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Increased adipose tissue hypoxia and capacity for angiogenesis and inflammation in young diet-sensitive C57 mice compared to diet-resistant FVB mice

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Abstract

Objective—High-fat diets result in increased body weight. However, this is not uniform and determining the factors that make some animals or individual more susceptible to this diet-induced weight gain is a critical research question. The expansion of white adipose tissue (WAT) associated with weight gain requires high rates of angiogenesis to support the expanding tissue mass. We hypothesized that diet-induced obese (DIO) mice have a greater capacity for WAT angiogenesis and remodeling than diet-resistant (DR) mice at a young age, prior to age or diet-induced obesity.

Design—We measured body weight and body composition by NMR. We compared the expression of genes related to lipid metabolism, angiogenesis and inflammation by RT-qPCR and PCR arrays. WAT morphology and distribution of adipocyte size were analyzed. The level of hypoxia and vascular density was assessed by immunohistochemistry in WAT of young mice.

Results—C57Bl/6 mice were DIO and FVB/N mice DR after 8 weeks on a low fat diet or high fat diet (HFD). However, C57Bl/6 mice had lower body weight, lower adiposity, smaller adipocytes and decreased leptin and lipogenic genes expression in AT than FVB/N mice at 9 weeks of age on a chow diet. Despite having smaller adipocytes, the level of hypoxia and the expression of pro-angiogenesis genes were higher in WAT of young C57Bl/6 mice than young FVB/N mice. In addition, expression of genes related to macrophages and their recruitment, and to

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Supplementary information is available at IJO's website.

Conflict of Interest

Dr. Seeley has served as a paid consultant for Novo Nordisk, Eli Lilly, Angiochem, Novartis, and Ethicon Endo-Surgery. Dr. Seeley has served as a paid speaker for Novo Nordisk, Pfizer, and Ethicon Endo-Surgery. Dr. Seeley has received research support from Novo Nordisk, Pfizer, Mannkind, Ablaris and Ethicon Endo-Surgery.

proinflammatory cytokines, was significantly higher in WAT of young C57Bl/6 mice than young FVB/N mice.

Conclusion—These data suggest that the potential for WAT remodeling in early period of growth is higher in C57Bl/6 mice as compared to FVB/N mice and we hypothesize that it may contribute to the increased susceptibility to DIO of C57Bl/6 mice.

Keywords

adipose tissue; angiogenesis; inflammation; hypoxia; diet-induced obesity; diet-resistant; body weight; high-fat diet

Introduction

Obesity is a complex and multifactorial disease. Early identification of obesity-prone humans may provide a strategy for preventing or reducing the incidence and severity of the disorder. While it is clear that environmental factors such as consumption of calorically-dense, high-fat diets (HFDs) can lead to increased adiposity, this response is not uniform. Rodent experiments make it clear that even when exposed to the same obesogenic diet and housed under the same conditions, there are important genetic differences that contribute to the susceptibility to gain weight on a HFD.

Modulation of genes related to thermogenesis, such as UCP-1, affects the development of diet-induced obesity (DIO) in mice,¹ and hypothalamic neural circuits involved with regulation of energy balance are altered in offspring of DIO rats.² Many hypotheses have been offered to explain the differences between DIO and diet-resistant (DR) animals. One common hypothesis is that differences in the key CNS circuits that regulate energy balance differ between DIO and DR animals. Consistent with this, there is an inborn genetic disposition to develop leptin or insulin resistance, altered glucose sensing, and disturbed metabolism of norepinephrine, serotonin, and dopamine in the hypothalamus of DIO rats.²

An alternative hypothesis is that a key difference between DIO and DR animals relates to the ability of white adipose tissue (WAT) to accommodate the storage of increased calorie intake. Analogous to tumors, WAT has the capability to quickly expand and proliferate throughout adulthood. Also similar to tumors, such expansion is highly dependent on the ability to generate new blood vessels^{3, 4} and as a result, WAT is highly vascularized and has angiogenic properties⁵ that are critical to its ability to expand.^{3, 4} Interestingly, treatments that inhibit WAT angiogenesis potently (but reversibly) reduce body weight and fat mass in rodents and non-human primates, indicating that WAT integrity and sustained weight gain depends on maintaining WAT vasculature.^{4, 6, 7}

WAT angiogenesis is highly correlated with adipogenesis, inflammation and the need for ongoing tissue remodeling. Many different cell types in WAT, including mature adipocytes, preadipocytes, pericytes, endothelial cells and inflammatory cells, are involved in the capacity for WAT to remodel itself under different circumstances.^{3, 8} Obese rodents and humans have elevated levels of angiogenic factors and macrophages in their WAT.^{9, 10} Such low-grade inflammation is progressively increased in WAT of mice fed a HFD and these

changes have been hypothesized to contribute to obesity and insulin resistance.¹¹ The surface area of adipocytes reflects their volume and is positively correlated with the number of macrophages.^{11,12} As obesity develops, there is a switch to a more inflammatory phenotype in macrophages in WAT.¹³

In the present work, we hypothesized that at a young age and before exposure to a HFD, the WAT of mice that are susceptible to weight gain on a HFD have increased capacity for tissue expansion, including the capability of rapid angiogenesis, and that this capacity may contribute to the enhanced susceptibility to develop obesity. To this end, we compared the ability of two mouse strains to gain weight and undergo WAT remodeling when fed a HFD. While C57Bl/6 (C57) mice gained substantially more weight and fat on the HFD than FVB/N (FVB) mice, the latter being almost completely resistant to weight gain on the HFD. Consistent with this, the capacity for WAT tissue remodeling was far greater in C57 compared to FVB mice, and this was the case prior to their exposure to the HFD.

