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Conjugated linoleic acid improves blood pressure by increasing adiponectin and endothelial nitric oxide synthase activity☆

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Abstract

Conjugated linoleic acid (CLA) has been reported to reduce blood pressure in obese insulin-resistant rats, but its mechanism of action has not been identified. The objective of this study was to determine whether CLA isomers can reduce obesity-related hypertension in the *fa/fa* Zucker rat in relation to adiponectin production and endothelial nitric oxide synthase (eNOS) activation. Obese *fa/fa* Zucker rats were randomly assigned to one of four groups: (1) *cis*-9,*trans*-11-CLA, (2) *trans*-10,*cis*-12 (t10,c12)-CLA, (3) control or (4) captopril. After 8 weeks, systolic blood pressure increased 30% in control obese rats. This increase was attenuated 11%–13% in the t10,c12-CLA isomer and captopril groups, respectively. The t10,c12-CLA isomer concurrently elevated adiponectin levels in both plasma and adipose tissue and increased phosphorylated eNOS in adipose tissue as well as the aorta. Although a direct effect of CLA was not observed in cultured endothelial cells, direct adiponectin treatment increased phosphorylation of eNOS. Endothelial nitric oxide synthase phosphorylation was also increased in adipose of *fa/fa* Zucker rats infused with adiponectin in parallel with improvements in blood pressure. Our results suggest that the t10,c12-CLA isomer attenuates development of obesity-related hypertension, at least in part, by stimulating adiponectin production, which subsequently activates vascular eNOS.
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Keywords: Adiponectin; Blood pressure; Adipose; Obesity; Conjugated linoleic acid; Nitric oxide

1. Introduction

Hypertension makes a significant contribution to the morbidity and mortality associated with cardiovascular disease, and obesity is a major factor promoting hypertension [1]. Although the relationship between obesity and hypertension is well established, the reason for this association remains poorly understood, but it is likely linked to endothelial dysfunction [2]. The increase in adipose tissue that leads to obesity initially results from an enlargement of adipocytes and is associated not only with changes in adipocyte metabolism but also their endocrine properties [3]. The resultant alteration in secretion of a variety of bioactive molecules may alter gene expression and cell signaling in various tissues, including the vasculature, and thus contributes to obesity-related hypertension [3].

The amount of adiponectin secreted by the adipose tissue decreases as fat accumulates [4], and an association between low plasma adiponectin levels and increased risk of cardiovascular disease is now recognized [5]. Adiponectin is a 30-kDa protein secreted from the adipose tissue that exhibits antiatherogenic and antidiabetic properties [6]. While it is typically found in the circulation at concentrations between 0.5 and 30 μg/ml [7], plasma levels are lower in obesity. Recently, hypoadiponectinemia has been associated with the development of obesity-related hypertension, specifically by altering expression of endothelial nitric oxide synthase (eNOS) [8], the enzyme responsible for the production of the vasodilatory molecule nitric oxide (NO). Adiponectin can also increase NO production through phosphorylation of eNOS via AMP-activated protein kinase (AMPK) [9,10]. Although a clear link between adiponectin and hypertension has been identified, the direct effects of adiponectin treatment on obesity-related hypertension remain to be demonstrated.

Nonpharmacological intervention with dietary compounds is receiving increased attention as a novel approach to treating obesity-related hypertension. Conjugated linoleic acid (CLA) is a term used to describe a mixture of positional and geometric isomers

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of linoleic acid, an 18-carbon polyunsaturated fatty acid. Nagao *et al.* [11] have shown that feeding the *trans*-10,*cis*-12 (t10,c12)-CLA isomer results in significantly lower systolic blood pressure in Otsuka Long–Evans Tokushima Fatty (OLETF) rats compared with those fed linoleic acid or *cis*-9,*trans*-11 (c9,t11)-CLA. Current work from our laboratory has shown that feeding *fa*/*fa* Zucker rats a mixture of CLA isomers reduces the levels of proinflammatory agents that are elevated in obesity, reduces adipocyte hypertrophy and increases serum adiponectin [12]. Given these multiple actions of CLA, this study was designed to examine for the first time the relationship between adiponectin and eNOS activity in the blood pressure lowering actions of CLA in an obese rat model. We also investigated the effects of recombinant adiponectin treatment on blood pressure of *fa*/*fa* Zucker rats and the contribution of eNOS to this process.

