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Adipose vascular endothelial growth factor B is a major regulator of energy metabolism

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Abstract

Excessive fat accumulation causes obesity and many diseases. Previous study demonstrates VEGFB universal knockout induces obese phenotypes including expansion of white adipose tissue, whitening of brown adipose tissue, increase of fat accumulation and reduction in energy consumption. However, roles of VEGFB in adipose tissues are not clear. In this study, we have generated a mouse model with adipose-specific VEGFB repression using CRISPR/dCas9 system (*Vegfb^{AdipoDown}*) and investigated the roles of VEGFB in adipose development and energy metabolism. VEGFB repression induced significant changes in adipose tissue structure and function. *Vegfb^{AdipoDown}* mice have larger body sizes, larger volume of white adipose tissues than its wild type littermates. Adipose-specific VEGFB repression induced morphological and functional transformation of adipose tissues toward white adipose for energy storage. Metabolic processes are broadly changed in *Vegfb^{AdipoDown}* adipose tissues including carbohydrate metabolism, lipid metabolism, nucleotide metabolism and amino acid metabolism. We have demonstrated that adipose VEGFB repression can recapitulate most of the phenotypes of the whole body VEGFB knockout mouse. Intriguingly, approximately 50% VEGFB repression in adipose tissues can almost completely mimic the effects of universal *Vegfb* deletion, suggesting adipose VEGFB is a major regulator of energy metabolism and may be important in prevention and treatment of obesity.

Key Words

- ▶ VEGFB
- ▶ adipose development
- ▶ energy metabolism
- ▶ obesity
- ▶ mouse model

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Introduction

Obesity, an epidemic and chronic disease induced by excessive energy storage and adipose expansion, affects millions of people (Kopelman 2000, WHO Expert Consultation 2004, James 2008). An imbalanced energy intake and expenditure leads to the excessive adipose expansion (Cai *et al.* 2010). This excessive adipose

expansion is associated with hypertension, type 2 diabetes, atherosclerosis, heart disease, infertility, stroke and certain types of malignancies (Madsen *et al.* 2010, Castro *et al.* 2014, Luna-Luna *et al.* 2015, Mission *et al.* 2015, Cedikova *et al.* 2016). Adipose tissues are classified as white adipose tissues (WATs) and brown adipose tissue

(BAT). White adipocytes develop from Myf5-negative mesenchymal precursor cells (Cinti 2008, Rodeheffer *et al.* 2008) and look spherical in morphology. White adipocytes are filled with a single, large lipid droplet, small cytoplasm and few mitochondria on the edge of the cell. WAT acts as an energy storage organ in form of triglycerides. At the same time, WAT is an important secretory organ and produces leptin, adiponectin, resistin and growth factors including vascular endothelial growth factors (VEGFs) (Mick *et al.* 2002, Fain *et al.* 2004, Kershaw & Flier 2004). Brown adipocytes, in contrast, originate from precursors that express Myf5 (Kopelman 2000) and contain a huge number of mitochondria and multiple small lipid droplets for efficient energy use. BAT is rich in vasculature and expresses high levels of uncoupling protein 1 (UCP-1) and can generate heat quickly from lipid oxidation through uncoupling respiratory chain (Richard *et al.* 2010, Richard & Picard 2011, Esteve Ràfols 2014, Shimizu & Walsh 2015). BAT function is critical in maintaining body temperature and body weight. An intermediate adipose tissue is also present that is called beige or brown-like adipose tissues (bATs). Beige adipocyte has mixed characteristics of both white and brown adipocyte. It usually contains multiple lipid droplets with variable size and is rich in mitochondria. The bAT is first identified from WAT under cold exposure or exercise (Enerbäck *et al.* 1997, De Matteis *et al.* 2013, Wang *et al.* 2013, Sun *et al.* 2015). At certain condition, bAT can develop from BAT as well (Kreuger *et al.* 2006). As bAT can transform energy to heat as efficiently as BAT, understanding of development and regulatory mechanism of bAT is important in fighting obesity.

