



L-arginine supplementation reduces blood pressure and plasma lipid levels in an animal model of perimenopause induced by 4-Vinylcyclohexene diepoxide

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Abstract

The incidence of women developing high blood pressure during perimenopause has been documented and is sustained till menopause. However, no study till date on beneficial effects of L-arginine supplementation on BP during perimenopause in humans and animal models of perimenopause. Female rats 28 days old were divided into 3 groups; Control group was injected (SC) daily with Corn oil (2.5 µl/g BW) for 15 days, allowed to grow till 12th week; VCD group was injected (SC) daily with 4-vinylcyclohexene diepoxide (160 mg/kg BW) diluted in Corn oil (2.5 µl/g BW) for 15 days, allowed to grow till 12th week; VCD + L-ARG group was injected as VCD group, allowed to grow till 8th week, then administered oral 100mg/kg L-arginine daily for additional 4 weeks. Caudal BP was measured with tail-cuff apparatus (Kent Scientific CODA system) at weeks eight, ten, and twelve. Terminal BP was also measured with a power-lab apparatus and blood samples were subsequently collected for measurement of plasma lipid profile. L-arginine supplementation significantly reduced systolic, diastolic and mean arterial BP parameters in the VCD + L-ARG group compared to the Control and VCD groups ($P < 0.05$). It also significantly reduced total cholesterol and LDL concentrations in the VCD + L-ARG group compared to the Control and VCD groups ($P < 0.05$). HDL concentration was significantly higher in the VCD and VCD + L-ARG groups compared to the Control group ($P < 0.05$). These results show that L-arginine supplementation ameliorates some cardiovascular risk factors during perimenopausal transitory period.

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INTRODUCTION

Major hormonal transition periods characterize a woman's life span, commencing at puberty with rising estrogen levels, which remain high during pregnancy followed by a rapid decline postpartum, gradual decrease in perimenopause, and even lower levels in the menopausal stage [1–3]. Perimenopause, a phase where the body transitions towards menopause involves changes in ovarian hormones, quantity and quality of ovarian follicles, feedback relationships and clinical experiences beginning mid-life in women around age 35 – 50 years with regular flow ending 1 year after the final menstrual flow [4–5].

In the human population, systematic evaluation of the biological processes associated with menopausal transition can be challenging, hence rodent animal models have become crucial in gaining an understanding to the essential elements driving the reproductive and aging processes [6]. The 4-Vinylcyclohexene diepoxide (VCD) rodent model of transitional menopause has a significant edge above the natural aging (ovary intact) or ovariectomized models, because it closely resembles the perimenopausal period, having comparable circulating steroid hormone profile and preserved ovarian tissue, although resting follicle pool is depleted [6]. The VCD model of perimenopause eliminates the sudden halt of circulating ovarian hormones as seen in ovariectomized rats while ensuring a decrease ovarian function in rats as early as around 60 days of age [6–8].

The reduced risk of development of coronary heart disease in women compared to men is nullified during the menopause transition [9–

10]. Numerous studies have shown the association between perimenopause and altered lipid profile including elevated levels of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C), and reduced high density lipoprotein cholesterol (HDL-C) levels, thereby increasing CVD risk [11–13]. It has been proposed that reduced estrogen concentrations in this period might be responsible for the alterations in lipid profile seen in perimenopause [11, 14].

L-arginine a precursor for the synthesis of nitric oxide (NO) is an amino acid that play multiple roles in the cardiovascular system largely through NO production [15–17]. Several studies have established the antihypertensive and antioxidant properties of L-arginine, showing the benefits in hypertension and hypercholesterolemia [15, 18–24]. L-arginine supplementation has been shown to reduce BP and related cardiovascular risk factors in humans and animals. The incidence of women at high risk of cardiovascular diseases during perimenopause has also been documented and there is evidence this is sustained till menopause. There is paucity of data on the beneficial effects of L-arginine supplementation on cardiovascular risk factors during perimenopause in humans and its animal models. Hence this study aimed to assess beneficial effects of oral L-arginine on some cardiovascular risk factors (hypertension and dyslipidaemia) during this transitory period using a VCD induced animal model.

Materials and Methods

1. Chemicals

4-vinylcyclohexene diepoxide (VCD) was purchased from Sigma-Aldrich (St. Louis, MO,

USA). Enzymatic colorimetric assay for the quantification of triacylglycerol and total cholesterol were from Biotécnica (Varginha, MG, Brazil). L-arginine was purchased from Nowfoods (USA).