2. Methods

2.1. Conjugated linoleic acid feeding intervention

The animal protocol was approved by the University of Manitoba Protocol Management and Review Committee. Thirty-six male, 5-week-old, *fa*/*fa* Zucker rats (Harlan) were acclimatized for 5–8 days and then randomly assigned to one of four groups for 8 weeks: (1) 0.4% (w/w) c9,t11-CLA, (2) 0.4% (w/w) t10,c12-CLA, (3) control and (4) captopril. The purity of the isomers used in the current study was 96% and 99% for the c9,t11-CLA and t10,c12-CLA, respectively. A dose of 0.4% (w/w) was previously shown to be effective for reducing hepatic steatosis, oral glucose tolerance and adipoctye size in *fa*/*fa* Zucker rats [13,14]. The control and CLA diets were based on the AIN-93 formulation (Table 1). Captopril was added to the drinking water at 50 mg/kg/day. Systolic blood pressure was measured in the conscious state by the indirect tail cuff method using the IITC Life Sciences system. Animals were trained with the blood pressure equipment, including restraints and tail cuffs for at least 3 days before taking measurements. Blood pressure measurements were always taken in the morning, and triplicate measurements were collected for each animal and averaged. Research shows that the tail-cuff method provides comparable blood pressure results to telemetry measurements [15,16], and current research uses this method for measuring blood pressure in rats [17]. At the end of the study, tissues were collected for additional analysis as described below. Other findings for these rats related to weight gain, hepatic steatosis, adipocyte size and inflammatory markers have been published elsewhere [13,14].

2.2. Cell culture

Human aortic endothelial cells (HAECs; Clonetics) were expanded in growth medium (EBM-2; Lonza) and passaged when 70% confluent. Cells were seeded at 3×10^4 cells/well on 12-well plates and experiments were performed with cells at passage 5. Human aortic endothelial cells were treated for 1 h and then lysed with 2× sodium dodecyl sulfate sample buffer. Cell lysates were subsequently analyzed by Western blotting.

Table 1
Composition of experimental diets

Ingredients (g/100 g)	Control/captopril	c9,t11-CLA	t10,c12-CLA
Cornstarch [a]	36.3	36.3	36.3
Maltodextrin [b]	13.2	13.2	13.2
Sucrose [b]	10.0	10.0	10.0
Egg white [b]	21.3	21.3	21.3
Cellulose [b]	5.0	5.0	5.0
AIN-93G mineral mix [b]	3.5	3.5	3.5
AIN-93VX vitamin mix [b]	1.0	1.0	1.0
Choline [b]	0.3	0.3	0.3
Biotin mix [b,c]	1.0	1.0	1.0
TBHQ [d]	<0.01	<0.01	<0.01
Soy oil [b]	8.5	8.1	8.1
c9,t11-CLA oil [e,f]	0.0	0.4	0.0
t10,c12-CLA oil [e,f]	0.0	0.0	0.4

[a] Purchased from Best Foods, Etobicoke, Ontario, Canada.
[b] Purchased from Harlan Teklad, Madison, WI.
[c] Biotin mix=200 mg biotin/kg cornstarch.
[d] *tert*-Butylhydroquinone; purchased from Sigma-Aldrich, St. Louis, MO.
[e] CLA dose as 0.4% (w/w) c9,t11-CLA oil and 0.4% (w/w) t10,c12-CLA oil purchased from Natural ASA, Hovdebygda, Norway.
[f] Purity of the c9,t11 and t10,c12-CLA oils was 96% and 99%, respectively.

2.3. Acute adiponectin injection

Detailed methods for cloning and purification of adiponectin can be found in the Supplemental Material. Sixteen-week old *fa*/*fa* Zucker rats (n=6) were implanted with telemetry transponders (C50-PXT; Data Sciences International) in the femoral artery and allowed 10 days to recover. Each rat was given a 20-μl bolus injection of saline, via the tail vein, followed 48 h later with an injection of adiponectin (50 μg). The period between injections was to ensure blood pressure had normalized. Blood pressure was measured for 120 min following the injections. Finally, the rats were given the NOS inhibitor L-NAME in their drinking water (80 mg/kg/day) for 24 h, at which time adiponectin was injected and blood pressure monitored for 120 min. Telemetry was used to monitor blood pressure in this study because measurements needed to be taken continuously over a 2-h period.

2.4. Chronic adiponectin infusion

Osmotic pumps (model 2001; ALZET) implanted subcutaneously infused either saline or adiponectin (50 μg/day) to 16-week-old *fa*/*fa* Zucker rats (n=6/group). Blood pressure was measured every 48 h by the tail cuff method (CODA-6; Kent Scientific). Rats were euthanatized on day 7 of infusion, and plasma samples were collected for detection of recombinant adiponectin (Figure S1, Supplemental Material) and tissues were collected for postmortem analysis by Western blotting.

2.5. Plasma and tissue collection

Rats were euthanized by CO_2 asphyxiation at the end of the treatment period, and trunk blood was collected, centrifuged and stored at −80°C. The aorta and epididymal adipose tissue were removed, weighed, frozen in liquid nitrogen or embedding medium (Cryo-Gel; Instrumedics) and stored at −80°C. Adipocytes were isolated from the epididymal adipose tissue according to Jernas *et al.* [18]. Adipocytes were lysed and analyzed by Western blotting.

2.6. Measurement of plasma adiponectin

Total plasma adiponectin levels were measured using a commercial rat ELISA kit (Alpco Diagnostics) following the manufacturer's guidelines (n=5/group).