VEGFB is a member of vascular endothelial growth factors (VEGFs) that regulate vasculogenesis and lymphogenesis during embryonic development and angiogenesis in adult (Kreuger *et al.* 2006, Stüttfeld & Ballmer-Hofer 2009). Recently, VEGFB has been implicated in playing important roles in energy metabolism and lipid consumption in adipose tissue (Karpanen *et al.* 2008, Hagberg *et al.* 2010). Our previous study has demonstrated that knockout of VEGFB induces WAT expansion, WAT-associated gene upregulation, BAT whitening and BAT-associated gene downregulation (Jin *et al.* 2018). However, the contribution of adipose-specific VEGFB expression in adipose development and energy metabolism is not clear. In this study, we have generated a mouse model with adipose-specific VEGFB repression using CRISPR/dCas9 system and investigated the roles of VEGFB in adipose structure and function.

Materials and methods

Animals, VEGFB-knockdown mice and genotyping

All animal studies were approved by Institutional Animal Care and Use Committee for Animal Experimental Ethics, Committee of Northeast Normal University (IACUC/NENU). VEGFB-knockdown mouse model was constructed by CRISPR/dCas9 system. Details are provided in Supplementary Materials and methods (see section on [supplementary materials](#) given at the end of this article).

Gene expression analysis and immunoblotting analysis

Standard protocols were followed. See Supplementary Materials and methods for details.

Hematoxylin and eosin staining (H&E), oil Red O staining, immunohistochemistry and fluorescein angiography

See Supplementary Materials and methods for details of *in vitro* studies.

Intraperitoneal glucose tolerance test (IPGTT), intraperitoneal insulin tolerance test (IPITT), body temperature, oxygen consumption measurement and metabolic chamber analyses

Standard protocol were provided in Supplementary Materials and methods.

Primary preadipocyte culture, mature adipocytes induction and cell transfection

Cells were cultured in DMEM/F-12 with 20% FBS. See Supplementary Materials and methods for details.

RNA-Seq and gene ontology (GO) analysis

Genome wide gene expression was analyzed using BGISEQ-500 platform. Details are provided in Supplementary Materials and methods.

Statistical analysis

All statistical analysis for two groups was performed with SPSS software, version 20.0 (IBM). Results are expressed as mean $\pm$ S.E.M. *P* value ≤ 0.05 (unpaired Student's *t*-test)

was considered statistically significant. All graphics were prepared with GraphPad Prism 7.0 (GraphPad Software).

Results

Generation of Adipose-specific VEGFB repression mouse model using CRISPR/dCas9

To knockdown VEGFB function, two sgRNA targeting sites were selected in exon 1 region of *Vegfb* gene. Two plasmids pU6-sgRNA1-mAdiponectin-dCas9-KRAB-T2a-GFP

(pVEGFB-sgRNA1) and pU6-sgRNA2-Adiponectin-dCas9-KRAB-T2a-GFP (pVEGFB-sgRNA2) were constructed (Fig. 1A). To confirm repressive efficacy of plasmids pVEGFB-sgRNA1 and pVEGFB-sgRNA2 in VEGFB-expressing adipocytes, primary preadipocytes were isolated from sWAT of C57/BL6 mice. The primary preadipocytes were grown to confluences and then induced to mature adipocytes in culture medium containing adipocyte inducers I, II, III consecutively. To confirm successful induction, the mature adipocytes were stained with Oil Red O and assayed for adipose markers by quantitative

Figure 1

Generation of VEGFB repression mouse model using CRISPR/dCas9. (A) VEGFB gene structure and sgRNA target sites. Boxes and lines indicate exons and introns. (B) Culture of primary preadipocytes from C57/BL6 mice. Primary preadipocytes was induced with 0.5 mM IBMX, 1 μ M Dex and 10 μ g/mL insulin (top panel) and stained with Oil Red O (bottom panel). The control is uninduced preadipocytes. (C) Adipose-specific gene expression in cultured C57/BL6 primary preadipocytes by qPCR. (D) VEGFB repression analysis in transfected preadipocytes after adipokine induction. Control cell is transfected with an empty vector (Addgene #71237) and in blue and pVEGFB-sgRNA1/2-transfected adipocytes in orange. (E) Illustration of transgenic injection of pVEGFB-sgRNA1 and pVEGFB-sgRNA2 (top panel) and adipose-specific VEGFB downregulation (bottom panel). (F) Genotyping of *Vegfb*^{AdipoDown} mice by PCR. (G) Adipose-specific VEGFB gene repression analysis by qPCR. (H) Immunoblotting analysis of VEGFB protein. (I) Quantification of immunoblotting in H ($n = 3$). *Vegfb*^{AdipoDown} bars in red. Data represented by mean $\pm$ s.e.m. n.s., not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