Materials and methods

Animals

Male C57 mice and FVB mice obtained from Jackson Labs at 8 weeks of age were housed individually in standard mouse cages with a 12-h light/12-h dark schedule. Sixteen of each genotype had ad lib access to a high-fat diet providing 40% calories as fat (HFD, D03082706, Research diet, Inc.) or a low-fat diet with 4% fat (LFD, D03082705) for 8 weeks. In two separate experiments, a total of 16 mice of each genotype were fed a chow diet and sacrificed at 9 weeks of age after adaptation to the cage for 1 week for gene expression or histologic studies. All animal protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Body composition

Body composition was analyzed by an NMR (Echo Medical Systems) on Weeks 0, 8, and 12 in the first experiment, and at 9 weeks of age in the second study.

Adipocyte tissue morphometry

To determine adipocyte size, the cross-sectional area of adipocytes was determined on paraffin-embedded hematoxylin eosin-stained sections of epididymal fat at a magnification of 200× by image processing with custom-designed software. The method of determining adipocyte size was modified from previous papers using a computer image analysis.¹⁴ The distribution of adipocyte size was determined by relative frequencies of adipocytes having a size within a regular interval. The number of adipocytes was determined by dividing the total fat mass by the mean adipocyte size. A total 500 to 1200 adipocytes were used for the analysis of the mean adipocyte area and the distribution of adipocyte size for each mouse.¹⁴

Immunohistochemistry

To assess the degree of hypoxia in WAT, a HypoxyprobeTM-1 Plus Kit (Hypoxyprobe, Inc.) was used. Five mice in each group were intraperitoneally injected with 60 mg/kg of a HypoxyprobeTM-1 (pimonidazole HCl) 30 min before being sacrificed. Epididymal fat was

fixed in alcoholic zinc formalin (Anatech LTD.) overnight. The AT was embedded in a paraffin in pairs containing one C57 and one FVB mouse each per block to minimize any differences that might arise during the process of immunohistochemistry and sectioned at the thickness of 5 μm . The immunohistochemistry was performed and a total of 200 images (5 images per section) were captured at a magnification of 100 \times (AxioVision 4.6 microscope; Carl Zeiss). To correlate hypoxia with adipocyte size, the brown color signifying hypoxia was selected by using HSL color system (Hue 0-25 and 253-255; Saturation 25-255; Luminescence 0-225) and the number and the cross-sectional area of adipocytes were concurrently measured in the same field with the software. The degree of hypoxia was determined by dividing the area of brown color by the number or the averaged cross-sectional area of adipocytes in the same field.

For the detection of PECAM1, sections were incubated overnight at 4 $^{\circ}\text{C}$ with a rat anti-mouse CD31 antibody (1:500; BD Pharmingen). After incubation with biotinylated anti-rat IgG (1:200; Jackson ImmunoResearch Laboratories), sections were revealed with avidin-biotin complex (1:500; Vector Laboratories). Sections were counterstained with hematoxylin and eosin. The numbers of adipocytes and vessels were analyzed by three independent individuals blinded to the experimental conditions.

PCR arrays

Mice (8 per condition) were sacrificed after a 1-h fast. The epididymal fat was quickly frozen in 2-methyl butane on dry ice. An RT² First Strand Kit and RT² ProfilerTM PCR Array System (SABiosciences Corporation) was used to perform PCR arrays related to angiogenesis. Total RNA was isolated using RNeasy columns (Qiagen) after processing with Tri-reagent (Molecular research center, Inc.). The first strand cDNA and the cocktail solutions of cDNA including RT² qPCR Master Mix were prepared for each mouse. PCR arrays (eight in each group) were performed using an iCycler (Bio-rad Laboratories). The quality control tests for genomic DNA control, reverse transcription control, and positive PCR control of each array plates were all passed below the criteria. The array data were analyzed using RT² Profiler PCR Array Data Analysis. The names of all genes are presented using official symbol of NCBI in this study (Figures and Supplemental table 2). Three housekeeping genes not influenced by the experimental condition (*Hsp90ab1*, *Gapdh* and *Actb*) were selected as an endogenous control. The normalization and analysis of data were performed according to the instructions (SABioscience Corporation).

Real-time quantitative PCR

Total RNA was isolated from epididymal fat of each mouse in the same way as above, and cDNA was synthesized using iScript (Bio-rad). PCR was performed in triplicate using an iCycler and the iQ SYBR Green Supermix (Bio-rad Laboratories) or 7900HT Fast Real-Time PCR system (Applied Biosystems) (Supplemental table 1).¹⁴ The expressions of each gene were normalized to constitutively expressed L32 and relative expression was quantified.¹⁴ The names of all genes were presented using official symbol of NCBI in this study (Figures and Supplemental table 2).

Statistics

All data are expressed as mean \pm SEM. Data were analyzed by Student's T-test or two-way repeated measures ANOVA followed by Bonferoni's multiple comparisons using Prism 5.0.

Results

C57 mice are prone to diet-induced obesity whereas FVB mice are resistant

To test whether FVB mice are resistant to diet-induced obesity relative to C57 mice, we compared the change in body weight and energy intake by feeding both genotypes a low-fat diet (LFD) or high-fat diet (HFD) for 8 weeks. The body weight of C57 mice was lighter relative to FVB mice at 9 weeks of age at the start of the experiment (Figure 1A). C57 mice increased their weight by 20% after 8 weeks on the HFD compared to those on the LFD. In contrast, the FVB mice fed the HFD gained comparable weight as those on the LFD (Figure 1B). Likewise, the fat mass of C57 mice on a HFD was greater than that on a LFD while that of FVB mice on a HFD was not significantly different from that of LFD mice after both 5 and 12 weeks (Figure 1C). The difference in cumulative energy intake between LFD and HFD was significantly greater in C57 mice compared to FVB mice (Figure 1D and 1E). Given the lower energy expenditure in C57 mice reported in previous studies,^{15, 16} these data support a greater energy surplus in C57 mice relative to FVB mice. These data confirm the differential susceptibility to a HFD between DIO C57 and FVB mice.

