Figures and figure supplements

Endothelial-specific FoxO1 depletion prevents obesity-related disorders by increasing vascular metabolism and growth

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Figure 1. Endothelial-specific depletion of Foxo1 induced in adult male mice effectively reduces FoxO1 levels in skeletal muscle and adipose microvascular beds. (A) PCR of genomic DNA from multiple organs of control (Cre+; Foxo1f/f) and EC-FoxO1 KD mice using primers for the floxed and deleted (E1ΔE3) alleles. (B–C) Gene expression analysis of microvascular EC and CD16/CD32+ cells isolated from white adipose tissue of Control (n = 6) and EC-FoxO1 KD (n = 3–5) mice. (D) Representative Western blot images and quantitative analysis of FoxO1 and β-actin levels in capillary fragments isolated from skeletal muscle (n = 3–4). Results are expressed relative to β-actin levels. Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, calculated with two-tailed unpaired t-test.

DOI: https://doi.org/10.7554/eLife.39780.003
Figure 2. Greater vascular density in visceral adipose tissue of normal chow-fed EC-FoxO1 KD mice. (A) Pecam1 mRNA levels in various tissues of Control and EC-FoxO1 KD mice after 16 weeks of normal chow (NC) diet (Control n = 6, EC-FoxO1 KD n = 5). (B) Representative confocal images of adipose tissue whole-mount staining with BODIPY 493/503 (green) and G. simplicifolia lectin (red) (×20 magnification; scale bar = 100 μm). (C–E) Lectin area (C), capillary branch density (D) and microvessel diameters (E) were quantified from confocal images (Control n = 5, EC-FoxO1 KD n = 6). Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, calculated with two-tailed unpaired t-test.

DOI: https://doi.org/10.7554/eLife.39780.004
Figure 3. EC-FoxO1 depletion strongly induces vascular growth within adipose tissue in response to HF diet. (A) Pecam1 mRNA levels in different adipose tissue depots of Control and EC-FoxO1 KD mice after 16 weeks of high-fat (HF) diet (Control n = 5–7, EC-FoxO1 KD n = 7). (B) Gene expression analysis of eWAT of HF-fed Control and EC-FoxO1 KD mice (Control n = 7, EC-FoxO1 KD n = 6–7). (C) Representative confocal images of adipose tissue whole-mount staining with BODIPY 493/503 (green) and G. simplicifolia lectin (red) (C - scale bar = 100 μm). (D,E and I). Lectin area (D) and capillary branch density were quantified from these images (Control, n = 6; EC-FoxO1 KD, n = 5 or 6). (F–G) G. simplicifolia lectin (green) and Wheat germ agglutinin (red) staining of paraffin-sectioned adipose tissue (F - scale bar = 100 μm) was used to assess capillary to adipocyte ratio (G). (H) Representative confocal images of adipose tissue whole-mount staining with Isolectin alone (grayscale; scale bar = 20 μm). (I) Microvessel diameters were quantified from confocal images (Control, n = 6, EC-FoxO1 KD, n = 6). Data in all panels are expressed as mean ± SEM; *p < 0.05, ***p < 0.001, calculated with two-tailed unpaired t-test.

DOI: https://doi.org/10.7554/eLife.39780.005
Figure 4. EC-FoxO1 depletion also favors microvascular expansion in skeletal muscle under HF diet feeding. (A) Pecam1 mRNA levels in liver and skeletal of HF-fed Control (n = 5–6) and EC-FoxO1 KD (n = 6–7) mice. (B) Images of EDL muscle stained with Isolectin-FITC to identify capillaries (scale bar = 50 μm). (C) Capillary to fiber (C:F) ratios were calculated from 3 to 4 independent fields of view per mouse (Control n = 6, EC-FoxO1 KD n = 6). (D) Representative EM images of capillaries within skeletal muscle from HF-fed Control and EC-FoxO1 KD mice (x6.5k magnification; scale bar = 2 μm). (E–F) EC cross-sectional area (E) and capillary luminal diameter were quantified from EM images from n = 4 mice per group, with individual capillary measurements shown (F). Data in all panels are expressed as mean ± SEM; *p < 0.05, calculated with two-tailed unpaired t-test.

