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

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 $\pm$ SEM ($n = 15$).
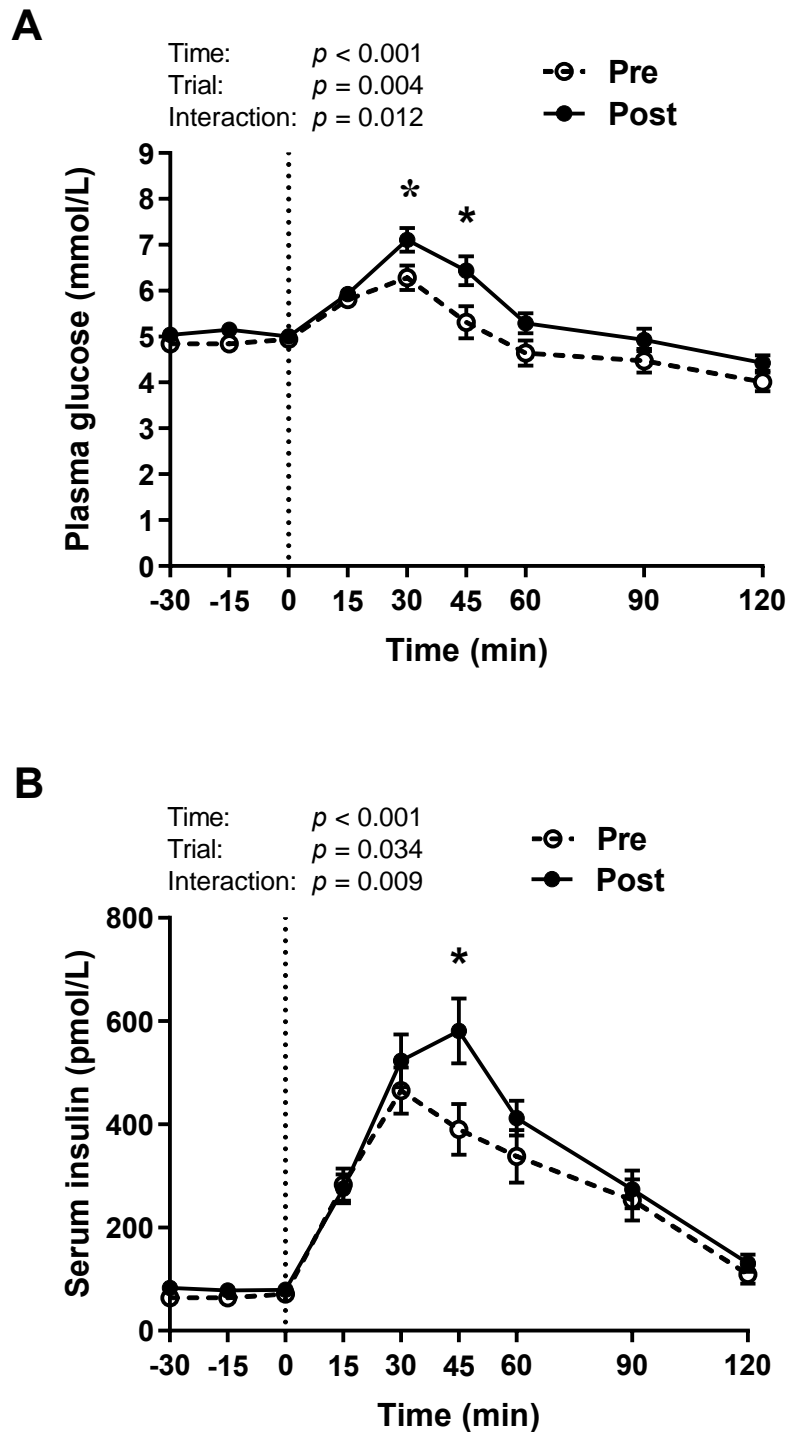
Fig 1. Plasma glucose (A) and serum insulin (B) before (pre) and after (post) 7 days of high-fat overfeeding. Time points -30 – 0 min represent the final 30 min of the 2-h pre-infusion period. All subsequent time points are following the ingestion of carbohydrate plus protein (indicated by dotted line). Data presented are means ± SEM ($n = 15$). *significantly different between trials at the annotated time point ($P < 0.05$).