2. Animals

2.1. Experimental animals and diet

Thirty female Sprague-Dawley rats at 28 days of age were obtained from the animal laboratory center, College of Medicine, University of Lagos. All the animals were housed in groups of 5 rats per clear polypropylene cage lined with wood shavings and acclimatized for one week. Rats were kept under normal light conditions (12 hours light/dark cycle) and normal room temperature (23 ± 1 °C). Pelletized normal rat chow and water was made available *ad libitum*, and all rats were weighed weekly. All experimental procedures were carried out in compliance with the international principles for laboratory animals as obtained in the Helsinki's declaration (NIH1985) guide for care and use of laboratory animals. The research protocol was also in line with the guidelines of the College of Medicine, University of Lagos, Health Research Ethics Committee.

2.2. Experimental design and animal groupings

Post-weaned rats were acclimatized for a week and then randomly divided into three groups as follows: Group I (Control) received daily subcutaneous injection of corn oil (2.5 μ l/g BW) for 15 consecutive days and allowed to grow till the 12th week. Group II (VCD) received daily subcutaneous injection of VCD (160 mg/Kg) [25–26] diluted in Corn oil (2.5 μ l/g BW) for 15 consecutive days and also allowed to grow till the

12th week. Group III (VCD + L-ARG) received daily subcutaneous injection of VCD (160 mg/Kg, BW) as in Group II above, allowed to grow till 8th week (i.e. 8 weeks after VCD administration), after which, 100mg/kg of L-arginine in distilled water was orally administered daily via oral cannula to rats in this group for additional duration of 4 weeks. The average age of rats in each group was 128 days and 12 weeks as stated above signified the number of weeks after either corn oil or VCD administration.

3. Measurement of Cardiovascular parameters

3.1. Non-invasive blood pressure measurement

Once every two weeks at the 8th week, 10th week and 12th week blood pressure parameters were recorded in all the three groups using tail-cuff (non-invasive) apparatus from Kent Scientific CODA system (Coda HT system with 4 activated channels, CODA-HT4). We have previously observed that female rats injected with VCD usually transit towards reproductive senescence from 80 to 100 days of age (yet to be published data). Beginning from the 8th week, animals in each group were aged within 80 to 100 days as stated above. Also non-invasive blood pressure parameters were measured from the 8th week till the 12th week to establish a pattern during this transitory period in female rats.

3.2. Invasive blood pressure measurement

Invasive BP parameters were measured using the Powerlab instrument. Briefly, at the end of the 12th week, rats were fasted overnight and anaesthetized with 1200mg/kg urethane injected intraperitoneally. The reflexes of the animals were checked, and subsequently placed on a suitable rodent surgical table. The femoral vein was

cannulated for saline administration with the skin on ventral side of the neck, right hind leg, and chest carefully shaved and disinfected. An incision (1–2 cm) was made in the epidermis (outer layer of the skin) of the right thigh, and the matrix of collagen fibers interlaced with elastic fibers of the dermis was cleaned carefully. A small incision (1.5–2 cm) was made in the neck of the rat for tracheostomy and carotid artery cannulation. The skin in the neck region was carefully cut open, and a slit incision made in the rat platysma muscles. The trachea was identified, small incision made on its cartilage tissue, and the tracheostomy was carried out using a small piece of rodent tracheal intubation tube.

The carotid artery was identified along with the vagus nerve and cannulated using a cannula pre-filled with heparinized normal saline (0.5 IU/ml) with the other end of the cannula connected to a three-way stopcock/saline filled tuberculin syringe and the animal connected to the power lab machine to record the BP parameters.

4. Blood sampling

Blood samples were collected from each rat immediately after invasive recording of BP parameters according to animal groupings and markings. The blood samples were collected from the left carotid artery using a capillary tube, collected into EDTA coated test tubes and centrifuged at 3000 (rpm) for 10 mins to extract the plasma. The plasma was stored at -80°C for measurement of the fasting lipid profile.

5. Fasting lipid profile assessment

Plasma concentrations of total cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL), and triglyceride (TG) were measured by enzymatic-colorimetric assay using commercial kits (Biotécnica, Varginha, MG, Brazil). Atherogenic fraction (AF) was calculated as the difference between TC and HDL. Increased AF is one of the indicators of cardiovascular risk.

6. Statistical analysis

All results were expressed as mean \pm SEM. Data were analyzed using GraphPad Prism version 8.0 (GraphPad software San Diego, California, USA). Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by student-Newman-Keuls post-hoc test. The differences among the three groups were analyzed using Tukey's Multiple Comparison test. Values of $P < 0.05$ were considered statistically significant.