DOI: https://doi.org/10.7554/eLife.39780.006
Figure 5. EC-FoxO1 KD mice exhibit a healthier adipose tissue expansion in response to HF diet. (A) Body weights during 16 weeks of HF feeding. (B) Summarized weight gain over the course of 0–14 weeks (Control n = 7, EC-FoxO1 KD n = 7). (C) Abdominal transverse micro-CT images of HF-fed Control (n = 5) and EC-FoxO1 KD (n = 6) mice (upper panel). Fat content (shown in white) was calculated as % of total trunk volume. (D) Representative hematoxylin and eosin-stained images of adipose tissue from the epididymal fat pad (scale bar = 100 μm). (E) Mean adipocyte cross-sectional area (Control n = 5 EC-FoxO1 KD n = 5). (F) mRNA for browning markers Ucp1 and Prdm16 relative to Hprt1 (Control n = 7, EC-FoxO1 KD n = 7). (G–H) Representative Western blot images and quantitative analysis (H) of pSer473-Akt and total Akt levels in eWAT after ex vivo incubation in the absence or presence of insulin. Results are expressed relative to total Akt levels (Control n = 4, EC-FoxO1 KD n = 7). (I–J) mRNA for adipokines (I, Adipoq and Leptin) and angiogenic markers (J, Vegfa and Apln) in eWAT relative to Hprt1 (Control n = 6–7, EC-FoxO1 KD n = 5–7). Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, calculated with two-tailed unpaired t-test. DOI: https://doi.org/10.7554/eLife.39780.007
Figure 5—figure supplement 1. Lower circulating and liver triglycerides in HF-fed EC-FoxO1 KD mice. (A) Average weekly caloric intake of mice determined between diet weeks 4 to 11 from only single housed mice (Control n = 4, EC-FoxO1 KD n = 5). (B–C) Non-fasting serum levels of triglycerides (B) and glycerol (C) of Control (n = 7) and EC-FoxO1 KD (n = 7–8) mice. (D) Triglyceride concentration in the skeletal muscle (gastrocnemius) and liver (Control n = 6, EC-FoxO1 KD n = 7).

DOI: https://doi.org/10.7554/eLife.39780.008
Figure 5—figure supplement 2. EC-Foxo1 depletion has no impact on adipose mitochondrial content and respiration, or sensitivity to isoproterenol. (A–B) Representative Western blot images (A) and quantitative analysis (B) of mitochondrial OXPHOS proteins in total homogenates from eWAT of HF-fed Control (n = 7) and EC-FoxO1 KD mice (n = 9). Results are expressed relative to β-actin. (C) Analysis of uncoupled (State 2) and ADP-stimulated (State 3) respiration rates in eWAT from HF-fed Control (n = 7) and EC-FoxO1 KD mice (n = 9) supported by Complex I (NADH from pyruvate/malate, PM, and glutamate, G) and Complex II (FADH2 from succinate, S). (D–E) Representative Western blot images (D) and quantitative analysis (E) of pSer563-HSL and total HSL levels in eWAT after ex vivo incubation in the absence or presence of 10 μmol/L isoproterenol for 30 min. Results are expressed relative to total HSL levels (Control n = 3, EC-FoxO1 KD n = 7). Data in all panels are expressed as mean ± SEM; *p < 0.05, ***p < 0.001, calculated with two-tailed paired t-test.