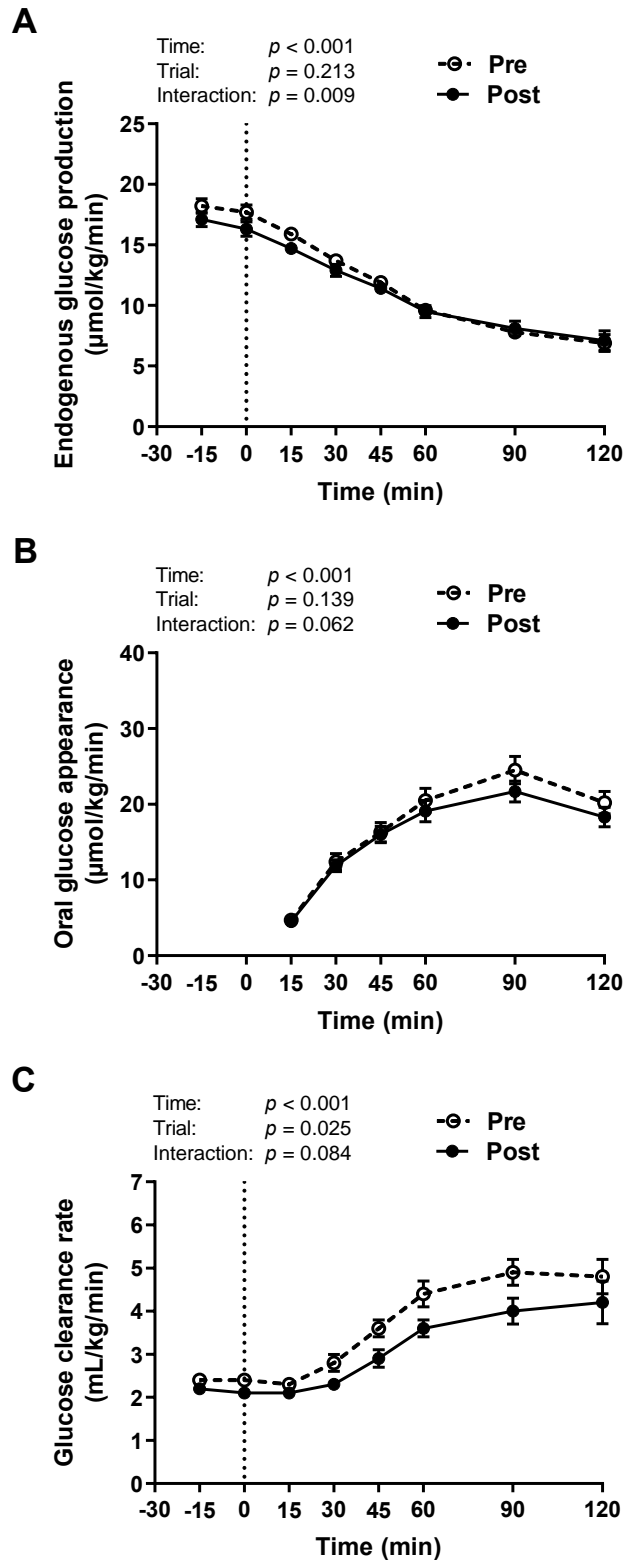
Figure 2. Endogenous glucose production (EGP) (A), oral glucose appearance (B), and whole-body glucose clearance rate (C) before (pre) and after (post) 7 days of high-fat overfeeding. Time points -30 – 0 min represent the final 30 min of the initial 2-h pre-infusion period. All subsequent time points are following ingestion of carbohydrate plus protein (indicated by dotted line). Data presented are means ± SEM ($n = 14$).