2.7. Western blotting

Protein was extracted from frozen aorta and adipose tissue samples and subjected to Western blotting [12] with antibodies (diluted 1:1000) to adiponectin (Calbiochem), and phosphorylated eNOS (Ser1177), eNOS, phosphorylated AMPK-α (Thr172) and AMPK-α (Cell Signaling). Membranes were stripped and reblotted for either mitogen-activated protein kinase (MAPK p44/42) (Cell Signaling) or β-tubulin (Abcam) to correct for variability in sample loading. Levels of mitogen-activated protein kinase are constant in *fa*/*fa* Zucker rats subjected to dietary intervention (Figure S2, Supplemental Material). Results are expressed as arbitrary units after being normalized to the intensity control (same sample on each gel) and loading control.

2.8. Immunofluorescence staining in epididymal adipose tissue

At room temperature, 10-μm-thick sections of adipose tissue were fixed in 4% paraformaldehyde for 10 min under UV light. The slides were blocked in 3% bovine serum albumin–phosphate-buffered saline for 1 h at room temperature then incubated overnight at 4°C with primary antibodies for adiponectin (3 μg/ml; Abcam) and phospho-eNOS (1:100; Cell Signaling). The slides incubated with secondary antirabbit antibody coupled to an Alexa Fluor 488 tag (2.5 μl/ml; Molecular Probes) and antimouse antibody with a Cy3 tag (1:250; Jackson ImmunoResearch) for 1 h at room temperature. The slides were then incubated with the nuclear stain, Hoechst 33342 (5 μl/ml; Sigma), for 1 min at room temperature and cover-slips were applied with mounting medium. Images of immunostained adipose sections were captured using a BH2-RFCA Olympus fluorescence microscope, a Q-Imaging digital camera and Q-Capture Pro 6.0 software.

2.9. Statistical analysis

Differences were considered significant at $P<.05$ and all results are expressed as mean±SEM. Data were analyzed by a repeated measures two-way ANOVA for main effects of treatment and time (blood pressure *fa*/*fa* Zucker rats) or one-way ANOVA for treatment effects (parameters in *fa*/*fa* Zucker rats, HAECs, acute adiponectin infusion) followed by means testing with Duncan's multiple range test. Student's t test was used to determine the effects of chronic adiponectin infusion (blood pressure and protein levels).

3. Results

3.1. Physiological actions of CLA

In our study, no differences in feed intake or body weight were observed due to either the dietary CLA supplementation or captopril treatment (Table 2). Epididymal adipose tissue weight was also similar among all groups (Table 2). These data indicate that neither the CLA isomers nor captopril altered these parameters. Over the 8-week study, body weight increased approximately threefold in all groups. In parallel, systolic blood pressure increased more than 30% in obese rats fed the control diet (Fig. 1A). This increase in blood pressure was significantly attenuated in all treatment groups, with t10,c12-CLA and captopril having the greatest effect (Fig. 1A). At the end of the study, systolic blood pressure was 7%, 11%, and 13% lower in the c9,t11-CLA, t10,c12-CLA and captoril groups than the control group, respectively. These data are the first to show that c9,t11-CLA affects blood pressure, albeit not as potently as t10,c12-CLA.

3.2. Effect of CLA feeding on adiponectin and eNOS

Circulating levels of total adiponectin were 1.7-fold higher in the group receiving the t10,12-CLA isomer compared with control (Fig. 1B). In contrast, neither c9,t11-CLA nor captopril significantly affected plasma adiponectin concentrations. Treatment with the t10,c12-CLA isomer also resulted in a twofold increase in adiponectin levels in adipose tissue relative to the control group (Fig. 1C). No effect was seen with either captopril or c9,t11-CLA on adipose adiponectin levels. These results suggest the increase in circulating adiponectin in the t10,c12-CLA group results from elevated production.

Nitric oxide is produced in adipose tissue by eNOS and causes vasodilation of blood vessels, thus the potential contribution of eNOS activity was examined. Phosphorylation of eNOS was increased 5- to 13-fold in the adipose of the t10,c12-CLA isomer group compared with all other groups (Fig. 2A), implicating eNOS as a target for the actions of t10,c12-CLA. There was no change in total eNOS levels in adipose with any treatment (Fig. 2B). To identify the source of eNOS that is susceptible to CLA treatment, microscopy (Fig. 3A) and Western blotting (Fig. 3B) were used to examine levels of eNOS in two abundant cell types present in adipose tissue. These data clearly show that eNOS is present in the endothelial cells and not in adipocytes (Fig. 3A and 3B) suggesting that NO is produced by the blood vessels that perfuse adipose tissue rather than by the adipocytes themselves.

To explore the possibility that the change in eNOS phosphorylation seen in adipose tissue could represent a systemic effect of CLA, we next examined eNOS in the aorta. A threefold to eightfold increase was observed in the levels of phosphorylated eNOS in the t10,c12-CLA isomer group compared with all other groups (Fig. 2A). However, in contrast to the adipose tissue, eNOS was also increased twofold to threefold in the aorta of the rats receiving the t10,c12-CLA isomer compared with all other groups (Fig. 2B). From these results, it is plausible that the physiological actions of t10,c12-CLA on blood pressure are mediated via eNOS and that t10,c12-CLA specifically targets the endothelial cells of adipose tissue capillaries and major arterial conduits.