real-time PCR (qPCR). Red color was readily visible after induction (Fig. 1B). Adipose-specific marker genes *ap2* and *Ppar γ 2* were significantly induced (Fig. 1C). The induced mature adipocytes were then transfected with plasmids pVEGFB-sgRNA1/pVEGFB-sgRNA2 and control plasmid (3 μ g each). The transfected cells were harvested 48 h later for qPCR. VEGFB mRNA was decreased by 32.4%, whereas VEGFA mRNA was increased by 32.3%. The VEGFB receptor *Vegfr1* was increased by 36.0% and *Nrp1* decreased by 24.3% respectively. No change was found of VEGFR2, the major receptor for VEGFA and angiogenesis (Fig. 1D). Results indicate that pVEGFB-sgRNA1/2 transfection successfully induced VEGFB repression and significant changes of adipose-related genes in mature adipocytes.

To generate VEGFB-knockdown mouse model, fragments span from U6 promoter to GFP about 11kb were isolated from pVEGFB-sgRNA1 and pVEGFB-sgRNA2 (Fig. 1A) and co-microinjected into C57/BL6J/DBA F1 zygotes (2.5 ng/ μ L each) (Fig. 1E). Transgenic founders with expression of sgRNAs and dCAS9-KRAB fusion proteins were identified by genomic PCR and qPCR (Fig. 1F). VEGFB mRNA expression levels of gonadal WAT (gWAT), s.c. WAT (sWAT), interscapular BAT (BAT), skeletal muscle, heart and kidney were analyzed by qPCR (Fig. 1G) and protein levels by immunoblotting (Fig. 1H). VEGFB mRNA was found decreased significantly by 46.2% in gWAT, 65.8% in sWAT and 30.6% in BAT (Fig. 1I). However, there were no significant changes of VEGFB mRNA expression in other tissues including skeletal muscle, heart and kidney. There is a similar decrease of VEGFB protein by immunoblotting assay (Fig. 1H and I). VEGFB protein showed a 72.7% decrease in gWAT, 78.3% in sWAT and 40% in BAT (Fig. 1I).

Adipose-specific VEGFB repression leads to increased energy storage

Mice with adipose-specific VEGFB repression (*Vegfb^{AdipoDown}*) had bigger body size than WT mice in the same litters (Fig. 2A). Volumes of gWAT and sWAT are significantly bigger and appear whiter. The color of BAT looks brighter as well (Fig. 2B), a phenotype of adipose whitening. Blood vessel density was decreased by 52.8% in gWAT (Fig. 2C and D) measured with cardiac perfusion of dextran-FITC and confirmed by immunohistochemistry with CD31 (Supplementary Fig. 2A and B). The mRNA levels of hypoxia-inducible transcription factor Hif-1 α was significantly decreased by 38.3% in gWAT, 39.3% in sWAT and 29.4% in BAT (Fig. 2E), indicating VEGFB knockdown did not induce oxygen stress due to reduced mitochondrial

activity and lipid metabolism. The average food intakes, food efficiency and body weight gain from week 5 to week 15 were not significantly changed in *Vegfb^{AdipoDown}* mice when compared to WT mice (Fig. 2G, H and I), while body weight was significantly higher from age 5 weeks to 15 weeks (Fig. 2F). Mice housed individually in metabolic chambers did not show significant changes in food intake, water intake, feces weight and urine volume (Supplementary Fig. 2C, D, E and F).

