# Preoperative diet impacts the adipose tissue response to surgical trauma

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***Background.*** *Short-term changes in preoperative nutrition can have profound effects on surgery-related outcomes such as ischemia/reperfusion injury in preclinical models. Dietary interventions that lend protection against stress in animal models (eg, fasting, dietary restriction [DR]) impact adipose tissue quality/quantity. Adipose tissue holds high surgical relevance because of its anatomic location and large 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 preoperative 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], preoperative DR) were modulated before 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 preoperative DR tended to reverse these changes. Age and lipopolysaccharide (LPS)-simulated bacterial contamination also impacted the adipose tissue response to trauma, with young adult animals and LPS treatment exacerbating the proinflammatory response.*

***Conclusion.*** *Surgical trauma dramatically impacts both local and distal adipose tissue biology. Short-term preoperative DR may offer a strategy to attenuate this response. (Surgery 2013;153:584-93.)*

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Dietary restriction (DR), or reduced food intake without malnutrition, is best known for lifespan extension in a variety of experimental organisms,[1] 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 operative ischemia–reperfusion injury.[2-8] DR thus holds potential as a clinically relevant strategy to alter the mammalian response to acute stress such as surgical trauma.[2-9]

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 networks.[10-13] Although obesity broadly correlates with metabolic and cardiovascular disorders, qualitative adipose tissue factors seem to be important determinants of health and disease beyond simple body mass index.[14-16] Dietary intake serves as a key determinant of adipose tissue quantity and quality in humans.[16] Importantly, the plasticity of adipose tissue in response to food intake makes it a prime potential mechanistic vehicle for dietary effects.[11,17]

Owing to anatomic proximity and relatively large tissue volume, adipose tissue is ubiquitously traumatized in operative procedures. Links between adipose tissue biology and clinically relevant surgical outcomes are emerging.[18] For instance, exacerbated adipose tissue interleukin-6 release in obese surgical patients correlates with

Binh Nguyen and Ming Tao contributed equally to this work.

Supported by the National Heart, Lung, and Blood Institute (T32HL007734), American Heart Association (12GRNT9510001), and the Carl and Ruth Shapiro Family Foundation. Y.E.W. is supported through the CCCB and the Dana-Farber Strategic Plan Initiative.

Accepted for publication November 1, 2012.

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0039-6060/$ - see front matter



http://dx.doi.org/10.1016/j.surg.2012.11.001

perioperative insulin resistance.[18] However, 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 operative trauma to adipose tissue, we sought to understand the impact of preoperative diet on the response to surgery and its modulation by clinically relevant variables, including age and bacterial wound contamination. We found that surgical trauma upregulates markers of inflammation, matrix remodeling, and angiogenesis, and that preoperative diet modulates this response.

## MATERIALS AND METHODS

**Murine surgical trauma model, local and distant adipose tissue collection.** Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were maintained on a 12-hour light–dark cycle for ≥1 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, Jena, Germany). A 2 × 1-cm L-shaped incision was made on the left flank of the animal. After retracting the skin, a 5 × 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 × 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 10 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, mice were again anesthetized. Adipose tissue was first harvested from the right flank of the animal after the same protocol outlined, representing harvest from a remote adipose tissue site. The left flank operative site was then reopened 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, Indianapolis, IN), a 60 kcal% fat diet (diet-induced obesity [DIO]; D12492; Research Diets Inc), or a high-fat diet switched to NC (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 operative trauma under various conditions.

*Mimicry of local wound infection.* Polysaccharide (LPS; 5 μg in 40 μL 40% w/v pluronic gel) was placed in the traumatized surgical field immediately before closure.

