tgfb1</i>	-5.2		4.1		
<i>Ctgf</i>		1.6	2.7	-1.5	-2.2
<u>Pathogen Recognition, Activation of Innate Immunity</u>					
<i>Tlr4</i>	-3.7		2.5	1.2	
<u>Angiogenesis</u>					
<i>Pgf</i>	-1.4	1.9	3.1	-1.9	
<i>Fit1</i>	-5.0		5.0		-1.9
<u>Aldosterone Signaling</u>					
<i>Nr3c2</i>	-6.9		5.7		
<i>Hsd11b2</i>		-1.4	-1.3	1.3	

Gene	NC 26-week vs. 11-week	DIO 11-week vs. NC 11- week	DIO 26-week vs. NC 26- week	DR 11-week vs. DIO 11- week	DR 26-week vs. DIO 26- week
<i>Agtr1a</i>	-3.7	-1.4	2.5	1.3	1.2
<i>Agtr1b</i>	-3.6	-1.9		1.9	
<i>Ace</i>	-3.9	-1.7		1.7	
<i>Cyp11b2</i>	-1.4				
<i>Sgkl</i>		-1.2	3.6	1.2	-1.3

Significance: $P < 0.05$; blank indicates lack of statistical significance

TABLE 2

Fold Induction of Adipose Tissue Gene Expression at Surgical Trauma Site

Gene	11 Week				26 Week				11 Week + LPS				26 Week + LPS			
	NC	DIO	DR	NC	DIO	DR	NC	DIO	NC	DIO	DR	NC	DIO	DR	NC	DR
<u>Proinflammatory</u>																
<i>Tnf</i>	23.8	7.2	20.1	11.9	14.4	8.1	103.5	107.1	114.6	290.3	43.8	48.2	290.3	43.8	48.2	48.2
<i>Il1b</i>	278.7	192.7	125.5	35.1	1177.6	79.2	1225.2	2261.0	1265.9	798.2	1406.7	1183.5	798.2	1406.7	1183.5	1183.5
<i>Ccl2</i>	47.2	13.9	33.8	24.9	35.7	20.3	99.0	80.5	133.7	107.4	39.3	51.1	107.4	39.3	51.1	51.1
<i>Il6</i>	26.4	56.2	44.3	20.9	551.9	34.4	228.3	448.8	331.4	69.9	250.1	208.0	69.9	250.1	208.0	208.0
<i>Icam1</i>	2.8	2.5	2.6	4.3	2.7	3.0	7.0	9.6	5.9	82.4	8.3	6.7	82.4	8.3	6.7	6.7
<i>Vcam1</i>																
<i>Cd68</i>	5.1	3.3	2.7	2.4	2.8	2.6	2.1	2.3	3.3	2.5	2.7	2.1	2.5	2.7	2.2	2.1
<i>Mgl1</i>																
							-16.6	-15.4	-12.7	-37.6	-10.3	-23.4	-37.6	-10.3	-23.4	-23.4
<u>Anti-Inflammatory</u>																
<i>Il10</i>	5.4	6.1	3.0	3.0	4.5	6.3	4.8	6.7	5.8	3.2	5.1	3.8	3.2	5.1	3.2	3.8
<u>Adipose Derived Hormones</u>																
<i>Adipoq</i>	-3.8	-2.4	-3.4	-2.6		-2.6	-5.2	-4.4	-6.7		-2.8	-4.1		-2.8		-4.1
<i>Lep</i>	-4.1	-3.8	-3.2				-7.3	-6.8	-5.4	3.2	-4.5		3.2	-4.5		
<u>Matrix Remodeling</u>																
<i>Mmp2</i>	-2.4		-2.4	-2.2												
<i>Mmp9</i>	2.0				18.7		18.4	93.8	17.0	5.9	22.9	9.1	5.9	22.9	5.9	9.1
<i>Tgfb1</i>	2.8	2.4	2.4	3.6	2.2	3.0		2.4	3.2	21.9			21.9			
<i>Ctgf</i>	5.3	4.9	7.5	6.8	3.2	5.0	3.5	3.0	3.1	4.0	2.3	3.9	4.0	2.3	3.9	3.9
<u>Pathogen Recognition, Activation of Innate Immunity</u>																
<i>Tlr4</i>	2.2	2.1		2.5	2.2	2.2			2.5	12.1						
<u>Angiogenesis</u>																
<i>Pgf</i>			2.0	3.1			5.0	3.6	3.0	4.1	2.4	4.3	4.1	2.4	4.1	4.3
<i>Fhl1</i>										4.2	-2.2		4.2	-2.2		
<u>Aldosterone Signaling</u>																
<i>Nr3c2</i>	-3.1		-2.8				-4.2	-4.1	-3.6	3.8	-4.4	-5.0	3.8	-4.4	3.8	-5.0
<i>Agtr1a</i>										5.0			5.0			
<i>Agtr1b</i>	-4.0		-4.1				-2.3									

Gene	11 Week			26 Week			11 Week + LPS			26 Week + LPS		
	NC	DIO	DR	NC	DIO	DR	NC	DIO	DR	NC	DIO	DR
<i>Ace</i>	-3.5		-3.1		-1.9		-3.3	-2.1	-2.6	2.8		-2.8
<i>Cyp11b2</i>									2.2			
<i>Sgk1</i>										5.2		
<u>Oxygen Sensing</u>												
<i>Nox4</i>							-2.2		-2.1			

Fold changes expressed relative to Day 0 baseline for each individual animal; Significance: $p < 0.05$; blank indicates lack of statistical significance; grey highlight indicates change in direction of gene expression toward the NC level by DR.