H&E staining demonstrated that VEGFB repression induced expansion of gWAT and sWAT (Fig. 2J). Lipid droplets were obviously fused and size increased in BAT of *Vegfb^{AdipoDown}* mice. Relative cell numbers per microscopic field were significantly decreased by 49.6% in gWAT, 58.8% in sWAT and 51.9% in BAT, and average cell size was increased by 2.0, 2.5 and 2.1 fold correspondingly (Fig. 2K and L). Expression of WAT-specific markers Leptin and Resistin were significantly increased by 74.4% and 60.7% in gWAT, 89.4% and 96.8% in sWAT, and 63.7% and 53.2% in BAT (Fig. 2M and N). In contrast, BAT-specific genes are all downregulated. Bone morphogenetic protein 7 (*Bmp7*), a growth factor of TGF family related to BAT development, was significantly decreased by 28.9% in gWAT, 58.9% in sWAT and 50.4% in BAT (Fig. 2O). The cell death-inducing DNA fragmentation factor- α -like effector (*Cidea*), a mitochondria-specific marker of BAT differentiation and thermogenesis (Nishimoto & Tamori 2017), was decreased by 35.3% in gWAT, 37.2% in sWAT and 25.6% in BAT (Fig. 2P). PRD1-BF1-RIZ1 homologous domain containing 16 (*Prdm16*), a regulator of thermogenic gene in UCP1-positive adipocytes (Lodhi *et al.* 2017), was decreased by 41.4% in gWAT, 38.5% in sWAT and 36.7% in BAT (Fig. 2Q). UCP1, a major mitochondrial inner membrane protein responsible for heat generation (Shimizu & Walsh 2015, Ikeda *et al.* 2017), was markedly decreased by 28.3% in gWAT, 52.2% in sWAT and 41.1% in BAT at mRNA levels (Fig. 2R) and 89% in sWAT and 31% in BAT at protein levels (Fig. 2S and T). These results demonstrate that adipose-specific VEGFB repression resulted in dramatic changes in adipose morphology and gene expression toward energy storage.

Adipose-specific VEGFB repression results in reduced energy expenditure

VEGFB plays important roles in energy metabolism. *Vegfb^{AdipoDown}* mice had a lower oxygen consumption and lower body temperature (Fig. 3A and B) along with increased body weight (Fig. 3C) and gWAT weight (Fig. 3D). These mice displayed a lower respiratory

Figure 2

Phenotyping of *Vegfb^{AdipoDown}* mice. (A) Representative macroscopic images of body sizes. (B) Adipose sizes. (C) Confocal images of gWAT after cardiac perfusion of dextran-FITC250000 (scale bar 100 μ m, $n = 3$). (D) Quantification of vessel density shown in C. (E) Relative mRNA levels of Hif-1a by qPCR. (F) Average body weight, (G) average food intake, (H) food efficiency and (I) average body weight gains of male mice on regular chow from week 5 to week 15 ($n = 8$). (J) Hematoxylin and eosin (H&E) staining of adipose tissues. Representative sections of adipose (original magnification 100 $\times$, $n = 6$). (K and L) Cell number and average cell area of adipose (μ m²). (M, N, O, P, Q and R) Relative mRNA levels of adipose-associated genes by qPCR, normalized to 18S mRNA ($n = 3$). (S) Immunoblotting analysis of UCP-1 ($n = 3$). (T) Quantification of immunoblotting shown in S. *Vegfb^{AdipoDown}* bars in red. Data represented by mean $\pm$ S.E.M. n.s., not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

exchange ratio (RER) as well as their WT littermate (Supplementary Fig. 2G). Body weight and gWAT weight were measured at age of 15 weeks. The average body weight and gWAT weight of *Vegfb^{AdipoDown}* to WT were 42.22 ± 0.85 g to 36.51 ± 1.24 g (Fig. 3C) and 0.86 ± 0.18 g to 0.51 ± 0.12 g (Fig. 3D) respectively. The gWAT weight rate was significantly increased as well (Fig. 3E). Triglyceride levels were significantly increased by 41.4% (Fig. 3F). Interestingly, blood glucose level and AUC of IPGTT were not significantly changed and showing a normal glucose response (Fig. 3G and H). After intraperitoneal insulin injection, blood glucose level did not change as well. Rate of glucose decay (KITT) was similar to WT, indicating insulin sensitivity was not affected (Fig. 3I and J). Results demonstrated that *Vegfb^{AdipoDown}* mice have reduced metabolic and thermogenic activities but preserved glucose sensitivity.