Table 2
Characteristics of *fa/fa* Zucker rats fed CLA isomers

	Control	c9,t11-CLA	t10,c12-CLA	Captopril
Total feed intake (g)	1524±29	1615±35	1619±24	1569±32
Initial body weight (g)	135±5.4	139±3.5	127±7.3	142±6.4
Final body weight (g)	569±9.3*	591±8.5*	580±11.3*	573±13.2*
Epididymal adipose (g)	17.1±1.1	18.7±1.8	19.7±0.7	19.3±1.0
Epididymal adipose (g/100 g body weight)	3.00±0.19	3.16±0.17	3.40±0.15	3.38±0.18

*Significant change ($P\leq.05$) within a group from initial to final body weight.

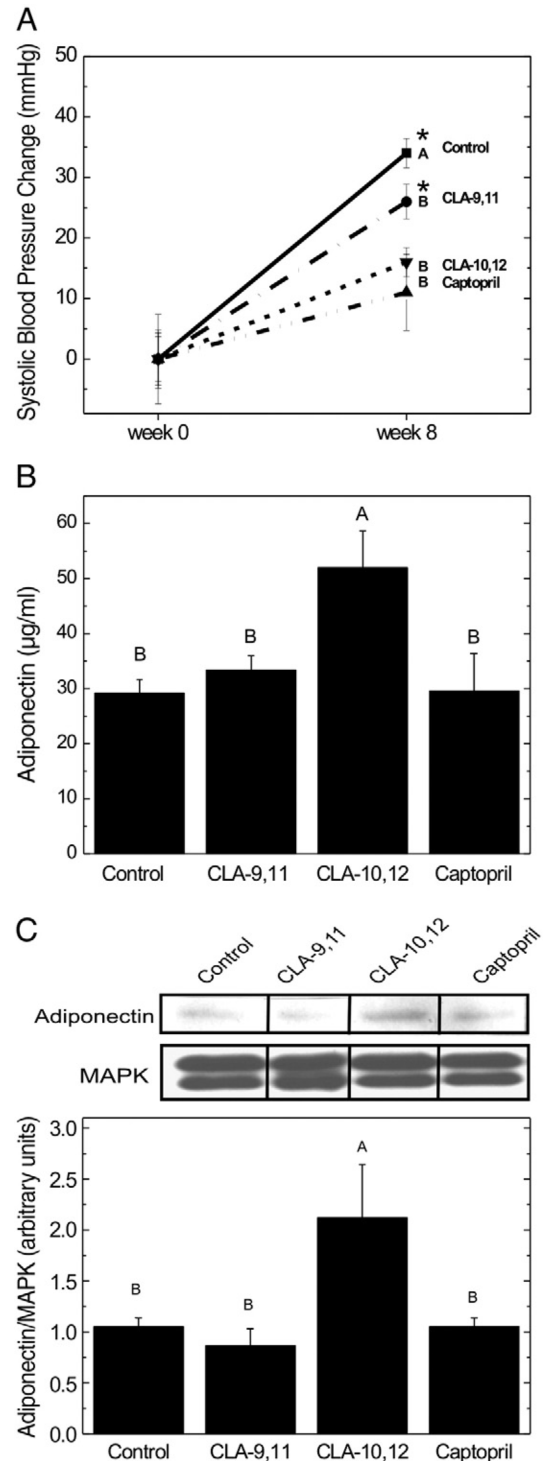

Fig. 1. Systolic blood pressure (A), plasma adiponectin (B) and adipose tissue levels of adiponectin (C) in *fa/fa* Zucker rats fed CLA diets. Overall mean systolic blood pressure was 114±2.4 mmHg at week 0 (n=36). Results for serum adiponectin in micrograms per milliliter are means±SEM (n=5 rats/group). Relative intensity of the bands was normalized to MAPK and protein levels of adiponectin expressed as arbitrary units are means±SEM (n=5 rats/group). Different letters denote significant differences ($P\leq.05$) at the same time point, and an asterisk (*) denotes significant change in systolic blood pressure from week 0.

3.3. Endothelial cell response to CLA

To determine whether CLA can promote NO production directly, HAECs were treated *in vitro* with CLA. No increase in phosphorylation of eNOS was detected with either CLA isomer (Fig. 4A), indicating the effects of CLA on vascular tissue is likely indirect. It was recognized that a circulating factor was the most probable intermediate since both local and systemic vessels responded to CLA. We therefore elected to determine whether adiponectin was this circulating factor responsible for increasing eNOS activity based on the fact that adiponectin secretion from adipocytes is promoted by CLA treatment. Endothelial cells were treated with adiponectin or the AMPK activator 5′-aminoimidazole-4-carboxamide riboside (AICAR). Both adiponectin and AICAR strongly stimulated eNOS phosphorylation (Fig. 4A). Furthermore, both treatments increased AMPK phosphorylation (Fig. 4B) without affecting total eNOS or AMPK levels (Figure S3, see Supplemental Material). Based on these observations, it was plausible that CLA-induced eNOS phosphorylation was achieved by enhancing the production of adiponectin in adipose tissue.

