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High-fat overfeeding impairs peripheral glucose metabolism and muscle microvascular eNOS Ser1177 phosphorylation.

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Article

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Table 1. Body mass, fasting biochemical blood parameters, and HOMA-IR before (pre) and after (post) 7-days of high-fat overfeeding

	Pre	Post	Significance
Body mass (kg)	77.65 ± 3.02	78.97 ± 3.06	<i>P</i> < 0.001
Glucose (mmol/L)	4.87 ± 0.08	5.06 ± 0.08	<i>P</i> = 0.027
Insulin (pmol/L)	67 ± 6	80 ± 7	<i>P</i> = 0.019
NEFA (mmol/L)	0.56 ± 0.08	0.35 ± 0.04	<i>P</i> = 0.003
TAG (mmol/L)	0.82 ± 0.07	0.60 ± 0.06	<i>P</i> < 0.001
Total cholesterol (mmol/L)	3.75 ± 0.15	3.88 ± 0.12	<i>P</i> = 0.034
HDL (mmol/L)	1.32 ± 0.08	1.56 ± 0.08	<i>P</i> < 0.001
LDL (mmol/L)	2.16 ± 0.14	2.06 ± 0.14	<i>P</i> = 0.113
HOMA-IR	2.1 ± 0.2	2.6 ± 0.2	<i>P</i> = 0.011

This file contains Table 1 and Figures 1-6 of the final manuscript accepted for publication in the *J Clin Endocrinol Metab.* 2019 Sep 12. pii: dgz018. doi: 10.1210/clinem/dgz018. [Epub ahead of print]

Table 1. NEFA, non-esterified fatty acids; TAG, triacylglycerol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance. Data presented are means ± SEM (*n* = 15).