The whole-body VEGFB knockout induced adipose whitening and altered gene expression of lipid transporters and glucose transporters (Jin *et al.* 2018). In this study, adipose-specific VEGFB repression induced expansion and whitening of gWAT, sWAT and BAT as well. *Fatp1*, *Fatp3* and *Fatp4* were analyzed and found significantly increased in gWAT and sWAT except *Fatp2*. *Fatp2*, *Fatp3* and *Fatp4* were decreased in BAT except *Fatp1* (Fig. 3K, L, M and N). Results suggest that VEGFB repression may lead to lipid mobilization from energy-consuming BAT to energy-saving WAT. *Fatp3* and *Fatp4* have no changes in liver, heart and muscle (Supplementary Fig. 2I and J). The oil red O staining of these tissues also support the results (Supplementary Fig. 2H), indicating that adipose VEGFB reduction does not alter the fatty acid mobilization in muscle and heart. Compare to lipid transporters, glucose transporters were less significantly affected. *Glut1* was

Figure 3

Physiological analysis of *Vegfb^{AdipoDown}* mice. (A) Oxygen consumption measured by CO_2 generation. (B) Body temperature. (C) Body weight. (D) gWAT weight. (E) Percentage of adipose tissues weight against body weight. (F) Triglyceride content ($n = 6$). (G and I) IPGTT and IPITT at age of 16 weeks ($n = 6$). (H) Area under the curve (AUC) of intraperitoneal glucose tolerance test (IPGTT). (J) Rate of glucose decay (KITT) of intraperitoneal insulin tolerance test (IPITT). (K, L, M and N) The mRNA levels of fatty acid transporters (Fatps) ($n = 3$). (O, P, Q and R) The mRNA levels of glucose transporters (Gluts) ($n = 3$). *Vegfb^{AdipoDown}* bars in red. Data represented by mean $\pm$ s.e.m. n.s., not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

slightly increased in sWAT but not altered in gWAT and BAT (Fig. 3O). Glut3 and Glut8 were upregulated in BAT but downregulated in gWAT (Fig. 3P and R). Glut4 was only downregulated in gWAT. Results indicate glucose transporters may partially account for the altered energy metabolism.

Adipose VEGFB repression leads to insulin resistance on HFD

Mice were fed on high-fat diet (HFD) and assayed for metabolic changes. *Vegfb^{AdipoDown}* mice showed bigger body size than WT mice in the same litters (Fig. 4A). Larger volume of gWAT and sWAT was observed (Fig. 4B). Weight of gWAT, sWAT, BAT and fat rate were dramatically increased in *Vegfb^{AdipoDown}* mice on HFD (Fig. 4C, D, E and F). *Vegfb^{AdipoDown}* mice had a significantly higher body weight on HFD (Fig. 4G). However, food intake, food efficiency and body weight gain was not significantly changed (Fig. 4H, I and J). Body temperature was lower as expected, although both *Vegfb^{AdipoDown}* and WT have slightly higher body temperature than that on regular food (Fig. 4K). Serum triglyceride and cholesterol levels were increased in *Vegfb^{AdipoDown}* mice (Fig. 4L and M). BAT was surrounded by white adipose tissues in addition to whitening. Consistent whitening and increased lipid

droplets accumulation in BAT was observed (Fig. 4N). As expected, cell sizes of gWAT and sWAT were significantly larger than control (Fig. 4N). After 12 weeks of HFD, blood glucose levels were higher and glucose clearance was impaired in IPGTT. This is also reflected by the high AUC values (Fig. 4O and P). After intraperitoneal insulin injection, blood glucose level was higher from 15 min to 120 min (Fig. 4Q). The rate of glucose decay (KITT) is lower than WT mice (Fig. 4R), indicating that *Vegfb^{AdipoDown}* mice became insensitive to insulin. Consistently, VEGFB repression decreased expression of *Bmp7*, *Prdm16* and *UCP-1*, and increased expression of *leptin* (Fig. 4S, T, U and V). These results suggest that adipose-specific VEGFB repression can increase risk of diabetic development on high-fat diet.

