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Acute endurance exercise increases *Vegfa* mRNA expression in adipose tissue of rats during the early stages of weight gain

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Abstract

The aim of this study was to determine the effects of acute exercise on key factors regulating angiogenesis in adipose tissue. Adipose tissue *Vegf-a* messenger RNA expression was upregulated immediately after acute exercise ($p < 0.05$) in rats consuming a high-fat diet, but was lower after exercise ($p < 0.05$) in rats consuming a low-fat diet. Our working hypothesis is that acute exercise augments angiogenic signaling under conditions when adipose tissue is expanding, and with repeated exercise sessions these signals can accrue to enhance vascularization.

Résumé:

Cette étude a pour objectif de déterminer les effets d'un exercice ponctuel sur les facteurs clés régulant l'angiogenèse dans le tissu adipeux. Immédiatement après un exercice ponctuel, on observe chez des rats au régime fort en gras une régulation à la hausse ($p < 0,05$) de l'expression de ARN messager du *Vegf-a* (*facteur de croissance de l'endothélium vasculaire*) dans le tissu adipeux, mais à la baisse ($p < 0,05$) chez des rats au régime pauvre en gras. Notre hypothèse de travail est que l'exercice ponctuel accroît la signalisation de l'angiogenèse dans des conditions selon lesquelles le tissu adipeux prend de l'expansion et que la répétition des séances d'exercice accroît par ses signaux la vascularisation. [Traduit par la Rédaction]

Keywords

white adipose tissue; exercise; angiogenesis; high fat diet; VEGF

Keywords

tissu adipeux blanc; exercice physique; angiogenèse; régime fort en gras; VEGF

Introduction

Adipose tissue is remarkably vascularized, lending to its important role in delivering fatty acids to the circulation according to metabolic need, as well as its increasingly appreciated

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Conflict of interest statement

There are no conflicts of interest to declare.

role as an endocrine organ. There is evidence that obese adipose tissue can become hypoxic (Pasarica et al. 2009). This hypoxia is often considered central to the adipose tissue dysfunction that contributes to obesity-related insulin resistance; including local inflammation, fibrosis, and dysregulated lipolysis (Sun et al. 2014). Elegant studies upregulating vascular endothelial growth factor (VEGFA, or VEGF; widely considered the master regulator of angiogenesis) have identified increased angiogenesis as a promising therapy to treat and/or prevent weight-gain-related impairments in adipose tissue and whole-body metabolism (Sun et al. 2012; Sung et al. 2013). This premise conflicts with the notion that suppressing adipose tissue expansion (by limiting angiogenesis) would improve obesity-related insulin resistance; a concept derived from preliminary studies of morbid obesity in human cell culture and mice (Bråkenhielm et al. 2004; Rupnick et al. 2002). Importantly, the regulation and consequences of angiogenesis in severe obesity may be very different from those that occur in the early stages of weight gain.

Exercise is a cornerstone treatment for insulin resistance, but very little is known about the role of exercise in increasing adipose tissue angiogenic capacity. Walton et al. (2015) found that exercise training increased vessel density in human subcutaneous adipose tissue, but this response was impaired in their obese, insulin-resistant subjects. Data concerning the regulation of the adipose tissue angiogenic response to acute exercise are mixed, with no consensus on the VEGF response to exercise in adipose tissue (Czarkowska-Paczek et al. 2011; Disanzo and You 2014; Van Pelt et al. 2017). Differences in ex vivo angiogenic capacity have been observed between depots (subcutaneous vs. visceral), and in response to weight gain and obesity in rodent and human fat (Gealekman et al. 2011; Song et al. 2016; Villaret et al. 2010). The aim of this experiment was to assess changes in adipose tissue expression of key angiogenic genes in response to a single session of exercise during early diet-induced weight gain in rats.