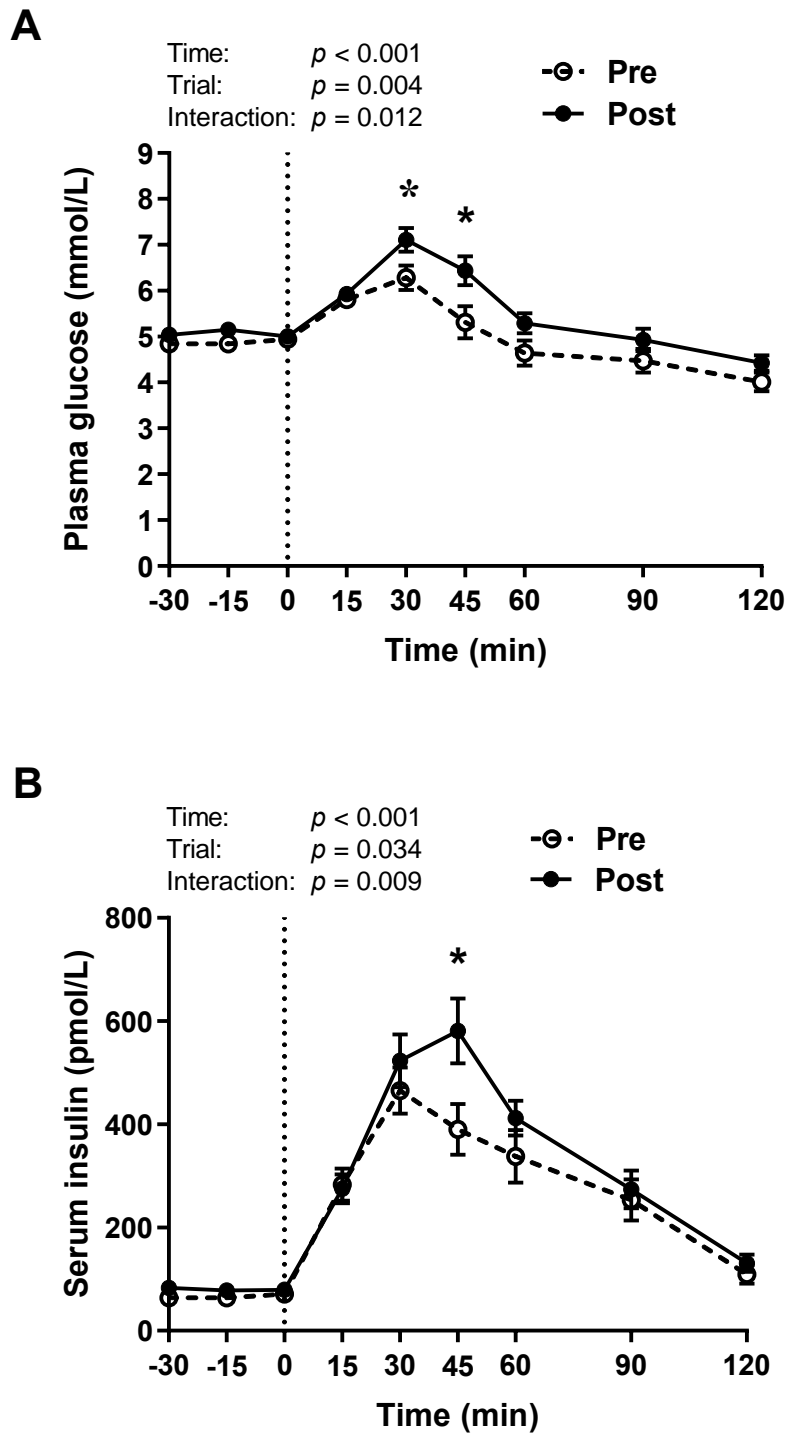


Fig 1. Plasma glucose (A) and serum insulin (B) before (pre) and after (post) 7 days of high-fat overfeeding. Time points -30 – 0 min represent the final 30 min of the 2-h pre-infusion period. All subsequent time points are following the ingestion of carbohydrate plus protein (indicated by dotted line). Data presented are means \pm SEM ($n = 15$). *significantly different between trials at the annotated time point ($P < 0.05$).