DOI: https://doi.org/10.7554/eLife.39780.009
Figure 6. EC-Foxo1 depletion improves glucose homeostasis in HF-fed mice. (A–D) O₂ consumption (A), CO₂ production (B), Respiratory exchange ratio - RER (C) and daily activity (D) were measured during indirect calorimetry tests using a comprehensive laboratory animal monitoring system (CLAMS, Control n = 5, EC-FoxO1 KD n = 6). (E) Glucose tolerance of HF-fed Control and EC-FoxO1 KD mice was examined by intraperitoneal glucose tolerance test after 15 weeks of HF diet and 16 hr fasting. (F) Area under the curve (AUC, Control n = 7, EC-FoxO1 KD n = 7). (G) Insulin sensitivity of HF-fed Control and EC-FoxO1 KD mice was assessed by intraperitoneal insulin tolerance test after 14 weeks of HF diet and 4 hr fasting. (H) Area over the curve (AOC, Control n = 14, EC-FoxO1 KD n = 16). (I) Plasma glucose levels of HF-fed Control (n = 14) and EC-FoxO1 KD (n = 16) mice after 4 hr fasting. (J–K) eWAT gene expression analysis by qPCR (Control n = 6–7, EC-FoxO1 KD n = 6). Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, calculated with two-tailed unpaired t-test (F, I, J, K) or post hoc Bonferroni-corrected t-tests when a statistical significance was detected by two-way ANOVA model (A,C,D).

DOI: https://doi.org/10.7554/eLife.39780.011
Figure 6—figure supplement 1. EC-FoxO1 depletion has no effect on muscle insulin sensitivity. Control and EC-FoxO1 KD mice fed NC or HF diet for 16 weeks were analyzed for muscle insulin sensitivity. Representative Western blot images (top) and quantitative analysis (bottom) of pSer473-Akt and total Akt levels in EDL muscle before and after in vivo insulin injection. Results are expressed relative to total Akt levels (NC-fed mice: Control n = 7, EC-FoxO1 KD n = 6; HF-fed mice: Control n = 6 and EC-FoxO1 KD n = 6). Data are expressed as mean ± SEM; *p < 0.05, calculated with two-tailed paired t-test.

DOI: https://doi.org/10.7554/eLife.39780.012
EC-Foxo1 depletion does not affect glucose homeostasis in NC-fed mice. Glucose metabolism was assessed in Control and EC-FoxO1 KD mice after 16 weeks of NC diet. (A) Glucose tolerance of Control and EC-FoxO1 KD mice was examined by intraperitoneal glucose tolerance test after 15 weeks of NC diet and 16 hr fasting (Control n = 7 and EC-FoxO1 KD n = 6). (B) Area under the curve (AUC). (C) Insulin sensitivity of Control and EC-FoxO1 KD mice was assessed by intraperitoneal insulin tolerance test after 14 weeks of NC diet and 4 hr fasting. (D) Area over the curve (AOC, Control n = 7 and EC-FoxO1 KD n = 6). (E) Plasma glucose levels after 4 hr fasting (Control n = 7 and EC-FoxO1 KD n = 6). Data in all panels are expressed as mean ± SEM.

DOI: https://doi.org/10.7554/eLife.39780.013
FoxO1 is a critical regulator of glucose metabolism in EC. (A, B, F) Gene expression analysis of EC fraction from adipose tissue from Control (n = 3–6) and EC-FoxO1 KD (n = 4–5) mice fed a HF diet for 7 weeks. (C–E) Increased glucose uptake (C) glucose consumption (D) and lactate production (E) in EC fraction from HF-fed EC-FoxO1 KD (n = 4) mice compared to Control (n = 4–5). Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, calculated with two-tailed unpaired t-test (A,B,C,F) or post hoc Bonferroni-corrected t-tests when a statistical significance was detected by two-way ANOVA model (D,E). DOI: https://doi.org/10.7554/eLife.39780.014
Figure 8. Pharmacological inhibition of FoxO1 in SMEC reproduces in vitro the endothelial phenotype observed with EC-Foxo1 depletion. (A–B) Representative Western blot images (A) and quantitative analysis (B) of FoxO1 and β-actin levels in primary EC from skeletal muscle cultivated under low (5 mmol/L) or high (25 mmol/L) glucose conditions for 48 hr (n = 5). Results are expressed relative to β-actin levels. (C–E) Transcript analysis by qPCR of microvascular EC from skeletal muscle cultivated under low (5 mmol/L, n = 8) or high (25 mmol/L, n = 8) glucose conditions for 48 hr in the presence or absence of the FoxO1 inhibitor (1 μmol/L AS1842856, n = 7) in the last 18 hr. (F–I) Representative Western blot images and quantitative analysis of hexokinase II (HK2, (F,G)), PFKFB3 (H, I) and β-actin levels in primary EC from skeletal muscle cultivated under high glucose (25 mmol/L) conditions and treated with 1 μmol/L AS1842856 for 24 hr (n = 6). Results are expressed relative to β-actin levels. (J) Glucose uptake after 18 hr treatment with 1 μmol/L AS1842856 of microvascular EC from skeletal muscle cultivated under high glucose conditions (n = 3). (K–L) Glucose consumption (K) and Lactate production (L) were assessed in SMEC in the absence or presence of 1 μmol/L AS1842856. Cells were pretreated with 1 μmol/L AS1842856 for 24 hr (n = 4). Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, calculated with two-tailed unpaired t-test (B,J), post hoc Bonferroni-corrected t-tests when a statistical significance was detected by two-way ANOVA model (C–E, K–L) or two-tailed paired t-test (G,I).