Materials and methods

Animal procedures

Male Wistar rats (Charles River Laboratories, Boston, Mass., USA) were received at 6–7 weeks of age and singly housed at 23 °C in the Unit for Laboratory Animal Medicine at the University of Michigan. After 1 week, animals were either placed on a high fat diet (HFD; 60% fat; Laboratory Diet No. D12492; ResearchDiets, New Brunswick, N.J., USA) for 2 weeks, or maintained on a low fat diet (LFD; 14% fat; Laboratory Diet No. 5L0D; LabDiet, St. Louis, Mo., USA) throughout. The night before experiments, animals were fasted starting at 1700 h. Beginning at 0730 h the following morning, rats either remained sedentary ($n = 12$ LFD-sedentary (SED), $n = 12$ HFD-SED), or underwent 2 h of swim exercise consisting of 4×30 -min bouts separated by 5 min of rest ($n = 12$ LFD-exercise (EX), $n = 12$ HFD-EX). Animals were anaesthetized at 0945 h, which was immediately after exercise in LFD-EX and HFD-EX. Inguinal white adipose tissue (iWAT), epididymal white adipose tissue (eWAT), and retroperitoneal white adipose tissue (rpWAT) fat depots were dissected. All procedures conformed to the *Guide for the Care and Use of Laboratory Animals* and were approved by the University of Michigan Committee on Use and Care of Animals.

Gene expression

Expression of genes involved in adipose tissue angiogenesis was measured by the 2^{-CT} method using Taqman reagents (Schmittgen and Livak 2008). Briefly, ~50 mg of whole adipose tissue was homogenized in 1-mL RNA-Stat 60 (Tel-Test Friendswood, Tex., USA), centrifuged (12 000g) to remove the top lipid layer, and RNA was isolated by chloroform treatment. Total RNA was measured by spectrophotometry, and 260/280 nm ratio was assessed to confirm sample purity. Primers were ordered from Integrated DNA Technologies and tested for linearity across the range of complementary DNA loaded. *Ppia* was used as a housekeeping gene and a No Template Control was loaded on every plate. Probes for a variety of angiogenic genes were purchased from Integrated DNA Technologies: *Vegf-a* (RefSeq NM_031836); angiopoietin-2 (*Ang2*) (RefSeq NM_134454); angiopoietin-like protein 4 (*Angptl4*) (RefSeq NM_199115); *Pecam1* (RefSeq NM_031591); *Vegf-b* (RefSeq NM_053549); *Flt1* (i.e., *Vegfr1*-RefSeq NM_019306); *Kdr* (i.e., *Vegfr2* - RefSeq NM_013062).

Statistical analyses

Effects of HFD on body weight and fat pad weight were compared by *t* tests. Exercise and diet effects on messenger RNA (mRNA) expression were assessed across depots by 2-way repeated-measures ANOVA with 1-factor (depot) repetition to assess depot $\times$ exercise and depot $\times$ diet interactions. Holm-Sidak post hoc test was used when required. Non-normal data were logor root-transformed. Data are expressed as means $\pm$ SE. Significance was considered $P < 0.05$.

Results

Although there was no difference in body weight after the 2-week HFD compared with LFD ($P = 0.42$), regional fat pad weights were significantly greater in HFD versus LFD (Table 1). Furthermore, the sum of all fat pad masses (a crude marker of total fat mass) was ~35% greater in HFD rats versus LFD rats ($P = 0.000003$). Therefore, the 2-week HFD stimulus represented a model for the early signs of weight-gain, featuring fat pad expansion before changes in body weight.

First examining the effects of the dietary intervention without prior exercise, adipose tissue mRNA expression of *Vegf-a* and *Pecam* (an endothelial cell marker) were not different between LFD-SED and HFD-SED animals in any depot (Fig. 1A and 1B). However, *Ang2* expression was significantly higher in HFD-SED versus LFD-SED within the intra-abdominal depots only (eWAT, $P = 0.023$; rpWAT, $P = 0.027$; Fig. 1C), while *Angptl4* expression was significantly higher in HFD-SED versus LFD-SED within iWAT and eWAT (iWAT, $P = 0.037$; eWAT, $P = 0.039$; Fig. 1D). Gene expression of *Vegf-b*, *Vegfr1*, or *Vegfr2* were not affected by the diet (data not shown).