3.4. Adiponectin infusion and blood pressure

Although improved blood pressure was associated with increased circulating adiponectin in obese rats treated with CLA isomers, it could not be resolved whether the increase in adiponectin was able to affect blood pressure. Therefore, we elected to test the effect of a known concentration of adiponectin on obesity-related hypertension directly. A bolus injection of adiponectin had no effect on systolic blood pressure compared with saline (Fig. 5A). Furthermore, adiponectin did not reverse the blood pressure increase obtained by placing L-NAME in their drinking water for 24 h (Fig. 5A). Diastolic blood pressure was also unchanged after adiponectin injection (Figure S4, see Supplemental Material). In contrast, when adiponectin was infused continuously for 7 days, systolic blood pressure was improved compared with saline-treated rats (Fig. 5B), although diastolic blood pressure did not change (Figure S4, see Supplemental Material). These data clearly establish that adiponectin is capable of modulating blood pressure, although chronic exposure over several days is necessary for its actions.

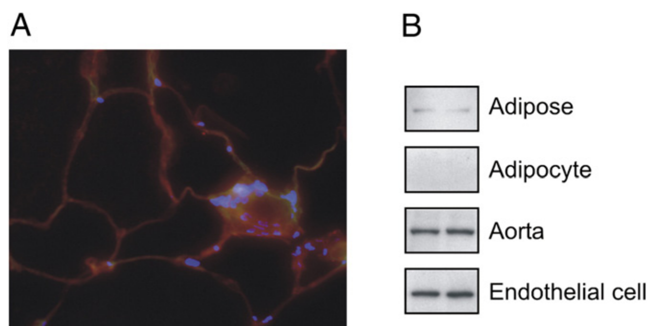

Fig. 3. Representative fluorescent image of epididymal adipose tissue from control *fa/fa* Zucker rats showing phospho-eNOS is associated with vascular structures and is absent in adipocytes (A). Each section was stained with Hoechst 33342 (blue), adiponectin (red) and phospho-eNOS (green). Comparative Western blot (B) to determine the presence of eNOS in different cell types and tissues including adipose tissue, adipocytes and aorta from *fa/fa* Zucker rats and aortic endothelial cells from human.

3.5. Adiponectin infusion on aortic and adipose proteins

In the aorta, levels of phosphorylated eNOS (Fig. 6A) and AMPK (Fig. 6B) were unchanged after the 7-day adiponectin infusion. Similarly, no changes were observed in the levels of total eNOS or AMPK (Figure S5, see Supplemental Material). These data indicate aortic endothelial cells are not the primary target of adiponectin. Since adipose tissue also contains endothelial cells, we tested if eNOS activity was altered in adipose tissue after the 7-day infusion and found adiponectin infusion increased phosphorylated eNOS levels more than fivefold compared with the saline-infused group (Fig. 6A). Interestingly, phosphorylation of AMPK was unaltered (Fig. 6B). Furthermore, no changes were observed in the levels of total eNOS or AMPK in adipose tissue (Figure S5, see Supplemental Material). Based

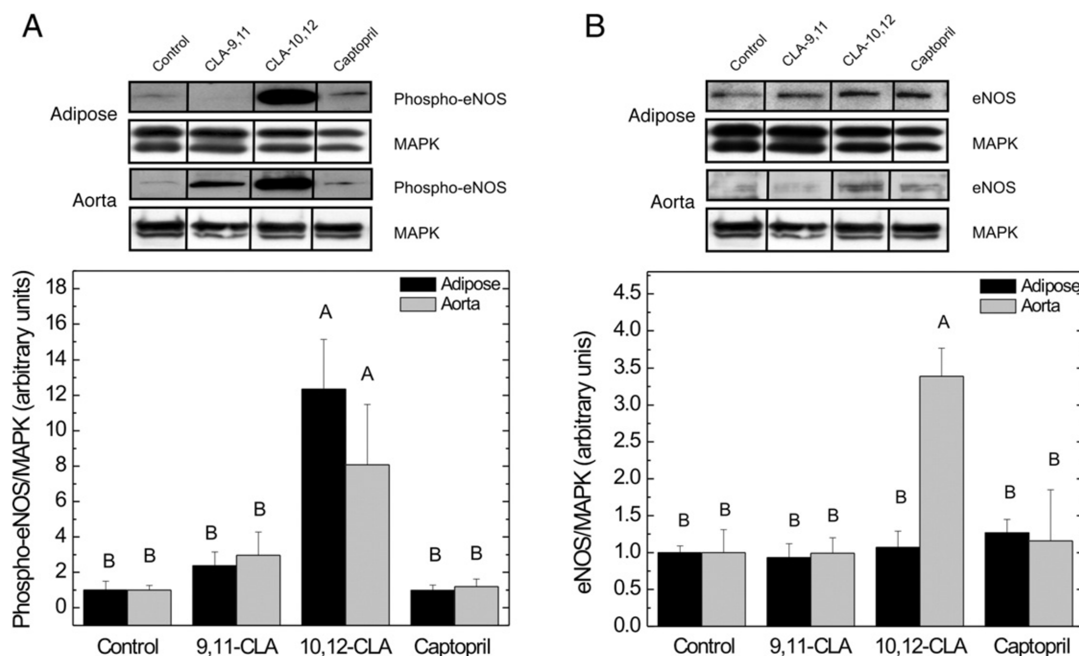

Fig. 2. Adipose tissue and aortic levels of phospho-eNOS (A) and eNOS (B) in *fa/fa* Zucker rats fed CLA diets measured using Western blot analysis (n=5 rats/group). Relative intensity of the bands was normalized to MAPK and protein levels expressed as arbitrary units are means±SEM (n=5 rats/group). For each tissue different letters denote significant differences ($P\leq.05$).