**Tissue analyses.** Total RNA was isolated from fresh frozen adipose tissue (RNeasy Mini Kit; Qiagen, Hilden, Germany), quantified with NanoDrop-1000 (Thermo Scientific, Waltham, MA), and qualified via Agilent 2100 Bioanalyzer (total RNA nanochip; Agilent, Santa Clara, CA). Quantitative real-time polymerase chain reaction (PCR; $RT^2$ qPCR Primer Assay, SYBR Green, SABiosciences, Frederick, MD) was completed for selected mediators (Supplemental Table I). The real-time PCR assays for the selected genes were performed on a 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) by using 400 nmol/L of forward and reverse primers, a 10-ng cDNA sample, and $RT^2$ qPCR SYBR Green/ROX Master Mix (Qiagen) in a 25-μL reaction volume. Each target gene was simultaneously run with housekeeping genes (Hsp90ab1, Hprt1, and 18SrRNA) on all investigated specimens. The comparative computed tomography method was used for experimental setup and data analysis. To minimize variations in sample loading and make comparisons 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 owing 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 trichrome.

**Statistical analyses.** Each of the 12 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 the mean values ± standard error of the mean. Clinically meaningful comparisons were then completed for specific scenarios, and gene expression identified as up- or downregulated if $P < .05$. Differential expression was determined using the LIMMA package.[19] All $P$ values reported were corrected for multiple comparisons testing using Benjamini and Hochberg correction.[20] We also performed multivariate analyses using a $P < .01$. Principle component analysis was performed across all selected genes, and Euclidean distances between all sample pairs were calculated using the first 3 principle components. All statistical and clustering analyses were performed using R and Bioconductor.[21]

## RESULTS

**Effect of high-fat diet (DIO) and short-term preoperative DR 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 6 weeks until surgery at 26 weeks of age (middle-aged mice). Animals on a purified NC 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 NC 3 weeks before surgery (DR group). Although allowed ad libitum access to control chow, these animals lost weight (15.7% ± 4.5%) relative to the DIO group over the 3-week preoperative period. To evaluate the effects of age, the same 3 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 2 weeks and control chow for 3 weeks before surgery. Whereas the 11-week-old NC and DR groups had a similar weight (28.1 vs 27.4 g, respectively; $P = .53$), 26-week-old animals that had undergone DR still had substantially higher weights (37.7 g) than NC controls (31.0 g; $P = .0006$).

We first surveyed a host of mediators linked to adipose tissue homeostasis and the response to surgical trauma via quantitative reverse transcriptase-PCR (Supplemental Table I) from adipose tissue samples obtained at baseline as a function of diet and age. Employing a statistical threshold of $P < .05$, dietary manipulations significantly impacted adipose tissue gene expression (Table I). In 26-week-old DIO animals (third data column), the proinflammatory 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 downregulated (*Il1b, Il6*). Expression of the anti-inflammatory cytokine *Il10* and the alternatively activated macrophage marker *Mgl1* were downregulated by DIO. Similar trends were seen for DIO in the 11-week-old animals (Table I, second data column), although the magnitude of the effects were smaller likely owing to the shorter time period on the high-fat diet (5 vs 20 weeks). Interestingly, there were a number of age-dependent changes observed on the control between young and middle-aged animals (Table I, first data column), including a decrease in some proinflammatory markers (*Tnf, Icam1, Lep*) and an increase in anti-inflammatory markers (*Mgl1, Il10*).

DR for 3 weeks pushed the adipose tissue phenotype back toward the NC baseline for 13 of 20 significant genes changed by the DIO diet at baseline in 26-week-old mice, including *Lep, Il1b, Il6, Mgl1, Il10,* and *Mmps* (Table I). The results were more profound still in young adult mice, in which 12 of 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 operative procedure. One day later, adipose tissue from the local surgical site and a remote site on the opposite flank were harvested. Quantitative reverse transcriptase-PCR revealed induction of all pro- and anti-inflammatory mediators examined except *Vcam1* and *Mgl1* (Table II) when preoperative adipose tissue from each animal was utilized as its own baseline. Adipose tissue–derived hormones *Adipoq* and *Lep* were both generally downregulated by operative trauma. *Mmp2* was modestly downregulated in younger animals, whereas the other matrix remodeling mediators *Tgfb1* and *Ctgf* were widely upregulated. Full results are detailed in Supplemental Figs 1–4. Although more subtle, both age groups did show a modest systemic response evidenced by alterations in gene expression at the remote tissue site (Supplemental Table II). Adipose tissue from 26-week-old DIO animals yielded an exaggerated proinflammatory response signature (increased *Il1b* and