Young C57 mice fed chow have smaller adipocytes and lower adiposity than young FVB mice

The body weight of C57 mice was actually lower due to both lower adiposity and less lean mass relative to FVB mice at 9 weeks of age on a chow diet (Figure 2A and 2B). The cross-sectional area of individual adipocytes was smaller in C57 mice than FVB mice and there were more small adipocytes and fewer large adipocytes in C57 mice (Figure 2C and 2D). There was also a trend toward a decrease in the number of adipocytes in C57 mice relative to FVB mice ($P=0.06$). Consistent with the lower adiposity and smaller adipocytes, the level of gene expression of *Lep* was lower in WAT of C57 mice relative to FVB mice (Figure 2E).

To determine whether the difference in adipocytes between young C57 mice and FVB mice is associated with differential adipose lipid metabolism, we compared the expression of genes involved in adipogenesis, utilization of fatty acid, lipogenesis and lipolysis in WAT of both groups. There was no significant difference between genotypes in expression of genes for transcriptional factors involved in adipogenesis such as *Pparg2* and *Srebf*. The expression of genes involved in lipogenesis, including *Fasn* and *Acaca* were significantly lower in C57 mice relative to FVB mice. *Lpl* and *Cd36* are associated with release of fatty acids from circulating lipoproteins and uptake by WAT.^{17, 18} These genes involved in fatty acid utilization were significantly lower in WAT of C57 mice relative to FVB mice (Figure 2G). The expression level of *Adrb3* (beta 3-adrenergic receptor) is associated with lipolysis and sympathetic activity in WAT,¹⁹⁻²¹ and is lower in WAT of obese rodents.²² Consistent with the lower adiposity of C57 mice, *Adrb3* gene expression was higher in WAT of C57 mice relative to FVB mice (Figure 2G).

Young C57 mice on a chow diet have higher angiogenic activity in adipose tissue than young FVB mice

The growth or regression of vasculature depends on the net balance between angiogenic stimulators and angiogenic inhibitors, and the process of angiogenesis comprises modulation of migration and proliferation of endothelial cells (ECs), conversion of ECs to pericytes, recruitment of pericytes and smooth muscle cells, and stabilization of vessels.^{23–25} Because the expandability of WAT highly depends on its vasculature,⁴ we hypothesized that C57 mice, with their tendency to develop DIO when fed a HFD, have a higher angiogenic potential in WAT at a young age, prior to exposure to a HFD. To test this, we determined the expression of 84 genes involved in the process of angiogenesis by RT-PCR array. A total of 32% of angiogenic genes were differentially expressed in WAT between C57 mice and FVB mice, with 78% of the significantly changed genes being more highly expressed in C57 relative to FVB mice.

Genes for growth factors that increase migration and proliferation of ECs or stimulation of VEGF such as *Vegfa*, *Egf*, *Fgf1*, *Figf*, *Hgf*, and *Igf1* were all expressed at higher levels in WAT of C57 mice relative to FVB mice, while *Pgf*, which stimulates angiogenesis only in pathologic conditions (Figure 3A),^{25, 26} was lower. *Angpt1*, which maintains a normalized state of vasculature, was highly expressed in C57 mice while *Tek*, a receptor for *Angpt1* and *Angpt2*, and *Angpt2*, an antagonist ligand for Tie-2, were not different, indicating that stabilization of WAT vasculature and pro-angiogenesis was increased in WAT of C57 mice relative to FVB mice.^{26, 27} However, *Ctgf*, which is associated with fibrosis, proliferation of mesenchymal cells including pericytes and vascular smooth muscle cells, and inhibition of VEGF-induced angiogenesis,^{28, 29} and *Pdgfra* which is related to proliferation of mesenchymal cells and an autocrine regulator of FGF-2 and VEGF, were lower in WAT of C57 mice (Figure 3A).³⁰ This may imply that the process of supporting actively sprouting vessels is less more active in WAT of C57 mice than FVB mice.

Transcriptional factors such as *Epas1* (Hif-2alpha), *Smad5*, *Tbx1*, and *Tbx4* were highly expressed in WAT of C57 mice whereas Hif-1alpha was not different between genotypes (Figure 3B). *Epas1* promotes angiogenesis in response to hypoxia and *Smad5* is associated with ECs proliferation.^{31, 32} *Tbx1* and *4* are associated with FGF signaling, angiogenesis or morphogenesis during development.^{33, 34} The difference in expression of these transcription factors is consistent with a more pro-angiogenic environment in WAT of C57 mice (Figure 3B).

The expression of *Thbs1* and *Thbs2*, endogenous anti-angiogenic factors, was lower in WAT of C57 mice (P=0.07 and P<0.05, respectively; Figure 3C). Other anti-angiogenic factors including *Coll8a1* and *Col4a3*, a major constituent of basement membrane cleaved by MMP-9, tended to be lower (P=0.07) or were significantly lower in WAT of C57 mice relative FVB mice (Figure 3C).^{25, 35} These lower anti-angiogenic factors are also consistent with the greater pro-angiogenesis in WAT of young C57 mice. In addition, genes regarding degradation of the extracellular matrix such as *Mmp9* and *Mmp19* were higher in C57 mice, favoring angiogenesis, but *Mmp2* was lower (Figure 3C).

Stab1 is expressed by sinusoidal endothelium or alternatively by activated macrophages and acts as a scavenger receptor for endocytosis of SPARC, a potent inhibitor of angiogenesis.³⁶ Expression of *Stab1* was higher in WAT of C57 mice. *Ptgs1*, which is related to enhanced tube formation or vasodilation, was also higher in WAT of C57 mice (Figure 3D).³⁷

Although most of the significantly changed genes were pro-angiogenic, some of the genes were either anti-angiogenic or have mixed function. *Flt-1* is increased by hypoxia, but it is not clear whether it mediates VEGF signaling in its intact form, or plays a role as a vascular permeability receptor, or sequesters VEGF and subsequently antagonizes VEGF action in a soluble form under some circumstances.^{24, 38, 39} In the present study, WAT of C57 mice had higher *Flt-1* gene expression relative to FVB mice. *Npr1* is related to vasodilatation via increased guanylyl cyclase activity and inhibits synthesis and function of VEGF activities.⁴⁰ *Lect1* contributes to growth of chondrocytes and inhibits angiogenesis (Figure 3D).^{41, 42} *Npr1* and *Lect1* were higher in WAT of C57 mice.