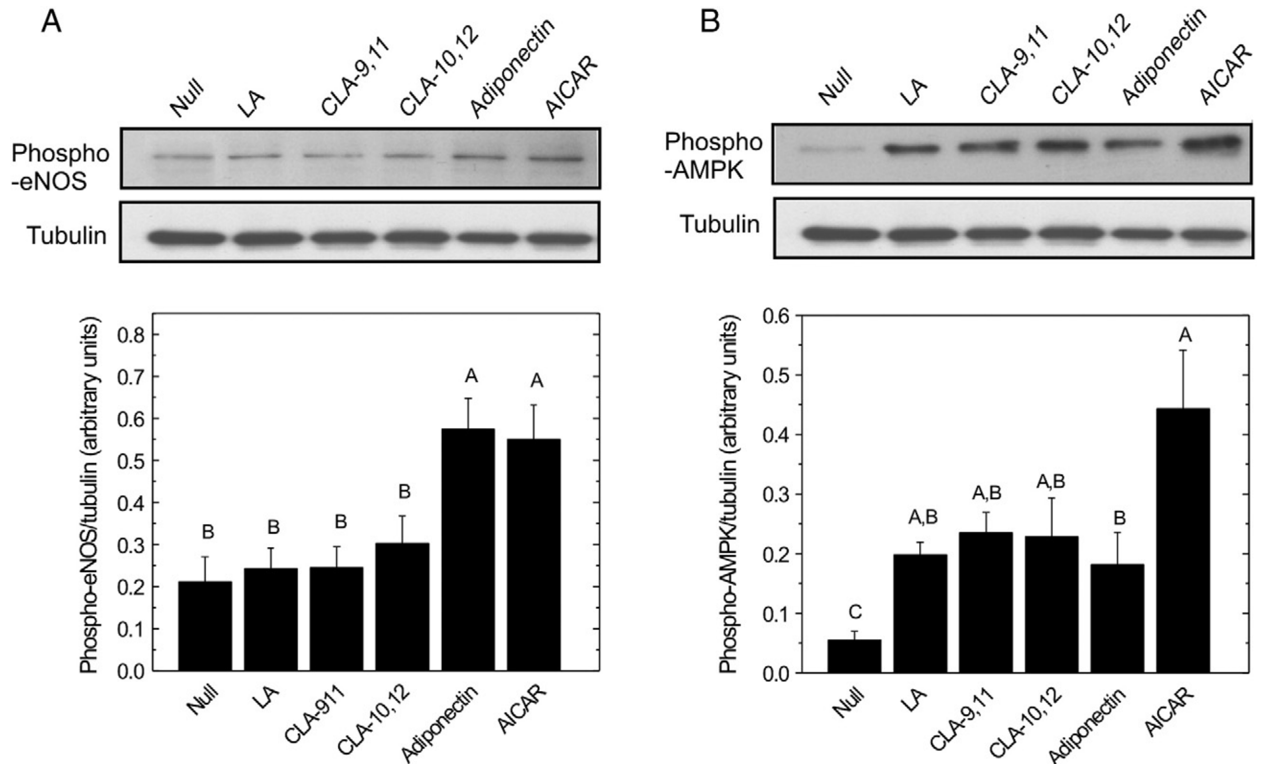

Fig. 4. Levels of phospho-eNOS (A) and phospho-AMPK (B) in HAECs treated with CLA isomers. Relative intensity of the bands obtained by Western blot analysis of 6 independent experiments were normalized to β-tubulin and protein levels expressed as arbitrary units are means±SEM. Cells were either untreated (null) or treated with 60 μM linoleic acid (LA), 60 μM individual CLA isomers (CLA-9,11 and CLA-10,12), 10 μg/ml adiponectin or 10^{-3} M AICAR. Different letters denote significant differences (P≤.05).

on these results, adiponectin appears to selectively target the microvascular endothelial cells that modulate blood pressure rather than endothelial cells of conduit vessels.

4. Discussion

The current study has demonstrated that feeding t10,c12-CLA to *fa/fa* Zucker rats prevents the increase in blood pressure associated with obesity. We have also shown that the t10,c12-CLA treatment was as efficacious as a standard antihypertensive drug in blocking the increase in blood pressure. Rats receiving the t10,c12-CLA isomer displayed increased levels of adiponectin in both plasma and adipose tissue. These changes were accompanied by increased levels of phosphorylated eNOS in both the adipose tissue and aorta, most likely in the endothelial cells of both tissues. However, a direct effect of CLA on eNOS phosphorylation in cultured endothelial cells was not observed, which suggests that the effect of CLA on blood pressure is indirectly mediated through adipose tissue-dependent production and secretion of adiponectin. In support of this conclusion, we are the first to show that infusion of obese rats with a known amount of adiponectin improves systolic blood pressure and increases eNOS phosphorylation in adipose tissue comparable to dietary supplementation with CLA.