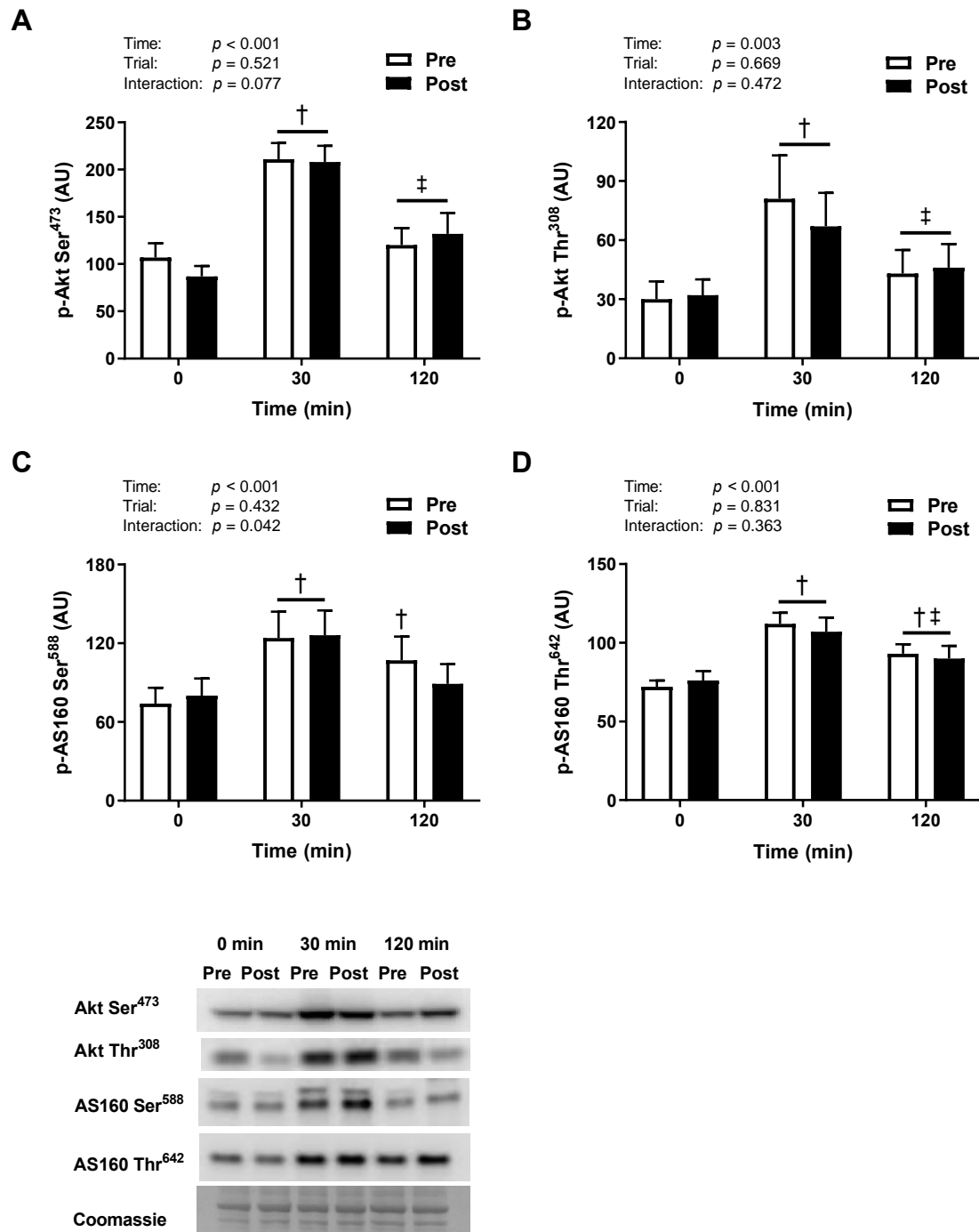
Figure 3. Phosphorylation of skeletal muscle Akt Ser$_{473}$ (A), Akt Thr$_{308}$ (B), AS160 Ser$_{588}$ (C), and AS160 Thr$_{642}$ (D) during fasting and following ingestion of carbohydrate plus protein, before (pre) and after (post) 7 days of high-fat overfeeding. Data presented are means $\pm$ SEM ($n = 13$). AU, arbitrary units. †significantly higher than 0 min ($P < 0.05$). ‡significantly lower than 30 min ($P < 0.05$).