**Table I.** Fold induction by age and dietary manipulations for baseline adipose tissue

| *Gene* | *NC 26 vs 11 weeks* | *DIO 11 weeks vs NC 11 weeks* | *DIO 26 weeks vs NC 26 weeks* | *DR 11 weeks vs DIO 11 weeks* | *DR 26 weeks vs DIO 26 weeks* |
|---|---|---|---|---|---|
| Proinflammatory | | | | | |
| *Tnf* | −1.9 | — | 2.4 | −1.4 | 1.5 |
| *Il1b* | 1.9 | — | −3.9 | 1.9 | 3.3 |
| *Ccl2* | | 2.1 | — | −1.9 | — |
| *Il6* | 1.8 | — | −3.3 | — | 2.1 |
| *Icam1* | −5.6 | −1.1 | 4.5 | — | — |
| *Vcam1* | — | — | — | 1.3 | — |
| *Cd68* | 1.4 | — | −1.4 | — | 1.6 |
| *Mgl1* | 1.7 | −1.4 | −2.9 | 1.6 | 1.9 |
| Anti-inflammatory | | | | | |
| *Il10* | 1.4 | 1.5 | −2.4 | 1.6 | 1.6 |
| Adipose-derived hormones | | | | | |
| *Adipoq* | −3.9 | — | 3.1 | — | — |
| *Lep* | −11.4 | 3.5 | 50.0 | −4.7 | −4.1 |
| Matrix remodeling | | | | | |
| *Mmp2* | 1.5 | −1.5 | −2.7 | 1.7 | 2.0 |
| *Mmp9* | — | −4.3 | −10.3 | 2.8 | 6.0 |
| *Tgfb1* | −5.2 | — | 4.1 | — | — |
| *Ctgf* | — | 1.6 | 2.7 | −1.5 | −2.2 |
| Pathogen recognition, activation of innate immunity | | | | | |
| *Tlr4* | −3.7 | — | 2.5 | 1.2 | — |
| Angiogenesis | | | | | |
| *Pgf* | −1.4 | 1.9 | 3.1 | −1.9 | — |
| *Flt1* | −5.0 | — | 5.0 | — | −1.9 |
| Aldosterone signaling | | | | | |
| *Nr3c2* | −6.9 | — | 5.7 | — | — |
| *Hsd11b2* | — | −1.4 | −1.3 | 1.3 | — |
| *Agtr1a* | −3.7 | −1.4 | 2.5 | 1.3 | 1.2 |
| *Agtr1b* | −3.6 | −1.9 | — | 1.9 | — |
| *Ace* | −3.9 | −1.7 | — | 1.7 | — |
| *Cyp11b2* | −1.4 | — | — | — | — |
| *Sgk1* | — | −1.2 | 3.6 | 1.2 | −1.3 |

$P < .05$; — indicates lack of statistical significance.
*DIO*, Diet-induced obesity; *NC*, normal chow.

*Il6*, although relatively little impact on *Tnf*) compared with NC controls. Short-term DR also significantly attenuated the *Il1b* ($P = .002$) and *Il6* ($P = .015$) induction in this age cohort (Fig 1). Four other genes were significantly differentially regulated (*Adipoq, Mmp9, Tgfb1, Ctgf*) by DR versus DIO, all in the direction of expression toward the corresponding NC group. Indeed, of all the genes tested, DR resulted in changes from DIO in the direction of the NC group in a large portion of the genes tested (Table II, * indicated data cells). As a control for differences in baseline expression (Table I), normalization of gene expression after surgical trauma to the corresponding NC group yielded similar results (Supplemental Fig 5).