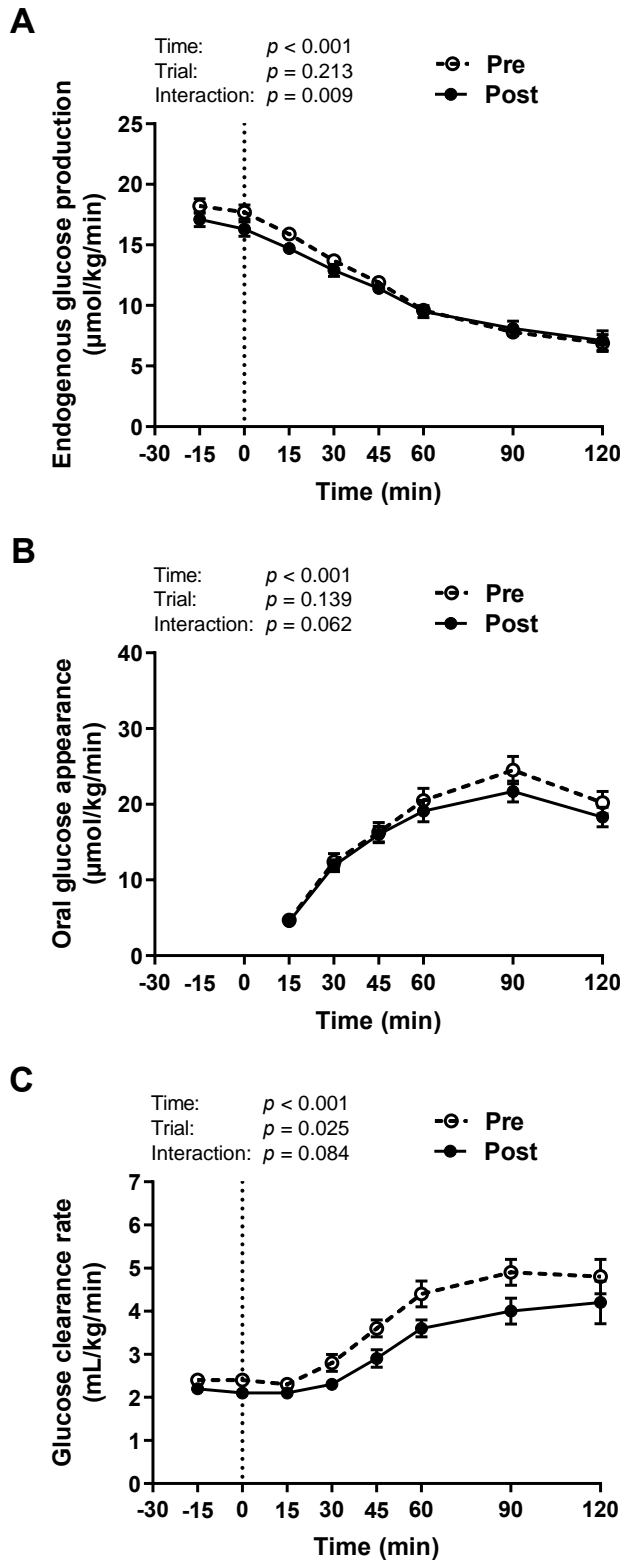


Figure 2. Endogenous glucose production (EGP) (A), oral glucose appearance (B), and whole-body glucose clearance rate (C) before (pre) and after (post) 7 days of high-fat overfeeding. Time points -30 – 0 min represent the final 30 min of the initial 2-h pre-infusion period. All subsequent time points are following ingestion of carbohydrate plus protein (indicated by dotted line). Data presented are means \pm SEM ($n = 14$).