In response to acute exercise, *Vegf-a* mRNA was greater in HFD-EX versus HFD-SED in all fat depots (all $P < 0.05$, Fig. 1A), but there was no depot $\times$ exercise interaction in the response of *Vegf-a* mRNA to exercise ($P = 0.952$). Conversely, *Vegf-a* mRNA was lower in LFD-EX versus LFD-SED in iWAT ($P < 0.01$) and eWAT ($P = 0.03$). In concert with *Vegf-a*

expression in LFD animals, *Pecam* mRNA expression was also lower in LFD-EX versus LFD-SED. *Pecam* expression in HFD animals was not significantly different after acute exercise but there was a trend for lower *Pecam* expression in rp-WAT from HFD-EX versus HFD-SED ($P = 0.06$, Fig. 1B). Acute exercise did not induce changes in expression of *Ang2* or *Angptl4* (Figs. 1C and 1D) in HFD-EX; which are genes that were already elevated without exercise in HFD-SED compared with LFD-SED. Acute exercise also did not induce changes in mRNA expression of *Ang2* or *Angptl4* in LFD animals except for an increase in *Ang2* expression in rpWAT ($P < 0.001$). Exercise reduced *Vegfr1* and *Vegfr2* expression in iWAT ($P < 0.03$) and rpWAT ($P < 0.03$) from LFD animals and rpWAT ($P < 0.05$) from HFD animals (data not shown). *Vegf-b* expression was not different between HFD-SED and HFD-EX, but expression was lower in LFD-EX compared with LFD-SED in all depots ($P < 0.001$).

Discussion

The goal of this study was to characterize changes in adipose tissue angiogenic gene expression in response to a single session of exercise in a model of early weight gain. Our main findings demonstrate that 2-h swim exercise rapidly (i.e., immediately postexercise) increased *Vegf-a* mRNA levels in both subcutaneous (iWAT) and intra-abdominal (eWAT, rpWAT) adipose tissue of male Wistar rats after 2 weeks of HFD but not in rats kept on LFD. The VEGF family is the only category of bona fide endothelial cell growth factors, and VEGF-A is by far the most well-characterized member of the VEGF family (Cao 2007; Corvera and Gealekman 2014). VEGF-A binding to VEGF-receptors on endothelial cells signals their proliferation and migration, as well as vasodilation and vascular permeability (Cao 2007), with the most potent and essential signaling thought to occur through tyrosine phosphorylation of the VEGFR2 (Olsson et al. 2006). The regulation of *Vegf-a* expression in adipose tissue is not well studied, but some work has found depot differences in angiogenic gene expression and angiogenic capacity in obesity (Gealekman et al. 2011; Song et al. 2016). There are very limited available data on the effects of exercise on VEGF in adipose tissue. Czarkowska-Paczek et al. (2011) found no change in *Vegf-a* gene expression in inguinal adipose tissue after a single session of exercise (1 h treadmill running at 28 m/min) in untrained male Wistar rats. Our findings suggest that nutrient status and fat mass may have an important impact on the effects of acute exercise on adipose tissue angiogenic signaling. Here, we report that acute exercise increased *Vegf-a* expression in the expanded adipose tissue mass of the HFD-fed rats, which is consistent with our recent findings in overweight-to-obese human subjects where *VEGF-A* gene expression was also elevated in abdominal subcutaneous adipose tissue sampled 1 h after an acute exercise session (Van Pelt et al. 2017). Our present finding that adipose tissue *Vegf-a* expression was lower immediately after exercise in animals maintained on LFD is consistent with previous reports on the acute effects of exercise in endurance trained rats (Czarkowska-Paczek et al. 2011), and may be interpreted to suggest that in the absence of an energy surplus and/or without expanded fat mass, exercise may reduce angiogenic signaling in adipose tissue.

Although we found *Vegf-a* expression increased with acute exercise in animals exposed to HFD, mRNA of other pro-angiogenic markers, *Ang2* and *Angptl4*, did not. One could speculate that elevated gene expression of *Ang2* or *Angptl4* in the HFD-SED (versus LFD-

SED) may explain the absence of a further increase in this signal after exercise. However, exercise did not increase the expression of these angiogenic genes in LFD rats with the exception of *Ang2* in rpWAT. Timing of sample collection (i.e., immediately after exercise) may also have contributed to this finding, as more time may be required after exercise for these factors to be elevated. VEGF-B is a less-studied endothelial growth factor that has recently been implicated in adipose tissue angiogenesis via indirectly increasing VEGF-A signaling (Robciuc et al. 2016). We found no exercise-induced elevation in the *Vegf-b* expression, or in 2 VEGF receptors *Vegfr1* and *Vegfr2*. In fact, the expression of *Vegf-b* was lower in all fat pads from LFD-EX versus LFD-SED animals, and *Vegfr1* and *Vegfr2* expression was lower after exercise in select fat pads from animals on both diets. Studies should extend these findings by more directly assessing VEGF signaling (e.g., measuring protein phosphorylation and receptor binding) in the hours and days after acute and chronic exercise. The genes assessed here are some of the most-studied genes in the regulation of adipose tissue angiogenesis (Corvera and Gealekman 2014; Gealekman et al. 2008, 2011); but there are other important pro- and anti-angiogenic genes that may contribute to the exercise-induced regulation of angiogenesis in adipose tissue.