DOI: https://doi.org/10.7554/eLife.39780.015
Figure 8—figure supplement 1. High-glucose conditions induce the expression of FoxO1 target genes. (A–B) Gene expression analysis of cultured skeletal muscle ECs in low (5 mmol/L, n = 8) and high glucose (25 mmol/L, n = 8) conditions. Data in all panels are expressed as mean ± SEM; **p < 0.01, ***p < 0.001 calculated with two-tailed unpaired t-test.
DOI: https://doi.org/10.7554/eLife.39780.016
Figure 9. EC-Foxo1,3 depletion increases vascular growth and upregulates endothelial glycolytic processes comparable to EC-Foxo1 depletion. (A) PCR of genomic DNA from eWAT of Control (Foxo1,3 flox/flox) and EC-FoxO1,3 KD mice using primers for the floxed and deleted alleles for each gene. (B) Foxo1 and Foxo3 mRNA levels in microvascular EC from white adipose tissue (Control n = 4, EC-FoxO1,3 KD n = 3). (C) Glucose levels after 14 weeks of HF feeding and 4 hr fasting (Control n = 6, EC-FoxO1,3 KD n = 6). (D) Endothelial cell marker Pecam1 mRNA level in skeletal muscle and eWAT of HF-fed Control and EC-FoxO1,3 KD mice (Control n = 5, EC-FoxO1,3 KD n = 5). (E–F) Gene expression analysis by qPCR of eWAT (E) and EC fraction from adipose tissue (F) of HF-fed Control (n = 3–5) and EC-FoxO1,3 KD (n = 3–5) mice. (G–H) Increased glucose uptake (G) and lactate production (H) in EC fraction from adipose tissue of EC-FoxO1,3 KD mice (n = 4) compared to Control counterparts (n = 4–5). Data in all panels are expressed as mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001, calculated with two-tailed unpaired t-test. 
DOI: https://doi.org/10.7554/eLife.39780.017
Figure 10. Schematic depicting the influence of FoxO1 in controlling the response of ECs to a HF diet. In wild-type mice, FoxO1 represses glycolysis, which prevents endothelial cell growth. This results in impaired angiogenesis during adipose tissue expansion as well as dysfunction of the adipose tissue, which consequently leads to decreased glucose tolerance and increased serum and intra-tissue levels of triglycerides. Conversely, when FoxO1 is depleted in endothelial cells, up-regulation of glycolytic genes accelerates glycolysis, which supports increased cellular metabolism, growth and proliferation. This, in turn, increases the nutrient demand of endothelial cells, resulting in higher uptake and consumption of glucose and an increased production of lactate. The accelerated endothelial cell growth ultimately preserves adipose tissue functions and promotes improved systemic glucose tolerance and lipid metabolism.

DOI: https://doi.org/10.7554/eLife.39780.018