Genes involved in modulation of cell-to-cell contacts, such as *Cdh5*, *Efna1*, *Efnb2*, *Ephb4*, and *Pecam1* were not different between C57 mice and FVB mice. In addition, the expression of *Itgav* and *Itgab3* genes that are highly expressed in activated ECs²⁵ was not different. Consistent with no difference in expression of these genes, the number of vessels per adipocyte was not different although the numbers of adipocytes or vessels per field were higher in WAT of young C57 mice relative to FVB mice (Figure 4A and B). These data are consistent with the hypothesis that the status of ECs does not differ between C57 mice and FVB mice maintained on a chow diet.

In sum, the analysis of these gene expression differences indicates little difference in the state of WAT ECs interacting with other ECs or pericytes between C57 mice and FVB mice. However, WAT of C57 mice had considerably greater expression of angiogenic factors compared to FVB mice. In particular, growth factors involved in proliferation and migration of ECs were mostly higher in WAT of C57 mice, and anti-angiogenic factors such as *Thbs1* and *Thbs2* were lower.

White adipose tissue of young C57 mice is more hypoxic than that of FVB mice

Within a 100 μm distance from capillaries, the exchange of oxygen or nutrients is the result of diffusion.⁵ Hypoxia is a key stimulator of angiogenesis. We therefore hypothesized that WAT hypoxia contributes to more pro-angiogenesis in WAT of C57 mice relative to FVB mice. To test this hypothesis, we compared the hypoxic area in WAT between C57 and FVB mice using immunohistochemistry. Because the staining area in WAT is highly dependent on the number and size of adipocytes, we also compared the hypoxic area after adjusting for number and size of adipocytes in the same field. The absolute area of hypoxia was significantly increased in WAT of C57 mice relative to FVB mice. Importantly, the area of hypoxia adjusted for either adipocyte number or size was still significantly increased in C57 mice relative to FVB mice (Figure 4C). These data suggest that WAT hypoxia may contribute to the increased expression of pro-angiogenic genes in WAT of C57 mice relative to FVB mice.

Young C57 mice on a chow diet have more inflammatory adipose tissue relative to young FVB mice

Because angiogenesis contributes to tissue remodeling, we hypothesized that low-grade inflammation may be associated with the greater weight gain on the HFD in C57 mice. To test this hypothesis, we compared the expression of genes related to inflammatory cytokines and macrophages in WAT between young C57 and FVB mice. A macrophage marker, *Emr1*, as well as genes related to recruitment of macrophages such as *Ccr2* and *Itgax*, were significantly increased in WAT of C57 mice relative to FVB mice. Proinflammatory cytokines including *Il1b*, *Il6*, *Ccl3* and *Cxcl2* were significantly increased in WAT of C57 mice relative to FVB mice while genes related to alternative activation of macrophages such as *Jag1*, *Tgfb1* and *Mgl2* were not significantly different. However, *Arg1* was significantly increased in WAT of C57 mice relative to FVB mice (Figure 5).

Discussion

The goal of these studies was to identify potential factors that might contribute to the differences observed in weight gain among animals exposed to a HFD. Long-term maintenance on a HFD increased body weight and adiposity of C57 mice whereas those of FVB mice were not significantly increased. The energy surplus in C57 mice was likely the result of both increased energy intake (Figure 1) and decreased energy expenditure.^{15, 16} This is consistent with previous data on these strains of mice (Figure 1).¹⁶ However, the difference of body weight between these strains at a younger age was not consistent with what was observed after 8 weeks on the HFD. Specifically, the body weight and adiposity were significantly lower in C57 mice relative to FVB mice at 9 weeks of age before exposure to a HFD. Consistent with the lower adiposity, leptin gene expression was lower in WAT of C57 mice relative to FVB mice and their adipocyte size was smaller. In addition, the amount of WAT correlated with expression of genes linked to lipid metabolism, including lower *Lpl*, *Cd36*, and *Fasn*, and higher *Adrb3* in C57 compared to FVB mice.

The key finding in this study is that young C57 mice have an expression profile consistent with greater inflammation and angiogenesis in WAT despite having less body fat than young FVB mice. Such data suggest that young C57 mice may already have a higher capacity for WAT remodeling.^{43, 44} The number of AT macrophages is positively correlated with increased size of individual adipocytes.¹² Interestingly, C57 mice have increased expression of inflammatory markers in WAT despite having reduced adipocyte size. WAT remodeling dynamically occurs both after acute weight loss or when WAT rapidly grows such as occurs in C57 mice exposed to a HFD.⁴³ Since expression of genes associated with WAT lipid metabolism correlated with the increased adiposity of young FVB mice (Figure 2G), neither fatty acid metabolism nor adipocyte death appears to contribute to increased macrophage and proinflammatory cytokine markers in young C57 mice.^{43, 45}

Numerous cell types in WAT, including immune cells, ECs, preadipocytes, and mature adipocytes, are involved in the process of tissue remodeling since WAT expansion and adipocyte differentiation require an adjusted vascular network and angiogenesis precedes adipogenesis.^{3, 5, 8, 44, 46} In addition, angiogenesis accompanies inflammation, and increased macrophage infiltration has been hypothesized to promote angiogenesis in

WAT.^{8, 47} During this process, hypoxia is a critical factor to initiate angiogenesis and it induces inflammation in WAT.⁴³ Larger adipocytes suffer from lack of oxygen and nutrient transfer because oxygen exchange usually occurs by diffusion through adipocytes.⁵ Given the smaller adipocytes of C57 mice relative to FVB mice, it is an interesting finding that levels of hypoxia evidenced by increased pimonidamole adduct staining and gene expression of Hif-2alpha are increased in WAT of C57 mice consistent with higher expression of pro-angiogenic and pro-inflammatory genes. WAT hypoxia in obesity has been observed in previous studies.⁴⁸ Those studies suggest that the increased WAT hypoxia in young DIO mice is important in initiating increased angiogenesis and the mild inflammation that contributes to the necessary tissue remodeling that allows C57 mice to gain more weight when exposed to a HFD.