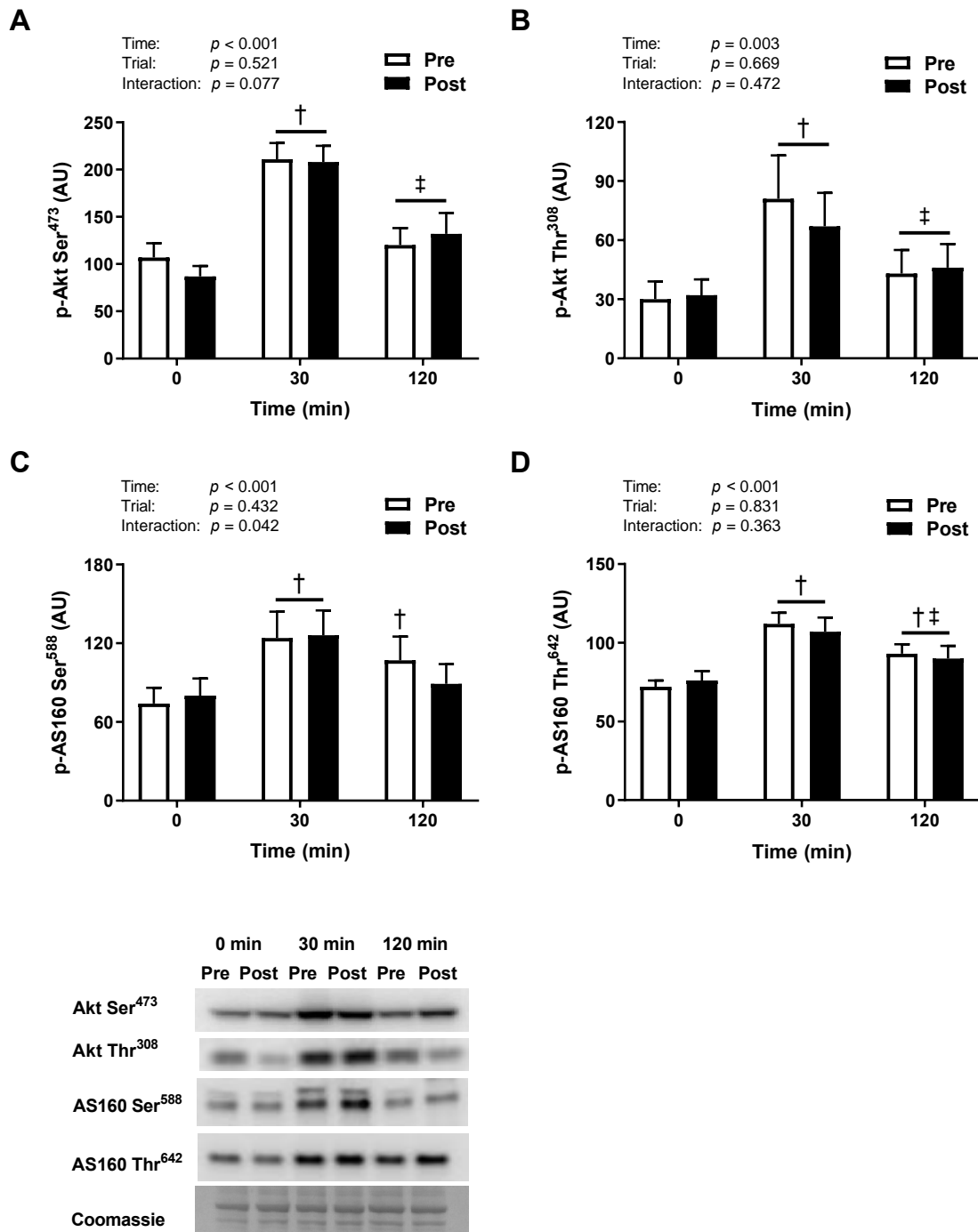


Figure 3. Phosphorylation of skeletal muscle Akt Ser⁴⁷³ (A), Akt Thr³⁰⁸ (B), AS160 Ser⁵⁸⁸ (C), and AS160 Thr⁶⁴² (D) during fasting and following ingestion of carbohydrate plus protein, before (pre) and after (post) 7 days of high-fat overfeeding. Data presented are means \pm SEM ($n = 13$). AU, arbitrary units. †significantly higher than 0 min ($P < 0.05$). ‡significantly lower than 30 min ($P < 0.05$).