VEGFB repression has broad effect on cell metabolism

RNA-Seq was used to investigate genome-wide effect of VEGFB repression. A total of 1579 DEGs (233 up and 1346 down) in WAT and 654 DEGs (282 up and 372 down) in BAT were identified. There are 1374 DEGs changed in gWAT only, 449 DEGs BAT only and 205 DEGs in both gWAT and BAT (Fig. 5A and B). KEGG pathway analysis showed that more diversified genes are involved in biological processes, including metabolism,

Figure 4

Phenotyping of *Vegfb*^{AdipoDown} mice on HFD. Representative macroscopic images of body sizes (A) and adipose sizes (B). (C) gWAT weight. (D) sWAT weight. (E) BAT weight. (F) Percentage of adipose tissues weight against body weight. Body weight gains (G), food intake (H), food efficiency (I) and body weight gains (J) of male mice on HFD from week 3 to week 12 ($n = 6$). (K) Body temperature. (L) Triglyceride content. (M) Serum cholesterol levels ($n = 5$). (N) H&E staining of adipose tissues. Representative sections of adipose (original magnification 100 $\times$, $n = 3$). (O) IPGTT and (Q) IPITT at age of 14 months ($n = 7$). (P) Area under the curve (AUC) of IPGTT. (R) Rate of glucose decay (KITT) in IPITT. (S, T, U and V) Relative mRNA levels of adipose-associated genes by qPCR, normalized to 18S mRNA ($n = 3$). *Vegfb*^{AdipoDown} bars in red. Data represented by mean $\pm$ s.e.m. n.s., not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

cellular process, environmental information processing, genetic information processing and organismal system (Fig. 5C and D). Many metabolism-related genes are differentially regulated under VEGFB (Fig. 6A, B, C, D, E and F). For example, epididymal-specific glutathione peroxidase 5 (GPX5), which is an important antioxidant and can protect cells from oxidative stress (Taylor *et al.* 2013), was dramatically increased in gWAT and decreased in BAT. Pyridoxal phosphatase (PDXP) that regulates expression of vitamin B6 (VB6) (Li *et al.* 2019) was decreased in gWAT and increased in BAT. Many mitochondrial genes were changed under VEGFB (Supplementary Fig. 1A). For example, ATP synthases were obviously decreased in gWAT and BAT (Supplementary Fig. 1A). Results demonstrate that broad range of genes involved in metabolism are changed under VEGFB.

Discussion

A new mouse model of adipose-specific VEGFB repression was generated using CRISPR-dCas9 technology to investigate VEGFB-regulated adipose development and function. Jin *et al.* generated *Vegfb*^{-/-} mice using CRISPR-Cas9 system and found that VEGFB deletion induces obese phenotype (Jin *et al.* 2018), similar to the study of Hagberg's *Vegfb*^{-/-} mice. But contribution of adipose-expressed VEGFB is not studied. *Vegfb*^{AdipoDown} mice showed larger body weight, body size and WAT expansion (Fig. 2A, B and F). Adipose-specific VEGFB repression induces morphologic and functional transformation of adipose tissues toward WAT development (Figs 2J and 4N), accompanied with upregulation of WAT-associated genes and downregulation of BAT-associated genes (Figs 2M, N, O, P, Q, R and 4S, T, U, V). In accordance with

Figure 5

KEGG analysis of DEGs. (A) Illustration of DEGs in Venn. (B) Numbers of DEGs of Vegfb-repressed to WT adipose tissues. KEGG pathway analysis of gWAT (C) and BAT (D). DEGs were selected based on expression levels ≥ 10 by fragments per kilobase of transcript per million ≥ 10 , fold change ≥ 2 ($\log_2 \geq 1$) and Q value < 0.05 .

BAT whitening, there is a reduced respiratory rate and low body temperature in *Vegfb^{AdipoDown}* mice (Fig. 3A and B). All the phenotypes basically recapitulated the ones in the universal VEGFB-knockout mouse model except insulin sensitivity in HFD condition (Fig. 4O, P, Q and R).

On regular diet, insulin sensitivity and glucose clearance are not affected by adipose VEGFB repression (Fig. 3G and I). Some studies have shown that intracellular fatty acid accumulation can directly impair insulin signaling and glucose uptake, causing insulin resistance (Schmitz-Peiffer *et al.* 1997, Boström *et al.* 2007, Schmitz-Peiffer 2010). It is seemingly healthy for adipose-specific VEGFB-knockdown mice. *Vegfb^{AdipoDown}* mice have similar food efficiency as WT mice (Fig. 2H). However, *Vegfb^{AdipoDown}* mice cannot take the high fat load (Fig. 4O, P, Q and R).