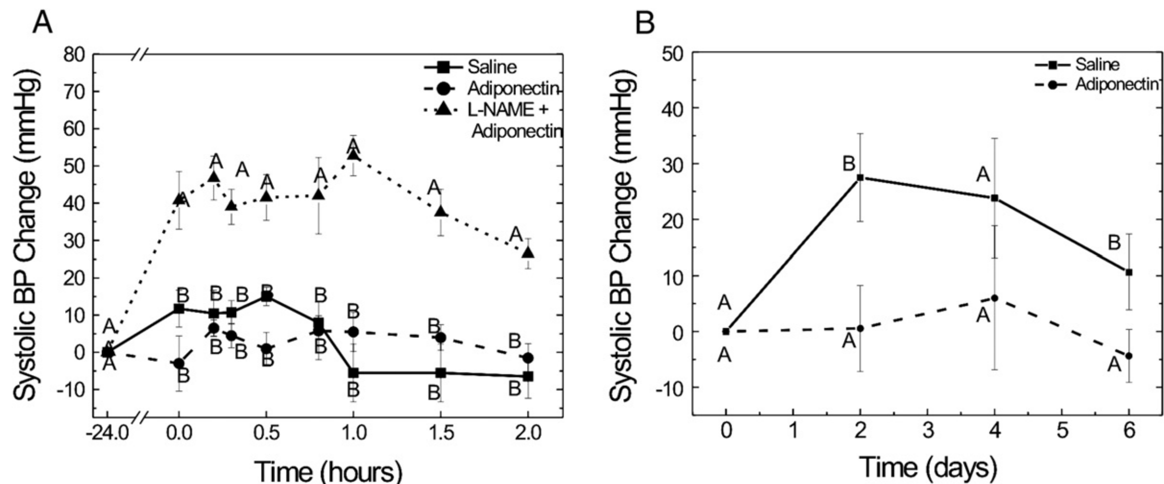

Fig. 5. Systolic blood pressure in *fa/fa* Zucker rats given adiponectin for 120 min (A) or 6 days (B). At the same time point, different letters denote significant differences (P≤.05) and results are means±SEM (n=4–6 rats/group).

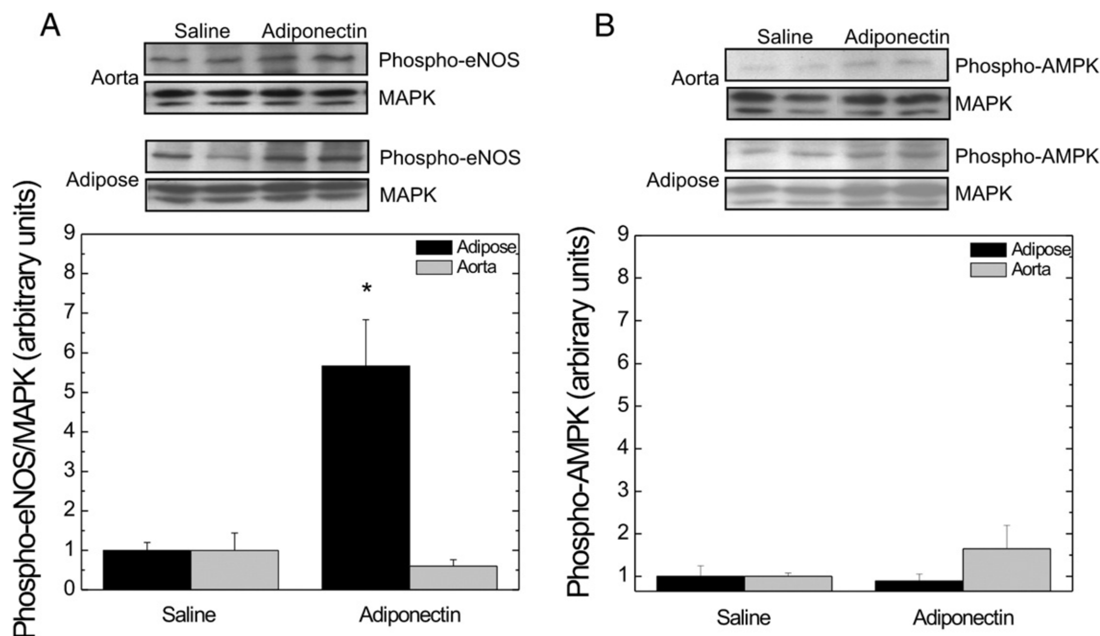

Fig. 6. Levels of phospho-eNOS (A) and phospho-AMPK (B) in adipose and aorta of *fa/fa* Zucker rats treated with adiponectin for 7 days. Relative intensity of the bands was normalized to MAPK and protein levels expressed as arbitrary units are means±SEM (n=6 rats/group). An asterisk (*) denotes a significant difference ($P\leq.05$) from saline-treated.