In this study, several important pro-angiogenic factors including *Vegfa*, *Fgf1*, *Egf*, *Hgf*, *Igf1*, *Figf1* and *Angpt1* were highly expressed and anti-angiogenic factors such as *Thbs1*, *Thbs2*, *Coll8a1* and *Col4a3* were lower in WAT of young C57 mice, suggesting an important difference in angiogenic potential between C57 and FVB mice. For example, *Vegfa* is a key target for HIF-1 alpha and plays a critical role in the angiogenic response to hypoxia. *Vegfa* stimulates angiogenesis in WAT and VEGF receptor-2 signaling contributes to both WAT angiogenesis and adipogenesis.^{3, 24} HGF is another WAT pro-angiogenic factor that does not directly promote adipocyte differentiation.⁴⁹ Both VEGF and HGF synergistically stimulate neovascularization, endothelial migration and proliferation.⁵⁰ ANGPT1, which stabilizes vessels, also plays a role in vascular remodeling by promoting angiogenesis in conjunction with VEGF.⁵¹

Of note, the gene expression level of *Fgf1* was dramatically upregulated in young C57 mice relative to young FVB mice while there was no difference of *Fgf2*, a potent and common angiogenic stimulator. FGF1 stimulates adipogenesis in WAT of humans,⁵² and FGFs are well known to stimulate angiogenesis in tumors by increasing VEGF-driven neovascularization.^{25, 26} Although a role of each of these genes in angiogenesis has been reported, many of these gene products have also been reported to regulate inflammation and adipogenesis.^{23, 43}

Given the growing prevalence of obesity, an important question is why individual's susceptibility to obesity varies. The present study used C57 mice and FVB mice to explore differences in WAT that may contribute to this variable response to obesogenic environments. While energy balance clearly involves CNS processes that regulate both food intake and energy expenditure, growing evidence points to an important role in signals from WAT that are related to the status of WAT vasculature.⁶ Consequently, the one way to view the current data is that C57 and FVB mice differ in the capacity of their WAT to expand and remodel and that this contributes to the propensity for increased intake and weight gain when mice are exposed to a HFD. Further work will be needed to assess the causal relationships between adipose tissue remodeling and energy balance (Figure 6).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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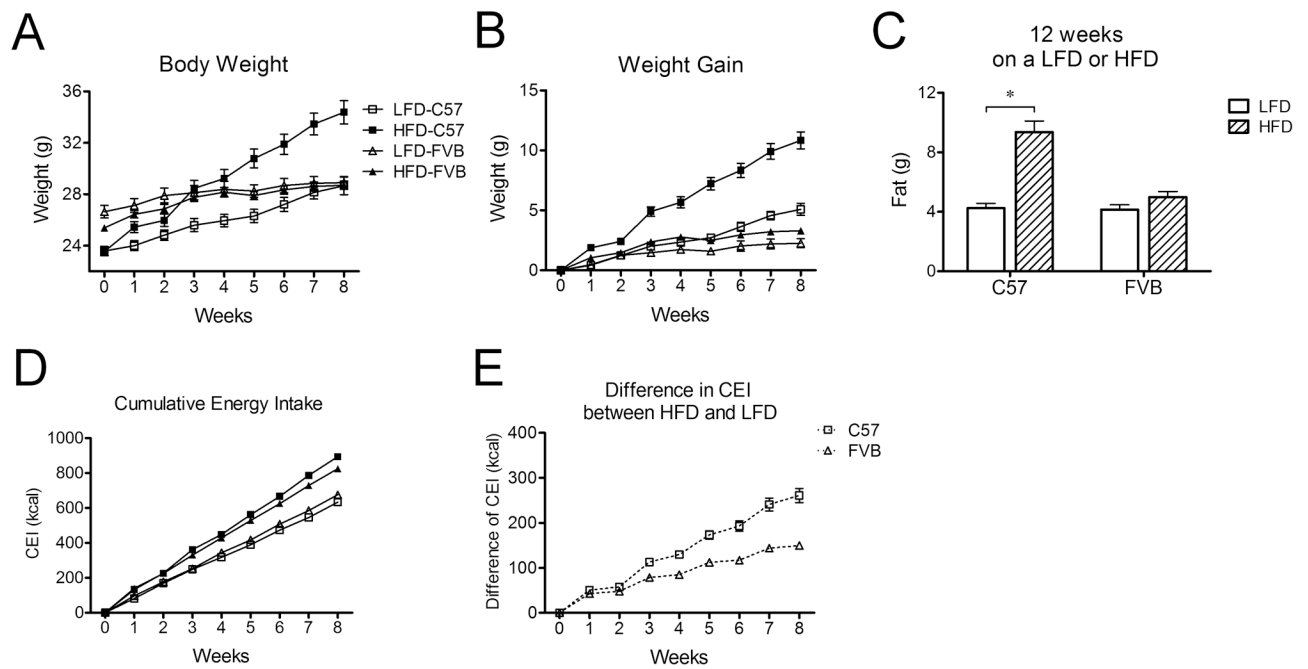


Figure 1. Changes in body weight, body composition, and energy intake of C57 mice (n=16) and FVB mice (n=16) in response to a LFD or a HFD

A) Change in body weight of C57 and FVB mice after 8 weeks on a LFD or a HFD

B) Change in weight gain of C57 and FVB mice after 8 weeks on a LFD or a HFD

C) Body composition of C57 and FVB mice after 12 weeks on a LFD or HFD

D) Change in cumulative energy intake of C57 and FVB mice after 8 weeks on a LFD or a HFD

E) Difference in cumulative energy intake between HFD and LFD in each group

* $P < 0.05$. CEI indicates cumulative energy intake.