FATPs are responsible for fatty acid transport to adipose tissues (Schaffer & Lodish 1994, Hirsch *et al.* 1998, Kazantzis & Stahl 2012). Significant increase of *Fatp1*, *Fatp3* and *Fatp4* mRNA levels in adipose tissues supports the role of VEGFB on fatty acid mobilization toward WAT (Fig. 3K, L, M and N). Hagberg *et al.* found that VEGFB deletion leads to downregulation of FATP3 and FATP4 in endothelial cells of muscle, heart and BAT. This reduced uptake leads to excessive accumulation of lipids in WAT (Hagberg *et al.* 2010). We have found that increase of FATP3 and FATP4 expression in WAT help to mobilize the

lipids toward storage as well (Jin *et al.* 2018). However, the high serum triglyceride may reflect an inefficient removal of lipid by FATP3 and FATP4 in white adipose tissues on HFD. In our previous study, *Vegfb* heterozygous deletion reduced *Vegfb* mRNA levels by up to 90% in mitochondria-rich tissues including heart, skeletal muscle and BAT (Jin *et al.* 2018). This result supports that mitochondria-rich tissues rely on fatty acids as main source for energy production and heat generation (Hagberg *et al.* 2010). Relatively passive WAT cannot efficiently handle the surplus lipid on HFD.

High-throughput RNA-Seq showed that not only VEGFB repression in adipose tissue affect lipid metabolism, but also metabolism of carbohydrate, amino acid, nucleotides, cofactors and vitamins (Fig. 6). These results confirm the important regulatory roles of VEGFB on broad aspect of metabolism. Robciuc and Cao respectively revealed that VEGFB/VEGFR1 acts as a decoy pathway for VEGFA/VEGFR2. Overexpressing VEGFB or tissue-specific loss of VEGFR1 on endothelial cells results in vascular expansion and improves metabolic outcome induced by HFD (Robciuc *et al.* 2016, Seki *et al.* 2018). This is consistent with our results that interaction between VEGFB/VEGFR1 and VEGF/VEGFR2 signaling is likely involved. When VEGFB was decreased, VEGFR1 binds more VEGF and inhibits VEGF/VEGFR2 pathway.

Studies have been focused on vasculature of adipose tissues considering VEGFB's roles on development and metabolism. However, it is clear beyond the role of vasculature. Adipose tissue adapts to its environment through two processes. One is quick response by shuffling fatty acid from energy storage (white adipose) to energy generation (heart, muscle and BAT). Another is long-term adaption by adjusting ratio of tissues that stores energy (white adipose) and tissues that consumes energy (heart, muscle and BAT). Although it is a prerequisite for adipose to have proper amount of vasculature for these functional demand, VEGFB is highly expressed in mitochondria-rich

tissue and has minimal effect on vasculature development. Its major role is regulating the balance of adipose development and energy metabolism with VEGFA. As far as we know, VEGFR2 is not expressed on adipocytes and VEGFR1 does not generate downstream signals. Cao's studies on mouse and zebrafish suggest that VEGFB function through Nrp1, independent of VEGFR1 (Jensen *et al.* 2015). This is consistent to our study that VEGFR1 and Nrp1 are oppositely regulated by VEGFB in adipocytes (Fig. 1D).

In conclusion, adipose VEGFB regulates adipose development and function through up and down

regulating wide range of genes from adipogenesis to metabolism. Approximately 50% VEGFB repression in adipose tissues (gWAT, sWAT and BAT) can almost completely recapitulate the effect of whole body VEGFB deletion (Supplementary Fig. 1D). All the results confirm that adipose-expressed VEGFB is a major regulator of adipose development and functions and plays indispensable roles in metabolism.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-19-0341>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Y W Z, Y C, X C F and L Q Z conceived and designed the experiments. Y C, M Y Z, C H W, Y T Z, T T Z, H Z W, M J Z, S A, M W G and H Y C carried out experiments. Y C, M Y Z and M X L analyzed the data. Y C and D L contributed reagents/materials/analysis tools. Y W Z and Y C wrote the paper. Y W Z, Y C and X D L edited and revised manuscript; Y W Z approved final version of manuscript. All authors were involved in revising the manuscript and had final approval of the submitted and published versions.

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