In separate cohorts of animals, very low-dose 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-old (*Ccl2* and *Icam1* upregulation; *Cd68* and *Mgl1* downregulation), and 26-week-old (*Tnf, Il1b, Ccl2, Il6, Mmp9, Tgfb1*, and *Vcam1* upregulation; *Mgl1* downregulation) animals. As discussed, DR brought the expression patterns in response to surgical trauma back toward those seen in the NC setting for several key mediators (Table II).

**Global adipose tissue gene expression analyses.** Figure 2 depicts principle component analyses of the two age groups. Although there were subtle differentials in Euclidean distances owing to the dietary perturbations, the greatest determinant of position was presence or absence of surgical trauma. LPS incited specific gene expression

**Table II.** Fold induction of adipose tissue gene expression at surgical trauma site

| | *11 weeks* | | | *26 weeks* | | | *11 weeks + LPS* | | | *26 weeks + LPS* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Gene* | *NC* | *DIO* | *DR* | *NC* | *DIO* | *DR* | *NC* | *DIO* | *DR* | *NC* | *DIO* | *DR* |
| Proinflammatory | | | | | | | | | | | | |
| *Tnf* | 23.8 | 7.2 | 20.1* | 11.9 | 14.4 | 8.1* | 103.5 | 107.1 | 114.6 | 290.3 | 43.8 | 48.2* |
| *Il1b* | 278.7 | 192.7 | 125.5 | 35.1 | 1177.6 | 79.2* | 1,225.2 | 2,261.0 | 1,265.9* | 798.2 | 1,406.7 | 1,183.5* |
| *Ccl2* | 47.2 | 13.9 | 33.8* | 24.9 | 35.7 | 20.3* | 99.0 | 80.5 | 133.7* | 107.4 | 39.3 | 51.1* |
| *Il6* | 26.4 | 56.2 | 44.3* | 20.9 | 551.9 | 34.4* | 228.3 | 448.8 | 331.4* | 69.9 | 250.1 | 208.0* |
| *Icam1* | 2.8 | 2.5 | 2.6* | 4.3 | 2.7 | 3.0* | 7.0 | 9.6 | 5.9 | 82.4 | 8.3 | 6.7 |
| *Vcam1* | — | — | — | — | — | — | — | — | — | — | 2.2 | 2.1 |
| *Cd68* | 5.1 | 3.3 | 2.7 | 2.4 | 2.8 | 2.6* | 2.1 | 2.3 | 3.3 | 2.5 | 2.7 | |
| *Mgl1* | — | — | — | — | — | — | −16.6 | −15.4 | −12.7 | −37.6 | −10.3 | −23.4* |
| Anti-inflammatory | | | | | | | | | | | | |
| *Il10* | 5.4 | 6.1 | 3.0 | 3.0 | 4.5 | 6.3 | 4.8 | 6.7 | 5.8* | 3.2 | 5.1 | 3.8* |
| Adipose-derived hormones | | | | | | | | | | | | |
| *Adipoq* | −3.8 | −2.4 | −3.4* | −2.6 | — | −2.6 | −5.2 | −4.4 | −6.7* | — | −2.8 | −4.1 |
| *Lep* | −4.1 | −3.8 | −3.2 | — | — | — | −7.3 | −6.8 | −5.4 | 3.2 | −4.5 | — |
| Matrix remodeling | | | | | | | | | | | | |
| *Mmp2* | −2.4 | — | −2.4 | −2.2 | — | — | — | — | — | — | — | — |
| *Mmp9* | — | 2.0 | — | — | 18.7 | — | 18.4 | 93.8 | 17.0* | 5.9 | 22.9 | 9.1* |
| *Tgfb1* | 2.8 | 2.4 | 2.4 | 3.6 | 2.2 | 3.0* | — | 2.4 | 3.2 | 21.9 | — | — |
| *Ctgf* | 5.3 | 4.9 | 7.5 | 6.8 | 3.2 | 5.0* | 3.5 | 3.0 | 3.1* | 4.0 | 2.3 | 3.9* |
| Pathogen recognition, activation of innate immunity | | | | | | | | | | | | |
| *Tlr4* | 2.2 | 2.1 | — | 2.5 | 2.2 | 2.2 | — | — | 2.5 | 12.1 | — | — |
| Angiogenesis | | | | | | | | | | | | |
| *Pgf* | — | — | 2.0 | 3.1 | — | — | 5.0 | 3.6 | 3.0 | 4.1 | 2.4 | 4.3* |
| *Flt1* | — | — | — | — | — | — | — | — | — | 4.2 | −2.2 | — |
| Aldosterone signaling | | | | | | | | | | | | |
| *Nr3c2* | −3.1 | — | −2.8 | — | — | — | −4.2 | −4.1 | −3.6 | 3.8 | −4.4 | −5.0 |
| *Agtr1a* | — | — | — | — | — | — | — | — | — | 5.0 | — | — |
| *Agtr1b* | −4.0 | — | −4.1 | — | — | — | −2.3 | — | — | | — | — |
| *Ace* | −3.5 | — | −3.1 | — | −1.9 | — | −3.3 | −2.1 | −2.6* | 2.8 | — | −2.8 |
| *Cyp11b2* | — | — | — | — | — | — | — | — | 2.2 | — | — | — |
| *Sgk1* | — | — | — | — | — | — | — | — | — | 5.2 | — | — |
| Oxygen sensing | | | | | | | | | | | | |
| *Nox4* | — | — | — | — | — | — | −2.2 | −2.1 | — | — | — | — |