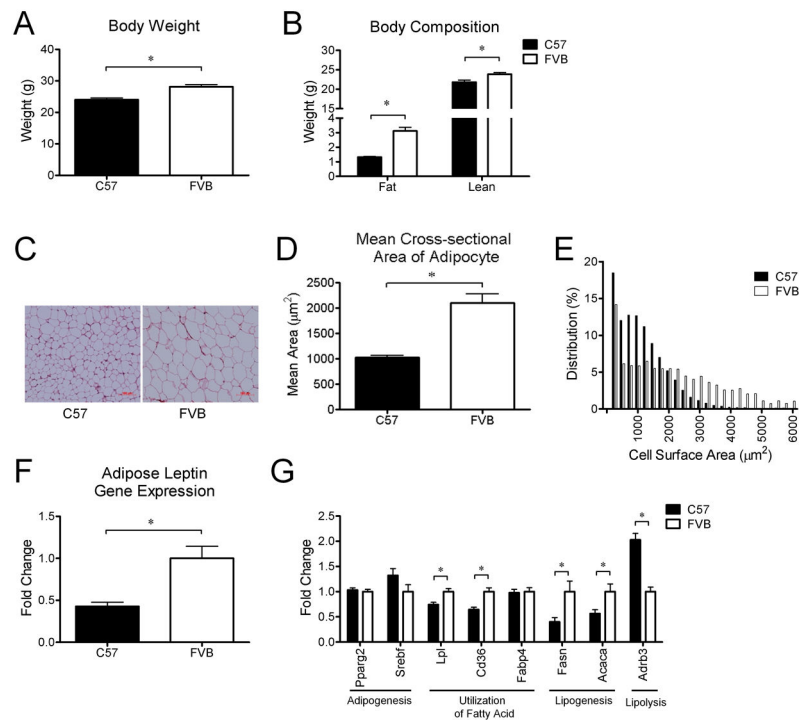


Figure 2.

Differences in body weight (A), body composition (B), mean cross-sectional area (D) and relative distribution of adipocyte size (E), adipose leptin gene expression (F), and differential expression of genes related to lipid metabolism in white adipose tissue (G) between C57 mice (n=8) and FVB mice (n=8) at 9 weeks of age on a chow diet.

Representative figures of adipocytes (C) in white adipose tissue of C57 mice and FVB mice at 9 weeks of age on a chow diet. * P<0.05. *Acaca*, acetyl-CoA carboxylase 1; *Adrb3*, beta 3-adrenergic receptor; *Cd36*, fatty acid translocase; *Fasn*, fatty acid synthase; *Lpl*, lipoprotein lipase; *Ppar2*, peroxisome proliferator activated receptor, gamma 2; *Srebf*, sterol regulatory element binding transcription factor.

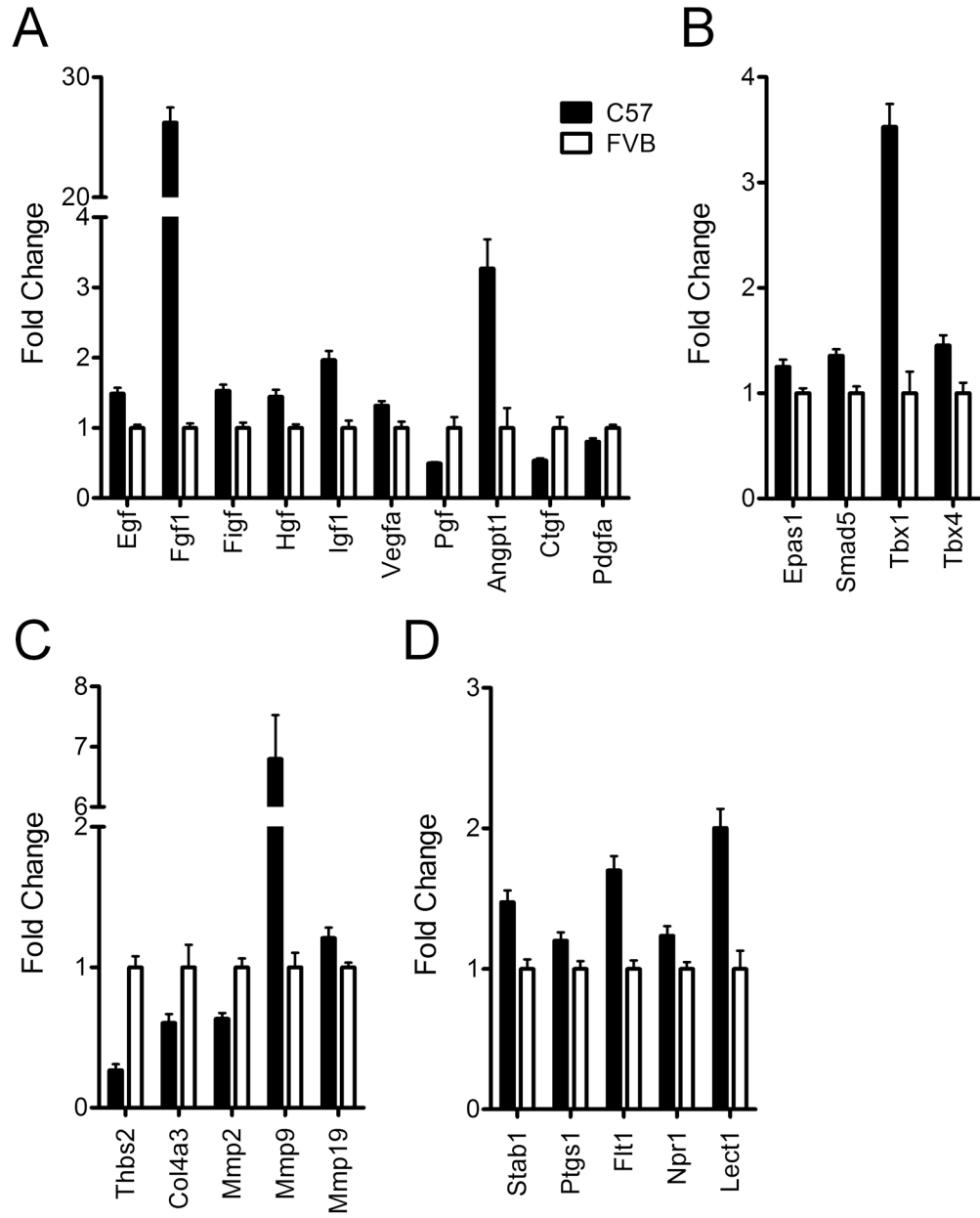


Figure 3.