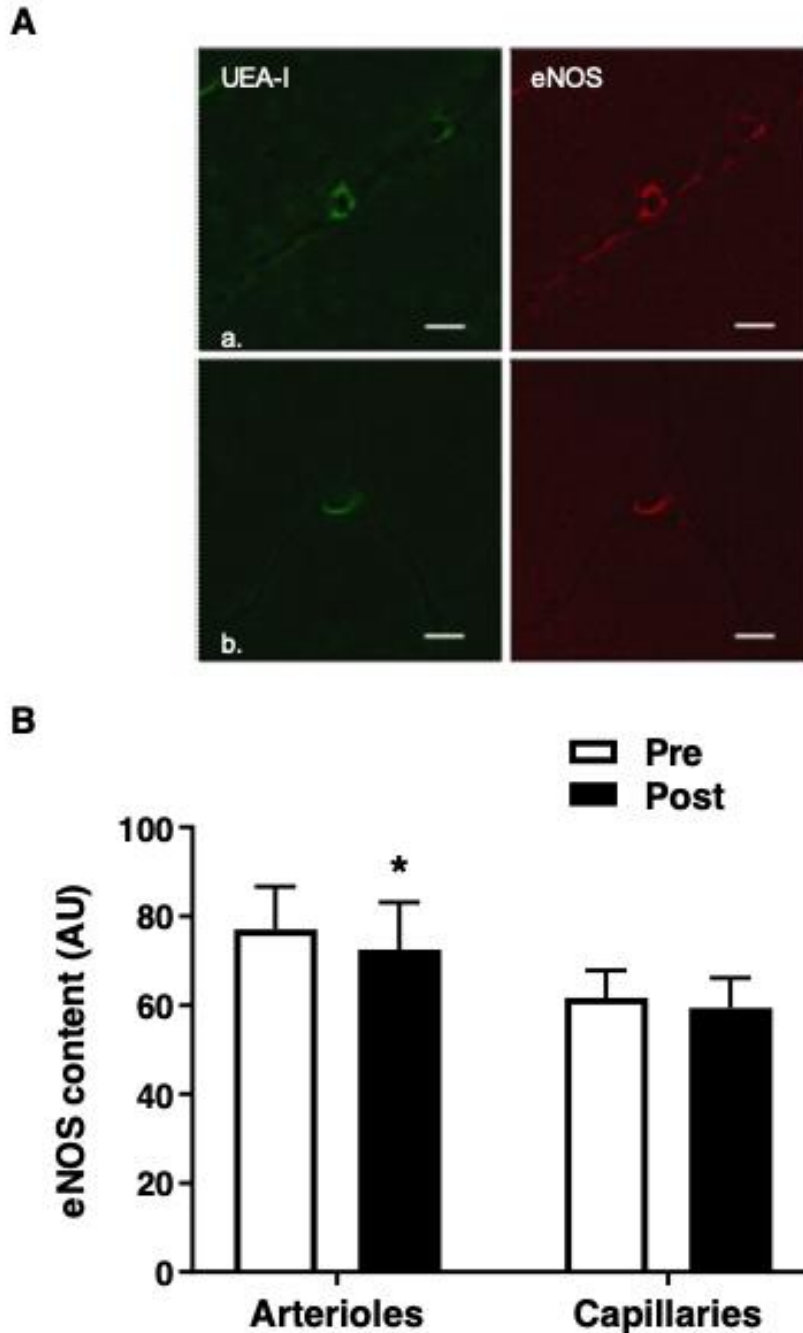


Figure 4. eNOS content in terminal arterioles and capillaries before (pre) and after (post) 7 days of high-fat overfeeding. A, representative confocal microscopy images of skeletal muscle arterioles from pre- (a) and post-high-fat overfeeding (b). The skeletal muscle microvascular endothelium was revealed using Ulex europaeus-FITC conjugated lectin (UEA-I) (green). Skeletal muscle eNOS expression was revealed using Alexa Fluor 546 conjugated secondary antibody (red). Images not shown, arterioles and capillaries were differentiated using anti- α smooth muscle actin in combination with Alexa Fluor 405 conjugated secondary antibody. Bar represents 10 μ m. B, mean fluorescence intensity of eNOS is summarized. Data presented as means \pm SEM ($n = 12$). *Significantly lower than before high-fat overfeeding ($P < 0.05$).