Results

Cardiovascular Parameters

Tables (1 – 3) show that Systolic blood pressure (SBP), Diastolic blood pressure (DBP), and Mean arterial pressure (MAP) were significantly higher ($P < 0.05$) in the VCD group compared to the control group. At the 8th week (commencement of L-Arginine administration), there was no significant difference in SBP, DBP and MAP between VCD and VCD + L-ARG groups. However, from the 10th week, SBP, DBP and MAP were significantly lower ($P < 0.05$) in VCD + L-ARG group compared to the VCD group.

Table 1. Systolic Blood Pressure (mmHg) in Control, VCD and VCD + L-ARG groups

Grouping (n = 10)	8 th week	10 th week	12 th week	Terminal
Control	131.5 ± 2.75mmHg	137.6 ± 1.41mmHg	127.8 ± 2.41mmHg	104.3 ± 2.01mmHg
VCD	155.71 ± 3.65*mmHg	150.9 ± 1.10*mmHg	160.0 ± 3.40*mmHg	119.2 ± 2.52*mmHg
VCD + L-ARG	157.17 ± 2.48*mmHg	116.9 ± 1.50 α mmHg	105.4 ± 3.87* α mmHg	101.2 ± 232 α mmHg

All results are expressed as mean ± SEM. One-way ANOVA with student-Newman-Keuls post hoc test. *P < 0.05 Vs.

Control, α P < 0.05 Vs VCD.

Table 2. Diastolic Blood Pressure (mmHg) in Control, VCD and VCD + L-ARG groups

Grouping (n = 10)	8 th week	10 th week	12 th week	Terminal
Control	102.1 ± 2.75 mmHg	109.6 ± 2.02 mmHg	92.2 ± 1.65mmHg	80.1 ± 2.42 mmHg
VCD	125.5 ± 2.12*mmHg	124.7 ± 3.46*mmHg	112.4 ± 2.14*mmHg	102.9 ± 3.12*mmHg
VCD + L-ARG	130.11 ± 2.48*mmHg	90.3 ± 2.35* α mmHg	83.8 ± 1.74* α mmHg	84.0 ± 3.87 α mmHg

All results are expressed as mean ± SEM. One-way ANOVA with student-Newman-Keuls post hoc test. *P < 0.05 Vs.

Control, α P < 0.05 Vs VCD.

Table 3. Mean Arterial Pressure (mmHg) in Control, VCD and VCD + L-ARG groups.

Grouping (n = 10)	8 th week	10 th week	12 th week	Terminal
Control	114 ± 2.75mmHg	119.8 ± 2.38 mmHg	96.6 ± 2.62mmHg	90.4 ± 2.12 mmHg
VCD	140.1 ± 2.48*mmHg	133.1 ± 2.12*mmHg	121.7 ± 4.67*mmHg	107.2 ± 3.64* mmHg
VCD + L-ARG	138.2 ± 2.08*mmHg	98.8 ± 2.63* α mmHg	92.9 ± 3.46* α mmHg	90.4 ± 3.06 α mmHg

All results are expressed as mean ± SEM. One-way ANOVA with student-Newman-Keuls post hoc test. *P < 0.05 Vs.

Control, α P < 0.05 Vs VCD.

Table 4. Fasting lipid profile

Table 4 shows that total plasma cholesterol was significantly lower (P < 0.05) in the VCD + L-ARG group compared to Control and VCD groups. The LDL and atherogenic factor were significantly higher (P < 0.05) in VCD group compared to Control and VCD + L-ARG groups. HDL was

significantly higher (P < 0.05) in VCD and VCD + L-ARG groups compared to Control group. There was no significant difference in triglycerides across the groups. Likewise, there was no significant difference in LDL concentrations between Control and VCD + L-ARG groups.

Table 4. Fasting lipid profile in Control, VCD and VCD + L-ARG groups

Grouping (n = 10)	Control	VCD	VCD + L-ARG
Total cholesterol (mmol/L)	2.42 ± 0.02	2.43 ± 0.04	2.15 ± 0.03* α
Triglyceride (mmol/L)	0.50 ± 0.03	0.50 ± 0.05	0.70 ± 0.07
HDL (mmol/L)	1.00 ± 0.01	1.30 ± 0.06*	1.40 ± 0.08*
LDL (mmol/L)	0.50 ± 0.03	0.90 ± 0.06*	0.53 ± 0.05 α
Atherogenic fraction (mmol/L)	0.83 ± 0.04	1.13 ± 0.07*	0.78 ± 0.06 α

Fasting lipid profile: Total cholesterol (mmol/L), Triglyceride (mmol/L), HDL (mmol/L), LDL (mmol/L), and Atherogenic factor (mmol/L). All results are expressed as mean ± SEM. One-way ANOVA with student-Newman-Keuls post hoc test. *P < 0.05 Vs. Control, α P < 0.05 Vs VCD.