The selection of a 2-week HFD was an important feature of the present study design, allowing us to assess the ability of exercise to signal increases in pro-angiogenic gene expression in the early stages of adipose tissue expansion. This 2-week HFD model has been previously used by Castorena et al. (2014, 2015) and Pataky et al. (2017) to study early onset insulin resistance (by in vivo homeostasis model assessment of insulin resistance and insulin-stimulated 2-deoxy glucose uptake by isolated skeletal muscles and single muscle fibers), and is characterized here to elicit increased iWAT, eWAT, and rpWAT mass, resulting in a 35% increase in the sum of fat pad masses. This model is of particular interest because many of the metabolic benefits accompanying experimental *Vegf-a* overexpression in mice are observed during early HFD exposure; while, in contrast, angiogenesis is thought to exacerbate the glucose intolerance in the context of advanced/morbid obesity (Sun et al. 2012). Although we found *Ang2* and *Angptl4* expression to be elevated in some adipose depots in HFD-SED versus LFD-SED, *Pecam* expression was not higher in HFD-SED versus LFD-SED in any of the depots studied here. While using *Pecam* mRNA expression as a marker for tissue vascularization should be interpreted with caution, this could indicate that capillarization has not increased at this early stage of weight gain.

It is important to note that the early response to weight gain is different between rodents and humans, with fat mass expansion in rodents achieved more through visceral adipose tissue expansion, while fat mass expansion in humans occurs more through subcutaneous adipose tissue expansion. Furthermore, histology as well as ex vivo analyses have demonstrated a greater angiogenic capacity of human visceral adipose versus human subcutaneous adipose tissue (Gealekman et al. 2011; Song et al. 2016). Therefore, an important secondary research question to extend applicability of the experiments here was whether a depot $\times$ exercise interaction existed in the angiogenic response to exercise. There was no depot $\times$ exercise interaction in the *Vegf-a* response to exercise, indicating the signal of exercise to increase adipose tissue *Vegf-a* might increase vascularization in multiple anatomically and metabolically distinct fat pads during the early stages of weight gain.

In summary, the main finding of this study is that acute aerobic exercise increases *Vegf-a* mRNA expression in subcutaneous and intra-abdominal adipose tissue from rats in the early stages of weight gain (i.e., 2-week exposure to HFD). This supports the potential for exercise to augment adipose tissue angiogenic signaling, which may in turn attenuate weight gain-related inflammation and insulin resistance through enhanced adipose tissue vascularization.

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Fig. 1.

Effects of 2-week high-fat diet (HFD) and 2-h swim exercise (EX) versus remaining sedentary (SED) on angiogenic gene expression in male Wistar rats. eWAT, epididymal white adipose tissue; iWAT, inguinal white adipose tissue; rpWAT, retro-peritoneal white adipose tissue. $n = 7-12$. *, Different ($P < 0.05$) from HFD-SED; †, different ($P < 0.05$) from LFD-SED.

Table 1.

Effects of 2-week high-fat diet on weight gain.

	LFD	<i>n</i>	HFD	<i>n</i>	<i>P</i>	Effect size
BW(g)	278±5	24	280±6	24	0.42	0.05
iWAT(g)	1.98±0.1	24	2.45±0.1	23	0.006	0.76
eWAT (g)	1.59±0.1	24	2.22±0.1	24	7E-06	1.40
rpWAT (g)	0.93±0.1	23	1.36±0.1	24	0.0002	1.12
Sum of fat pads (g)	4.49±0.2	23	6.05±0.3	23	3E-05	1.32

Note: Values are represented as means ± SE. BW, body weight; eWAT, epididymal white adipose tissue; HFD, high fat diet; iWAT, inguinal white adipose tissue; LFD, low fat diet; rpWAT, retro-peritoneal white adipose tissue.