Comparison of genes related to angiogenesis in white adipose tissue of C57 mice (n=8) and FVB mice (n=8) at 9 weeks of age on a chow diet. All genes displayed are differentially expressed between C57 mice and FVB mice ($P < 0.05$). Significantly different growth factors (A), transcriptional factors (B), anti-angiogenic factors and matrix metalloproteinases (C), and two pro-angiogenic genes and three anti-angiogenic genes (D). *Angpt1*, angiopoietin 1; *Col4a3*, collagen type IV alpha 3 (tumstatin); *Ctgf*, connective tissue growth factor; *Egf*, epidermal growth factor; *Epas1*, endothelial PAS domain protein 1 (Hif-2alpha); *Fgf1*, fibroblast growth factor 1; *Figf*, c-fos induced growth factor (VEGF-D); *Hgf*, hepatocyte growth factor; *Flt-1*, FMS-like tyrosine kinase 1 (VEGFR-1); *Igf1*, insulin-like growth factor

1; *Lect1*, leukocyte cell derived chemotaxin 1; *Mmp2*, matrix metalloproteinase 2; *Mmp9*, matrix metalloproteinase 9; *Mmp19*, matrix metalloproteinase 19; *Npr1*, natriuretic peptide receptor 1; *Pdgfa*, platelet derived growth factor alpha; *Pgf*, placental growth factor; *Ptgs1*, prostaglandin-endoperoxide synthase 1 (Cox-1); *Smad5*, MAD homolog 5; *Stab1*, Stabilin-1; *Tbx1*, T-box 1; *Tbx4*, T-box 4; *Thbs2*, thrombospondin 2; *Vegfa*, vascular endothelial growth factor A.

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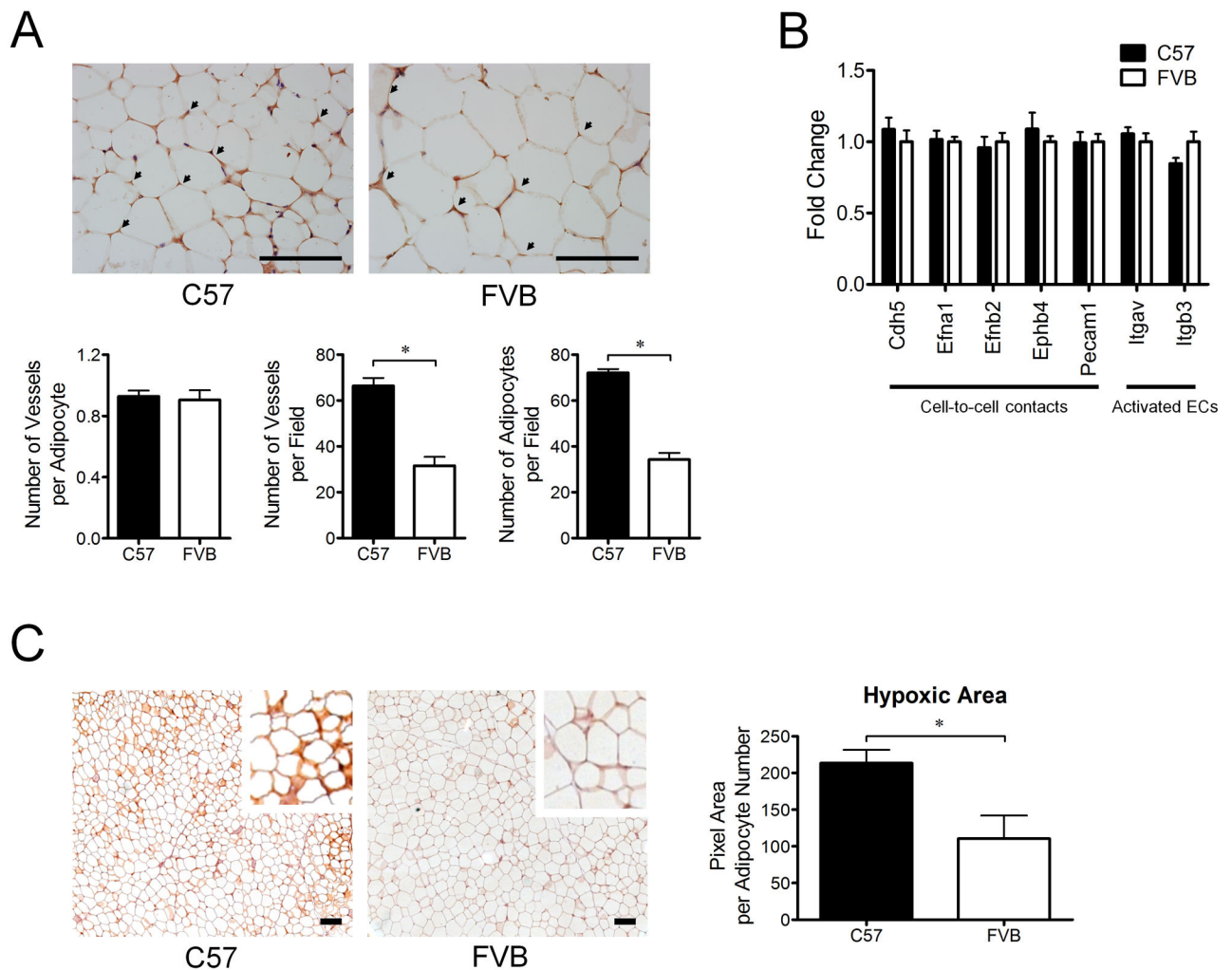


Figure 4. Comparison of vessel density and adipose tissue hypoxia in white adipose tissue between C57 mice and FVB mice at 9 weeks of age on a chow diet

A) Difference in vessel density in white adipose tissue of C57 mice (n=5) and FVB mice (n=4) at 9 weeks of age on a chow diet. Vessel density was calculated by dividing total number of vessels per field by total number of adipocytes per field ($\times 40$). Arrows indicate CD31 (PECAM1) staining. Scale bar, 100 μ m.