*Change in direction of gene expression toward the NC level induced by DR.
Fold changes expressed relative to day 0 baseline for each individual animal. Significance: $P < .05$; — indicates lack of statistical significance.
*DIO*, Diet-induced obesity; *LPS*, lipopolysaccharide; *NC*, normal chow.

signatures subtly different from those owing 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. After surgical trauma, tissue from mice fed a normal diet exhibited prominent inflammation, edema, and fat necrosis. Surprisingly, these inflammatory changes were significantly mitigated in the context of a DIO high-fat diet, and dietary reversal before surgery only partially restored the baseline inflammatory phenotype. Exposure to LPS magnified the response to operative intervention in mice fed a normal diet, but showed modest relative effect in mice that had been fed a high-fat diet with or without DR.

## DISCUSSION

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

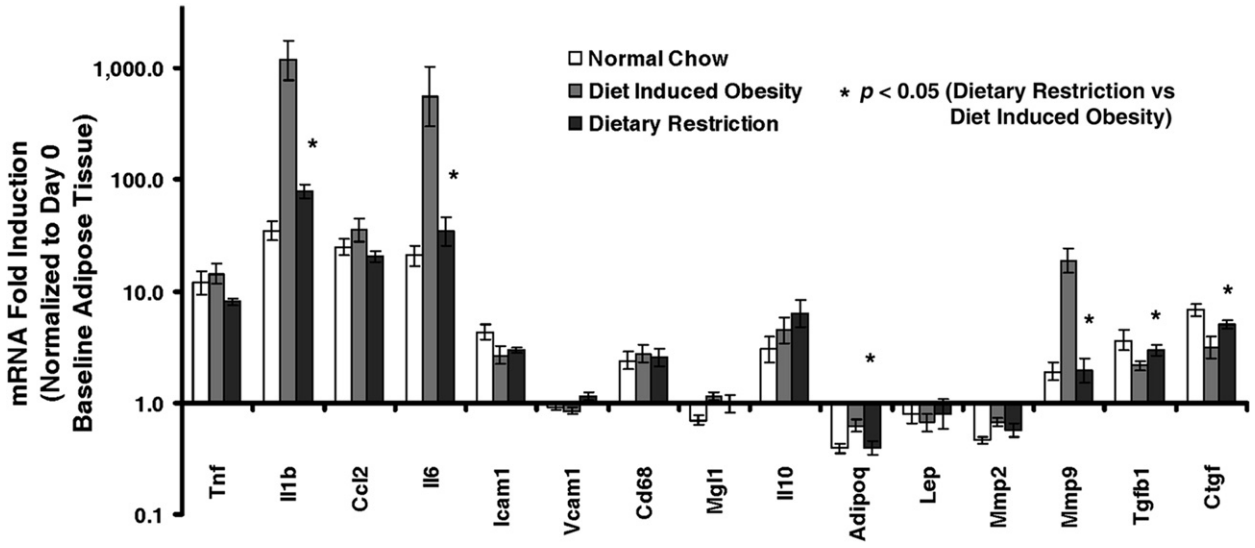


**Fig 1.** Selected adipose tissue gene expression patterns in 26-week-old mice under 3 different dietary conditions and subjected to operative trauma. Fold induction is presented relative to the baseline value of the corresponding individual animals in that group.

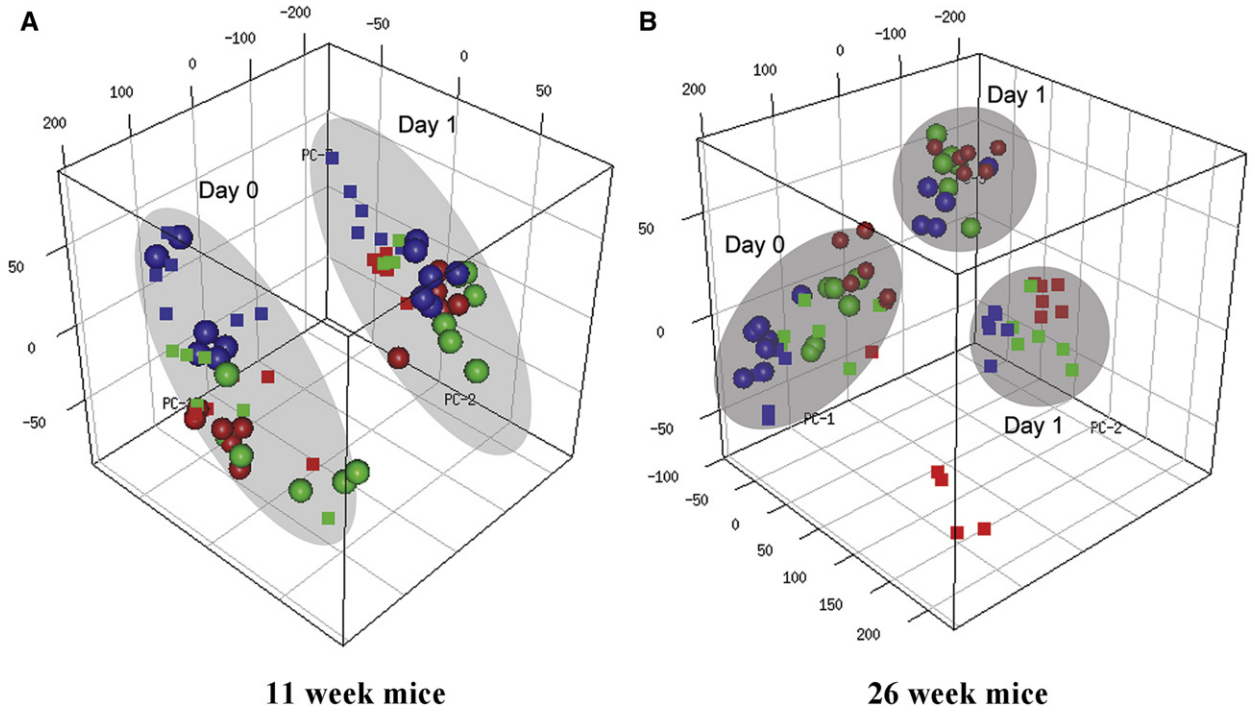


**Fig 2.** Principle component analysis of the assayed genes for the young and old age groups. *Red* = normal chow; *blue* = diet-induced obesity; *green* = dietary reversal; *spheres* = animals baseline (day 0) and after operative trauma (day 1); *squares* = animals receiving LPS in addition to the operative trauma. Although the dietary perturbations and LPS modestly impacted global gene expression, the operative trauma itself dominated, in both young and old mice. *PC-1*, *PC-2*, and *PC-3* represent the first, second, and third principle components, correspondently.