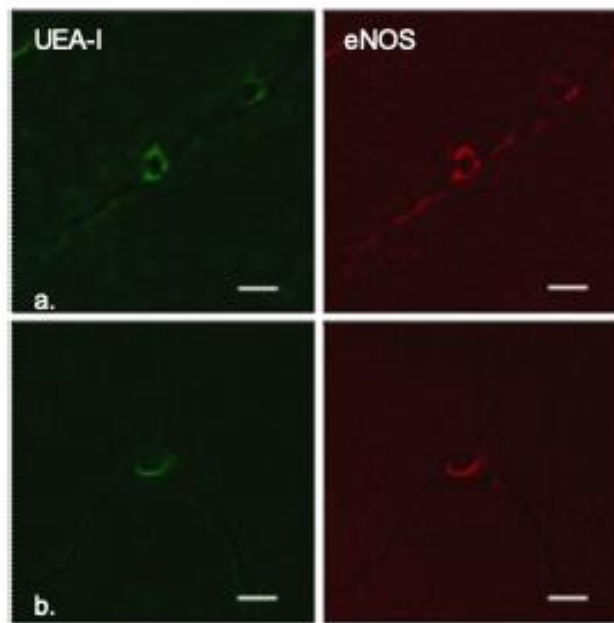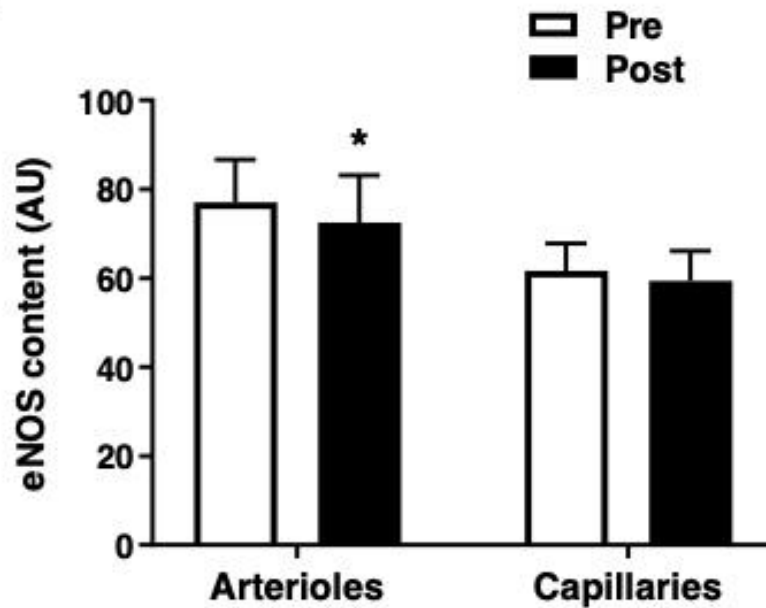
Figure 4. eNOS content in terminal arterioles and capillaries before (pre) and after (post) 7 days of high-fat overfeeding. A, representative confocal microscopy images of skeletal muscle arterioles from pre- (a) and post-high-fat overfeeding (b). The skeletal muscle microvascular endothelium was revealed 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 μm. B, mean fluorescence intensity of eNOS is summarized. Data presented as means ± SEM ($n = 12$). *Significantly lower than before high-fat overfeeding ($P < 0.05$).