B) Comparison of genes related to the status of endothelial cells (ECs) in white adipose tissue of C57 mice (n=8) and FVB mice (n=8) at 9 weeks of age on a chow diet. No significant differences in the gene expression were observed between C57 mice (n=8) and FVB mice (n=8).

C) Representative figures of adipose tissue hypoxia. Dark brown color indicates the staining area of hypoxia. All samples from C57 mice (n=5) and FVB (n=5) mice were processed in pairs during all the procedures of immunohistochemistry to minimize the occurrence of errors. Hypoxic area was adjusted for either adipocyte number or size in the same field. Scale bar, 100 μ m.

* $P < 0.05$. *Cdh5*, cadherin 5; *Efna1*, ephrin A1; *Efnb2*, ephrin B2; *Ephb4*, Eph receptor B4; *Pecam1*, platelet/endothelial cell adhesion molecule 1; *Itgav*, integrin alpha V; *Itgb3*, integrin beta 3.

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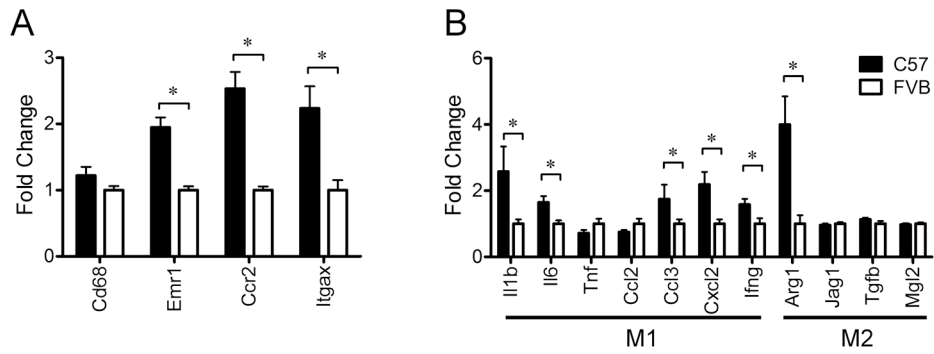


Figure 5. Differential expression of genes related to inflammation in white adipose tissue of C57 mice (n=8) and FVB mice (n=8) at 9 weeks of age on a chow diet. * P<0.05

A) The expression of genes of markers for macrophage and recruitment of macrophage

B) Different polarization of macrophage in white adipose tissue of C57 mice and FVB mice. M1 and M2 mean the type of macrophage polarization. M1-type macrophages are more involved in pro-inflammation and recruitment of macrophage. M2-type macrophages are involved in tissue remodeling.

* P<0.05. *Arg1*, arginase; *Ccl2*, chemokine (C-C motif) ligand 2; *Ccl3*, chemokine (C-C motif) ligand 3; *Ccr2*, chemokine (C-C motif) receptor 2; *Cxcl2*, chemokine (C-X-C motif) ligand 2; *Cd68*, CD68 antigen; *Emr1*, efg-like module containing, mucin-like, hormone receptor-like 1; *Ifng*, interferon gamma; *Il1b*, interleukin 1, beta; *Il6*, interleukin-6; *Itgax*, integrin, alpha X (complement component 3 receptor 4 subunit); *Jag1*, jagged 1; *Mgl2*, macrophage galactose N-acetyl-galactosamine specific lectin 2; *Tgfb*, transforming growth factor, beta 1; *Tnf*, tumor necrosis factor

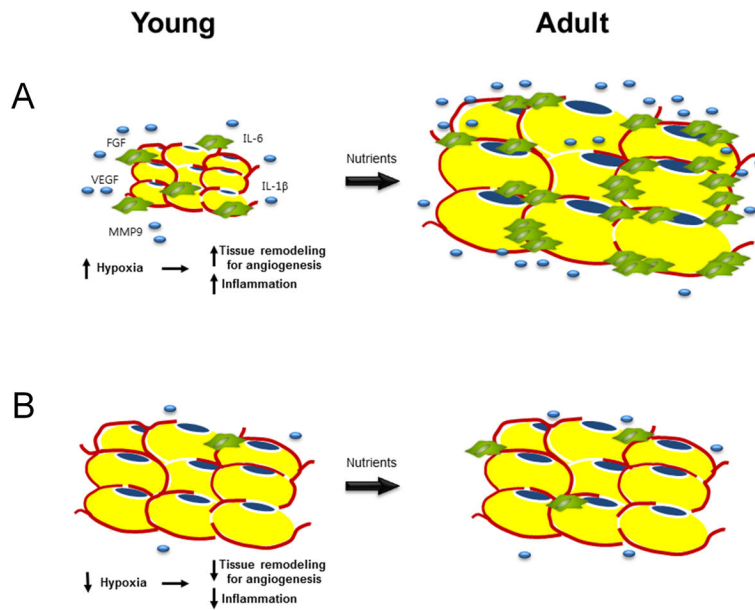


Figure 6. “Potential” hypothesis of increased inflammation and pro-angiogenesis presumably caused by adipose tissue hypoxia despite smaller adipocytes and lower adiposity in young mice

A) The increase in capacity for tissue remodeling resulting from hypoxia may determine the development of obesity in the future. Reduced vascular function responding to dynamic change of nutrients or gas exchange surrounding adipocytes may result in increased hypoxia and subsequent pro-angiogenesis in white adipose tissue despite smaller adipocytes. Inflammation is accompanied by angiogenesis.

B) The adequate ability of vasculature to meet the requirements may cause resistance to obesity. Reactivity of vasculature responding to the environments may be enough to compensate for the rapid increase of need for supplying nutrients or gas exchange. Therefore, adipocytes do not need the tissue remodeling for angiogenesis.