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 3 weeks of feeding on a low-fat control diet before surgery, mimicking a mild DR with potential clinical relevance. Note that the dietary perturbation was switching from a high-fat diet to a NC in mice,

and thus differs subtly from many of the traditional definitions of DR. However, these data provide proof of principle that changes in adipose tissue induced by short-term dietary modification may contribute to its ability to improve outcomes after operative 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 because a variety of clinically significant sequelae hold clear links to this host response.[22] These sequelae range from local reactions such as vascular restenosis to systemic multiple organ dysfunction and failure.[23-25] Humans typically have 20–30% body fat, and adipose tissue depots are directly traumatized in most operative 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 grafting.[26] 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 been made. Even minimal trauma such as catheter insertion has been reported to result in cytokine release from subcutaneous adipose tissue.[31] Gletsu et al[18] 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 persons.[18]

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 obesity,[32] and there is increasing recognition of metabolically healthy[14] 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 with a low-fat control diet, we were able to analyze 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 animals.[35] These animals were generally "primed" for a hyperacute response to the operative trauma.

We explored the effect of short-term DR, an emerging strategy to attenuate the hyperacute 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 of caloric 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 animals.[37] It is much more challenging to define caloric content and dietary composition of elective surgery candidates, who are often obese individuals ingesting 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 3 weeks in a DIO model. Importantly, this nontraditional preoperative DR showed modest reversibility of the DIO-induced changes, particularly in the young adult group. Histologic 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 (eg, total calories and weight change, change in fat content) that are important for this effect, and the biologic mediators.

Interestingly, older age was generally associated with an attenuated inflammatory gene response to operative 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 after surgical trauma was similarly paradoxical. Despite evidence of increased inflammation in the adipose tissue of the DIO model after operative trauma at the level of gene expression, DIO seemed to protect adipose tissue from infiltration of leukocytes, edema, and necrosis after operative trauma on a histologic 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 outcomes in some surgical scenarios, such as carotid endarterectomy.[38] Future studies are required to resolve these paradoxical findings.

We also modeled the impact of a low-grade bacterial wound contamination. For complex operative 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 upregulation (in both NC and DIO animals). LPS did have a clear downregulatory effect on *Mgl1*.

Owing 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 diet.[2] Furthermore, adipose tissue phenotypes can be modulated by pharmaceutical compounds in the absence of dietary interventions. Ikeoka et al[42] infused an intravenous lipid-heparin compound to increase nonesterified fatty acids in humans, leading to adipose tissue cytokine production. The multitude of emerging pharmacologic compounds that impact adipose tissue biology might have a role in altering the mammalian response to trauma.[41]

Despite rigorous adherence to standardized animal acquisition, care, anesthetic, and tissue harvest, four of the baseline samples for the LPS NC cohort were outliers when examined via principle component analysis (Fig 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 operative 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 represent well the variety of human operative interventions, because 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 perioperative 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 operative procedures.

The authors appreciate the technical assistance of Godfrey Ilonzo, Shuai Hao, Tianyu Jiang, and Ian Gao for care of animals and surgery assistance.

## CONFLICT OF INTEREST

James Mitchell has a provisional patent on dietary strategies to protect against ischemia reperfusion injury, and has been a consultant for L-Nutra, a company that develops medical food to fight disease, including cancer.

## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at 10.1016/j.surg.2012.11.001.

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