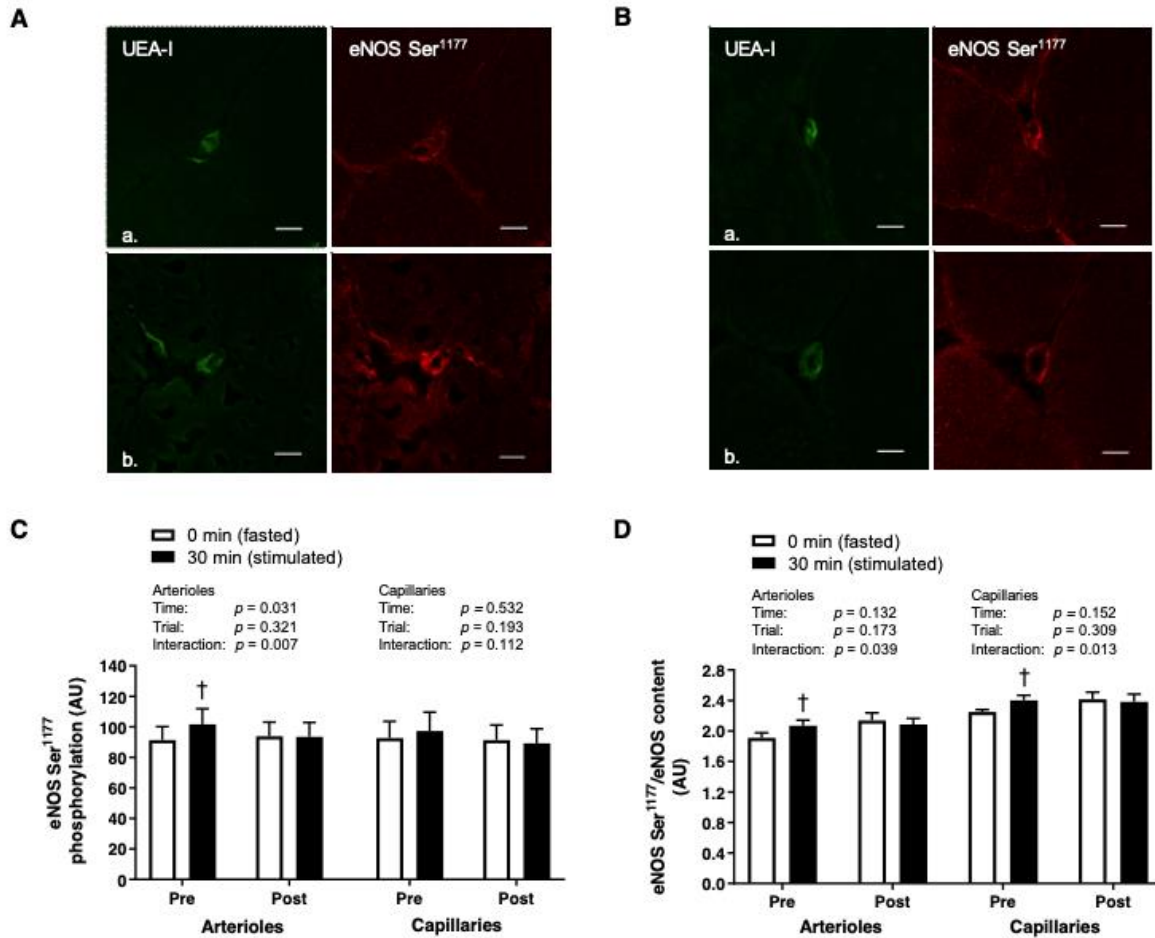


Figure 5. eNOS phosphorylation in terminal arterioles and capillaries during fasting (0 min) and 30 min after consuming carbohydrate plus protein, before (pre) and after (post) 7 days of high-fat overfeeding. A and B, representative confocal microscopy images of skeletal muscle arterioles from pre- (A) and post-high-fat overfeeding (B), in the fasted (a) and stimulated (b) state. The skeletal muscle microvascular endothelium was revealed using Ulex europaeus-FITC conjugated lectin (UEA-I) (green). Skeletal muscle eNOS Ser₁₁₇₇ phosphorylation was revealed using Alexa Fluor 633 conjugated secondary antibody (red). Images not shown, arterioles and capillaries were differentiated using anti- α smooth muscle actin in combination with Alexa Fluor 405 conjugated secondary antibody. Bar represents 10 μ m. C, mean fluorescence intensity of eNOS Ser₁₁₇₇ is summarized. D, eNOS Ser₁₁₇₇ phosphorylation normalized to eNOS content. Data presented as means \pm SEM (n = 12). †Significant increase from 0 min (fasted) ($P < 0.05$).