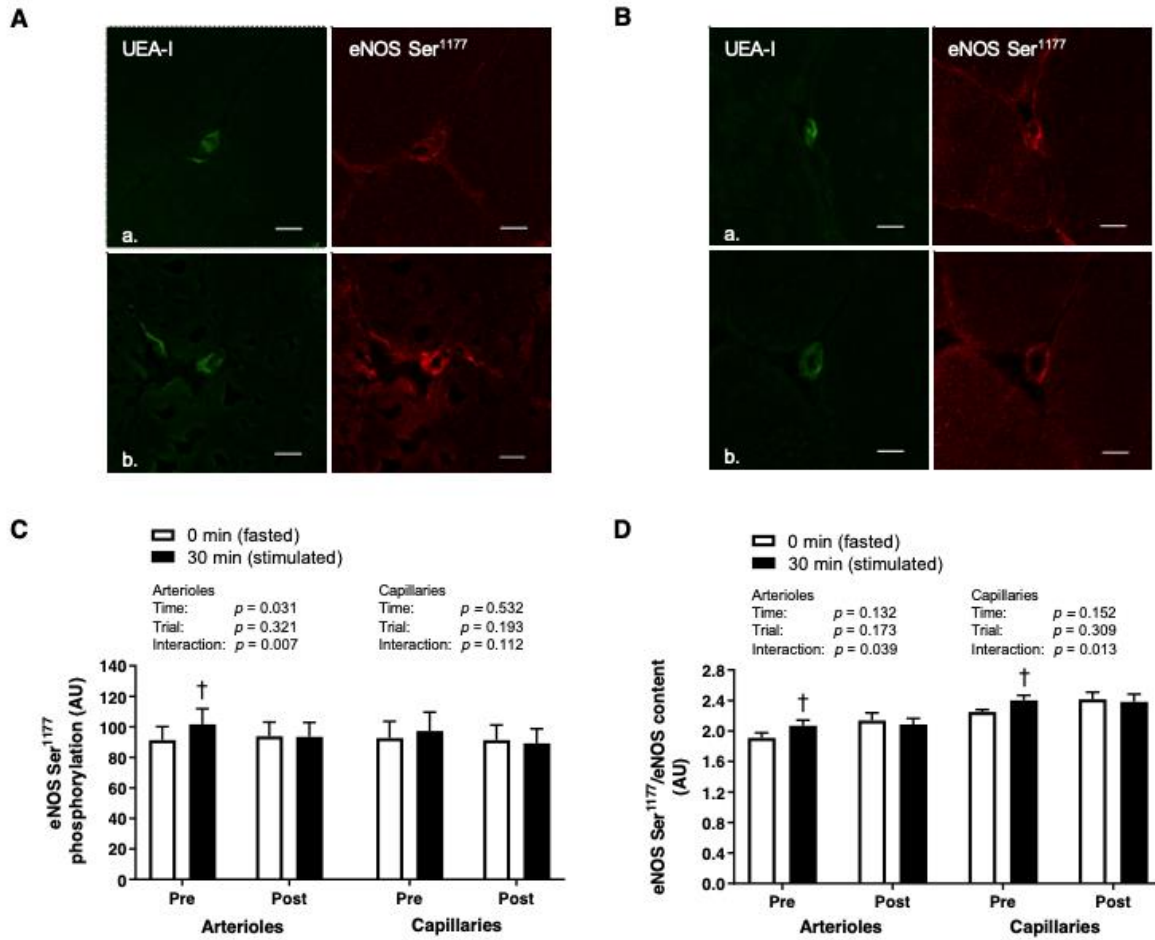
Figure 5. eNOS phosphorylation in terminal arterioles and capillaries during fasting (0 min) and 30 min after consuming carbohydrate plus protein, before (pre) and after (post) 7 days of high-fat overfeeding. A and B, representative confocal microscopy images of skeletal muscle arterioles from pre- (A) 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 Ser$_{1177}$ 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 μm. C, mean fluorescence intensity of eNOS Ser$_{1177}$ is summarized. D, eNOS Ser$_{1177}$ phosphorylation normalized to eNOS content. Data presented as means ± SEM (n = 12). †Significant increase from 0 min (fasted) ($P < 0.05$).
