# Angiotensin II receptor blockade and skeletal muscle metabolism in overweight and obese adults with elevated blood pressure

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# Abstract

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**Objectives:** Whether angiotensin II receptor blockade improves skeletal muscle fatty acid oxidation in overweight and obese humans is unknown. The purpose of the study was to test the hypothesis that the angiotensin II receptor blocker, olmesartan, would increase fatty acid oxidation and the activity of enzymes associated with oxidative metabolism in skeletal muscle of overweight and obese humans.

**Methods:** A total of 12 individuals (6 men and 6 women) aged 18–75 and with a body mass index  $\geq$ 25 kg/m<sup>2</sup> were assigned to olmesartan or placebo for 8 weeks in a crossover fashion. Fatty acid oxidation was measured before and after each intervention by counting the <sup>14</sup>CO<sub>2</sub> produced from [1-<sup>14</sup>C] palmitic acid in skeletal muscle homogenates.

**Results:** Fatty acid oxidation was not significantly different between treatment periods at baseline and post intervention. In addition, the enzyme activities of citrate synthase and  $\beta$ -hydroxyacyl-coenzyme A dehydrogenase in skeletal muscle homogenates did not differ between treatment periods at baseline or post intervention.

**Conclusions:** Treatment with olmesartan for 8 weeks does not improve fatty acid oxidation or the activity of enzymes associated with oxidative metabolism in skeletal muscle from overweight and obese individuals. Taken together, our results indicate that improvements in skeletal muscle metabolism are not among the additional benefits of olmesartan that extend beyond blood pressure reduction.

Keywords: angiotensin II receptor blockade, obesity, skeletal muscle metabolism

### Introduction

Obesity is associated with activation of the renin angiotensin system and an increased risk of development for the metabolic syndrome, type 2 diabetes (T2D) and hypertension [Davy and Hall, 2004; Rahmouni *et al.* 2005; Després *et al.* 2008]. Accumulating evidence has implicated angiotensin II (Ang II) in the development of the metabolic syndrome and T2D in obese individuals with hypertension [Henriksen *et al.* 2001; Abuissa *et al.* 2005; Sloniger *et al.* 2005; Wei *et al.* 2008]. The results from studies in rodents indicate that Ang II infusion reduces fatty acid oxidation (FAO) in skeletal muscle [Mitsuishi *et al.* 2009]. In turn, angiotensin II receptor blockade (ARB) normalizes FAO in rodent skeletal muscle. Furthermore, Moors and colleagues recently reported reductions in the saturation of triacylg-lycerol (TAG) and diacylglycerol (DAG) fractions in skeletal muscle following ARB treatment in patients with impaired glucose tolerance [Moors *et al.* 2013]. The reductions in intramuscular lipid saturation were not accompanied by alterations in the uptake of  $[^{2}H_{2}]$ -palmitate or very low density lipoprotein TAG, suggesting that increased FAO following ARB treatment might play a role.

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However, whether ARB improves skeletal muscle FAO in overweight and obese humans is unknown. The purpose of the present study was to test the hypothesis that the ARB, olmesartan, would increase FAO and the maximal activity of enzymes associated with oxidative metabolism in skeletal muscle of overweight and obese humans.

## Methods

#### Study participants

A total of 12 (6 men and 6 women) overweight and obese [body mass index (BMI)  $\ge 25 \text{ kg/m}^2$  or a body fat of  $\geq 20\%$  for men and  $\geq 25\%$  for women] adults (18-75 years) who were included our previous trial [Marinik et al. 2013] comprised the study sample since an adequate amount of muscle sample was acquired from these individuals for subsequent metabolic analyses (see below). All participants had elevated blood pressure (BP) (≥120/80 mmHg but <160/100 mmHg), but were free from overt cardiovascular disease, as assessed by physical examination and medical history. In addition, all participants were sedentary (defined as moderate to hard physical activity  $\leq$ 3 days/week), weight stable (±2.0 kg) for the prior 6 months, nonsmokers, and were not taking any medications that would influence any study outcomes (including lipid lowering medications and drugs with anti-inflammatory actions). The nature, purpose, risks and benefits were explained to each subject before obtaining informed consent. The experimental protocols were approved by the Virginia Tech Institutional Review Board.

#### Intervention

Subjects were first randomized to one of two treatments: olmesartan (ARB; Benicar, Daiichi Sankyo, Inc., Parsippany, NJ, USA) or control (no medication) for 8 weeks. After the 8 week intervention, study subjects participated in follow-up testing. After a 2 week washout period, subjects completed baseline testing, followed by the other intervention for 8 weeks, and ended the study with follow-up testing. Participants assigned to olmesartan continued treatment during the follow-up period, which lasted approximately 2 weeks. During the olmesartan intervention, participants were provided with 20 mg once daily (OD) for the first 2 weeks, followed by 40 mg OD for the remaining 6 weeks of the intervention. If a subject's BP fell below 110/70 mmHg during the first 2 weeks, the dose remained at 20 mg OD for the remainder of the study. Subjects were instructed to maintain their habitual physical activity level, dietary intake and body weight throughout the study period. All study participants completed baseline and follow-up testing within a 10 week period for each intervention treatment.