A positive relationship between obesity and hypertension has clearly been established [19], which explains the blood pressure increase observed with *fa/fa* Zucker rats in this report. Previous studies using obese rats have shown that improvements in blood pressure with t10,c12-CLA treatment are achieved simultaneously with reduced epididymal adipose tissue mass [11]. In contrast, the current study demonstrates that dietary treatment with t10c12-CLA attenuates systolic blood pressure in *fa/fa* Zucker rats despite the fact that these rats had similar epididymal adipose mass to the untreated controls. One reason for the differing results may be the use of different rat models; the OLETF rat model responds rapidly to dietary CLA intervention, with changes in adipose weight and blood pressure evident after only 3 weeks of feeding [11]. Another reason that may have influenced the outcome on adipose mass may be the type of background oil used in the diet: corn oil by Nagao *et al.* [11] *vs.* soy oil in the current study. On the other hand, the *fa/fa* Zucker rat may be resistant to weight changes since recent work by Martins *et al.* [20] confirms earlier work from our laboratory showing no reduction in adipose weight even though other physiological benefits have been noted at the same dose of CLA isomers [12–14]. This is also the first study to show attenuation of systolic blood pressure with the c9,t11-CLA isomer, which is naturally present in ruminant meats and dairy products, although it is not as effective as the t10,c12-CLA isomer. The mechanism for the c9,t11-CLA isomer-mediated effects requires further investigation as plasma and adipose adiponectin were unchanged.

In humans, only a few studies examining CLA and blood pressure have been reported. One study in healthy nonobese normotensive males showed no change in blood pressure after consuming CLA-enriched butter (predominantly c9,t11-CLA) [21], whereas a mixture of CLA isomers significantly enhanced the effect of ramipril on reducing blood pressure in obese men and women with hypertension [22].

Evidence from human and animal studies [8,23,24] has demonstrated a link between low levels of adiponectin and hypertension. We were able to show that adiponectin infusion in obesity can improve blood pressure. Furthermore, beneficial changes in the blood pressure of rats receiving the t10,c12-CLA isomer occurred in conjunction with increases in adiponectin levels in both plasma and adipose tissue. In agreement with our findings, Nagao *et al.* [25] showed CLA isomers increase plasma adiponectin in Zucker diabetic fatty rats, and they attributed the changes in adiponectin to enhanced gene expression in white adipose tissue. Likewise, we previously showed increases in serum adiponectin and mRNA levels in epididymal adipose tissue of *fa/fa* Zucker rats when given a diet containing mixed CLA isomers [12]. However, direct treatment of adipocytes with CLA does not increase adiponectin production (DeClercq *et al.*, unpublished), emphasizing the importance of cross-talk between different adipose-derived cells in mediating the effects of CLA on adiponectin production.

Impaired endothelial function is evident in obesity [26] and low levels of circulating adiponectin are associated with impaired endothelium-dependent vasorelaxation [24,27]. This evidence suggests that plasma adiponectin levels might be a useful indicator of endothelial dysfunction. In agreement with this concept, we demonstrated that adiponectin infusion improves blood pressure and increases eNOS phosphorylation in adipose tissue. Previous work suggests adiponectin can activate eNOS in endothelial cells, thus we explored the possibility that CLA isomers may influence endothelial cells within the adipose tissue and thereby alter eNOS activity. In contrast to all other groups, the t10,c12-CLA isomer increased eNOS phosphorylation in adipose tissue of *fa/fa* Zucker rats, suggesting that the t10,c12-CLA isomer may influence blood pressure by directly affecting endothelial cells within the adipose tissue. Our study examined the effects of CLA isomers on HAECs and showed that neither isomer affected eNOS activity. Furthermore, direct treatment of HAECs with adiponectin increased both phosphorylated eNOS and AMPK. Similar observations were made by Hattori *et al.* [9] in human umbilical vein endothelial cells.

Obesity-related hypertension is prevalent in today's world, so understanding the mechanisms causing this condition will help to develop new therapeutic strategies for prevention and treatment. The current study adds to the body of literature through *in vivo* and *in vitro* approaches to show that the t10,c12-CLA isomer has beneficial effects on endothelial cell function that can manifest as lower blood pressure. Furthermore, we have for the first time established that

direct treatment with adiponectin improves blood pressure in obese rats, thus indicating that altered cross-talk between adipocytes and endothelial cells within adipose tissue is a major underlying factor in the development of obesity-related hypertension. Consequently, it may be concluded that improvements in adipocyte function through consumption of t10,c12-CLA isomer attenuates blood pressure in part by increasing adiponectin, which improves endothelial function by increasing eNOS phosphorylation. Additionally, the t10,c12-CLA isomer lowers blood pressure through a different mechanism than the c9,t11-CLA isomer and captopril. Further work will be required to elucidate the actions of CLA on blood pressure regulatory systems and to uncover the details of the interactions between endothelial cells and adipocyte-derived molecules.

Supplementary materials related to this article can be found online at doi:10.1016/j.jnutbio.2011.02.003.

Acknowledgments

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