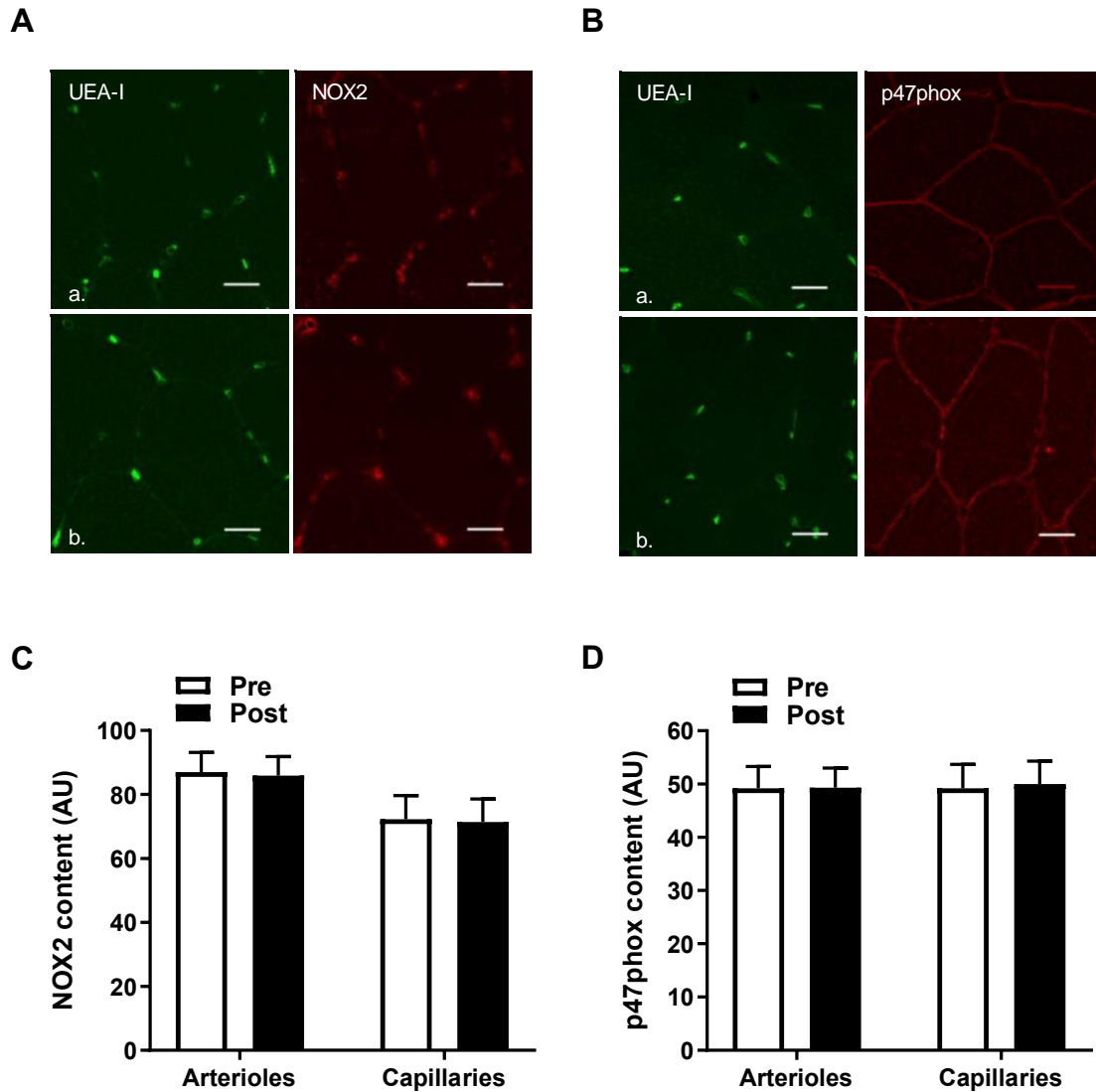
Figure 6. NOX2 and p47phox content in terminal arterioles and capillaries before (pre) and after (post) 7 days of high-fat overfeeding. A and B, representative confocal microscopy images of skeletal muscle from pre- (a) and post-high-fat overfeeding (b), illustrating NOX2 (A) and p47phox (B). The skeletal 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 μm. C, mean fluorescence intensity of NOX2 is summarized. D, mean fluorescence intensity of p47phox is summarized. Data presented as means ± SEM (n = 12).