## Experimental testing

All testing took place between the hours of 7 am and 1 pm. Subjects were fasted for the prior 12 hours (including the exclusion of caffeinated and alcoholic beverages), performed no vigorous physical activity for the prior 48 hours, and were free from acute illness or infection for 2 weeks prior to testing. Subjects also abstained from ingesting nonsteroidal anti-inflammatory drugs or any medications that may have interfered with study measurements for the prior 72 hours to testing sessions.

Resting BP measurements were performed according to American Heart Association guidelines and described in detail elsewhere [Marinik *et al.* 2013]. Body mass and composition was measured as described in our previous report [Marinik *et al.* 2013]. Plasma lipid and lipoprotein concentrations were measured by a commercial laboratory (Solstas Lab Partners, Roanoke, VA, USA) using conventional enzymatic methods.

Skeletal muscle samples were obtained from the vastus lateralis muscle using a modified Bergstrom needle biopsy technique as previously described [Marinik *et al.* 2013]. Samples used for FAO measures were weighed and placed on ice for analysis within 30 minutes. Samples used for analysis of enzyme activities were snap frozen in liquid nitrogen for later homogenization and analysis.

FAO was assessed in whole muscle homogenates by measuring  ${}^{14}CO_2$  production from the oxidation of  $[1-{}^{14}C]$ -palmitic acid as previously described [Hulver *et al.* 2005, Cortright *et al.* 2006].

Maximal enzyme activity was assessed in whole muscle homogenates. Snap frozen skeletal muscle was homogenized on ice (20-fold dilution) in a modified sucrose ethylenediaminetetraacetic acid (EDTA) medium (SET) containing 250 mM sucrose, 1 mM EDTA, 10 mM tris-HCl, and 1 mM adenosine triphosphate (ATP) pH 7.4 as previously described [Cortright *et al.* 2006]. Citrate

	Control		ARB		
Variable	Pre	Post	Pre	Post	Main effect/interaction
Body weight, kg	91.6 ± 4.6	93.0 ± 4.8	92.4 ± 4.5	93.0 ± 4.4 <sup>+</sup>	<sup>†</sup> Time effect
Body mass index, kg/m <sup>2</sup>	31.2 ± 1.8	32.5 ± 1.9	32.1 ± 1.8	$32.3 \pm 1.8^{+}$	<sup>+</sup> Time effect
Body fat, %	39.8 ± 3.4	41.7 ± 3.8	$40.7 \pm 3.2$	$41.1 \pm 3.3^{+}$	<sup>+</sup> Time effect
Total fat mass, kg	$35.4 \pm 4.1$	37.6 ± 4.4	36.4 ± 3.7	$37.0 \pm 3.9^{+}$	<sup>+</sup> Time effect
Fat-free mass, kg	55.3 ± 3.3	$54.3 \pm 3.7$	55.1 ± 3.5	$55.0 \pm 3.4$	
Systolic BP, mmHg	133.3 ± 3.0	137.4 ± 2.3	137.4 ± 3.5	$127.3 \pm 3.0^{+\pm}$	<sup>+‡</sup> Treatment $ imes$ time interaction
Diastolic BP, mmHg	77.5 ± 1.9	79.0 ± 1.3	79.8 ± 2.0*	71.8 ± 1.8 <sup>+‡</sup>	<sup>†‡</sup> Treatment × time interaction *Group difference
Triglycerides, mmol/l	$1.2 \pm 0.2$	1.3 ± 0.2	1.4 ± 0.2*	$1.4 \pm 0.2$	*Group difference
Total cholesterol, mmol/l	$5.2 \pm 0.1$	$5.5 \pm 0.2$	$5.2 \pm 0.2$	$5.1 \pm 0.1$	
HDL cholesterol, mmol/l	$1.3 \pm 0.2$	1.3 ± 0.2	$1.2 \pm 0.2$	$1.3 \pm 0.2$	
LDL cholesterol, mmol/l	$3.4 \pm 0.2$	3.6 ± 0.2	$3.3 \pm 0.2$	$3.2 \pm 0.2$	
Glucose, mmol/l	5 ± 0.2	5.1 ± 0.2	5.2 ± 0.2	5 ± 0.2	
Age: 51.5 ± 3.7 years					

Table 1. Subject characteristics before and after the control and ARB treatment.

All values are expressed as mean  $\pm$  standard error of the mean (SEM).

\*Effect of treatment

<sup>\$</sup>Time effect.

 $\ddagger$ Treatment  $\times$  time interaction.

p< 0.05

ARB, angiotensin II receptor blockade; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

synthase (CS) and  $\beta$ -hydroxyacyl-coenzyme A dehydrogenase (BHAD) maximal activities were determined spectrophotometrically as previously described [Hulver et al. 2005; Frisard et al. 2010].