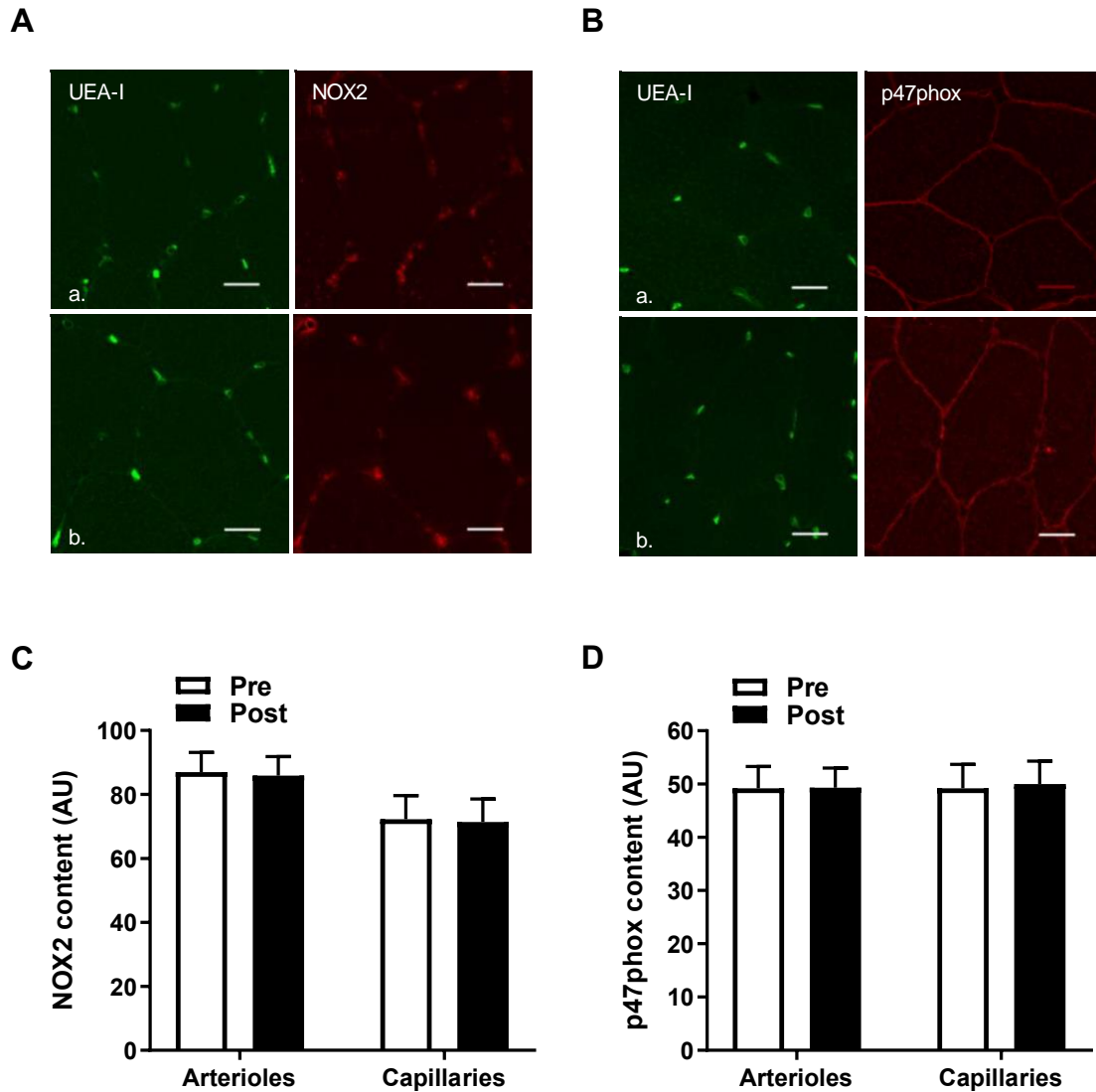


Figure 6. NOX2 and p47phox content in terminal arterioles and capillaries before (pre) and after (post) 7 days of high-fat overfeeding. A and B, representative confocal microscopy images of skeletal muscle from pre- (a) and post-high-fat overfeeding (b), illustrating NOX2 (A) and p47phox (B). The skeletal muscle microvascular endothelium was revealed using Ulex europaeus-FITC conjugated lectin (UEA-I) (green). Skeletal muscle NOX2 and p47phox expression were revealed using an Alexa Fluor 546 conjugated secondary antibody (red). Images not shown, arterioles and capillaries were differentiated using anti- α smooth muscle actin in combination with Alexa Fluor 405 conjugated secondary antibody. Bar represents 25 μ m. C, mean fluorescence intensity of NOX2 is summarized. D, mean fluorescence intensity of p47phox is summarized. Data presented as means \pm SEM (n = 12).