### Statistical analysis

A repeated measures analysis of variance was used to test the effects of treatment (ARB versus control), time and treatment by time interaction on dependent variables of interest. There was no effect of gender on the key study outcomes and therefore men and women are presented as a single group. Paired sample t-tests were used to compare the changes in dependent variables of interest. Results are expressed as mean ± standard error of the mean (SEM). Significance was set a priori at p < 0.05.

#### Results

Subject demographics and characteristics before and after each treatment are shown in Table 1. As we have previously reported, there were no significant differences between the ARB and control treatment periods at baseline, with the exception that diastolic BP and triglycerides were higher in the ARB treatment group compared with the control group at baseline [Marinik et al. 2013]. There was a significant group and interaction effect for the reduction in both systolic and diastolic BP with ARB treatment. There were no significant differences in body weight, BMI, body fat %, total body fat mass, lipid and lipoprotein, or fasting plasma glucose concentrations between treatments, while triglyceride levels remained higher during the ARB treatment compared with control.

 $CO_2$  produced from palmitate oxidation and the maximal enzyme activities of CS and BHAD before and after each treatment are depicted in Figure 1. There were no significant differences in CO<sub>2</sub> produced from palmitate oxidation as measured from [1-14C]-palmitate (Figure 1a) between the ARB and control treatments at baseline and following the intervention periods. In addition, there were no significant differences in maximal enzyme activities for CS (Figure 1b) and BHAD (Figure 1c) between the ARB and control treatments at baseline and following the intervention periods.

## Discussion

The main finding from the present study is that, in contrast to our hypothesis, skeletal muscle

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**Figure 1.** Effects of olmesartan on fatty acid oxidation and oxidative enzyme activity after 8 weeks of treatment. (a)  $CO_2$  produced from palmitate oxidation as measured from [1-<sup>14</sup>C]-palmitate oxidation in skeletal muscle homogenates, (b) citrate synthase and (c)  $\beta$ -hydroxyacyl-coenzyme A dehydrogenase (BHAD) enzyme activity as determined spectrophotometrically in skeletal muscle homogenates.

Values are expressed as mean  $\pm$  standard error of the mean (SEM) with p < 0.05. Data represent n = 12 for angiotensin II receptor blockade (ARB) and n = 10 for control treatments, respectively.

FAO was not increased following 8 weeks of ARB with olmesartan in overweight and obese adults

with elevated BP. In addition, the maximal activities of enzymes associated with oxidative metabolism were not altered following treatment in these individuals, supporting the observed lack of change in  $CO_2$  produced from palmitate oxidation following treatment. Our findings are consistent with a previous report [Moors *et al.* 2013] of a lack of change in skeletal muscle mRNA expression of target genes associated with oxidative metabolism following ARB in humans with impaired glucose metabolism.

ARB has been reported to have numerous cardiovascular and renal pleiotropic actions independent of BP lowering properties [Suzuki et al. 2002, 2004; Miyoshi et al. 2011]. Some [Fogari et al. 2005; Vitale et al. 2005; Nishimura et al. 2008] but not all [Moan et al. 1996,; Goossens et al. 2012; Lteif et al. 2012] studies suggest that ARBs improve insulin sensitivity. The discrepancy may be due, at least in part, to differences in peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) agonist activity of some of the medications within the ARB class. We previously reported [Marinik et al. 2013] that olmesartan, an ARB devoid of PPARy agonist activity [Benson et al. 2004], did not improve insulin sensitivity in overweight and obese adults. Taken together with our previous observations, our findings suggest that improvements in insulin sensitivity and skeletal muscle FAO are not among the pleiotropic actions of olmesartan that extend beyond its BP lowering capability.

We should emphasize a few limitations of our study. Our sample size was small, comprised of primarily Caucasian participants, and limited to overweight and obese individuals with elevated BP. In addition, we and others have reported that FAO is suppressed in skeletal muscle of obese humans relative to lean controls [Kim et al. 2000; Hulver et al. 2003]; however, this may be viewed as controversial as others have not observed the same [Holloway et al. 2007]. As such, our inclusion of overweight/obese individuals who may have had little to no impairment in FAO could have impacted our findings. However, we should emphasize that our subjects were sedentary, middle-aged and older adult adults, and thus would have reduced oxidative capacity as a function of age and physical inactivity [Johnson et al. 2013]. Importantly, ARB treatment has been shown to improve skeletal muscle lipid metabolism in a similar population to ours [Moors et al. 2013]. Nonetheless, future studies will be necessary to comprehensively address this issue.

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In conclusion, the findings of the present study suggest that ARB with olmesartan does not impact skeletal muscle FAO or oxidative maximal enzyme activities in overweight and obese adults with elevated BP.

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## **Conflict of interest statement**

The authors declare no conflicts of interest in preparing this article.

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