Discussion

The study showed that oral L-arginine administration significantly reduced blood pressure and improved lipid parameters in perimenopausal rats. Significant increases in blood pressure seen in VCD rats in this study was in accordance with previous studies [27–28]. Hypertension is the major risk factor for cardiovascular diseases and increases in blood pressure during perimenopausal has been attributed to decreased estrogen levels [29]. Estrogen has been demonstrated to have a regulatory effect on the blood pressure both centrally and peripherally. It binds to estrogen receptors in the cortex to modulate the action of the RAAS system thus causing a reduction in blood pressure. It also acts via the nitric oxide pathway to stimulate the production of NOS and gender differences in the production of endothelial nitric oxide has been reported [30].

In the periphery, estrogen stimulates the production of nitric oxide by the upregulation of endothelial nitric oxide synthase. Nitric oxide is a vasodilatory molecule and its deficiency is implicated in the in the pathogenesis of hypertension [30].

Studies have shown that nitric oxide levels are significantly lower in menopausal women with hypertension compared to premenopausal hypertensive women, thus implicating reduced nitric oxide in the pathogenesis of menopausal related hypertension [31]. Apart from reduced levels of nitric oxide occurring due to reduced synthesis, there is also increased consumption as a result of increased oxidative stress [32, 33]. Estrogen is protective against oxidative stress and increased oxidative stress parameters have been

documented with reduced estrogen levels [31]. Thus, an agent that possesses the dual properties of increasing nitric oxide levels and improving antioxidant potential will be a useful therapeutic agent in reducing the risk of hypertension in the perimenopausal period.

L-arginine possesses both properties [34]. It increases the levels of NO by serving as a substrate for NO as well as upregulating the production of endothelial nitric oxide synthase, the enzyme responsible for conversion of L-arginine to NO [35]. Our study showed that L-arginine supplementation significantly reduced the blood pressure of perimenopausal rats. Results seen in this study was in line with previous studies that showed that oral L-arginine administration is beneficial in hypertensive patients [36–39].

Another important cardiovascular disease risk is high levels of LDL-cholesterol. Increased levels of LDL cholesterol have been reported in the perimenopausal period. This study showed that LDL was significantly higher ($P < 0.05$) in VCD group compared to Control group and this supports previous reports of high levels of LDL in perimenopausal women [14]. Estrogen has been shown to produce a less atherogenic lipid profile with reduced levels of LDL and increased levels of HDL [40–42].

L-arginine administration reduced LDL levels of the perimenopausal rats to comparable levels as seen in control animals. These findings are in line with previous studies that showed the benefits of L-arginine in decreasing LDL levels [43–44]. Similarly, L-arginine supplementation significantly reduced total cholesterol levels as reported in previous studies [43–44].

Unlike findings with total cholesterol and LDL, there was no significant difference in the levels of HDL in the VCD and VCD + L-ARG groups. However, the HDL levels were higher in these perimenopausal groups compared to control group. This suggests that the perimenopausal period is associated with increased HDL levels. This is in line with prior studies [45–46] although other studies reported no change in HDL during the menopausal transition [14, 47 and 48]. In addition, L-arginine supplementation was associated with a significantly lower atherogenic fraction.

L-arginine has been shown to downregulate the expression of important enzymes in lipid biosynthesis such as fatty acid synthase and 3-hydroxy-3-methylglutaryl-CoA reductase [35]. On the other hand, the effect of estrogen on lipids appears to be mediated by increasing the hepatic expression of LDL receptors and decreasing abdominal lipoprotein lipase activity [49]. Although both agents act via the nitric oxide pathway, it appears that the effect of estrogen and L-arginine on lipid pathway may occur through independent pathways [34]. The interrelationship between L-arginine, estrogen and nitric oxide in lipid regulation during perimenopause is a potential area for further studies.

This study showed that L-arginine supplementation improved blood pressure and lipid profile in animal models of perimenopause. This may be protective against atherosclerosis during perimenopause [50]. Both hypertension and high levels of cholesterol are strong markers for cardiovascular disease. Lower levels of LDL have been shown to reduce the risk of atherosclerotic disease progression by causing a

downregulation of inflammatory molecules responsible for this phenomenon [51–53].

Conclusion

In summary, our study supports the hypothesis that daily oral L-arginine administration is protective against cardiovascular diseases by preventing hypertension and derangement of lipid profile during perimenopause due to the L-arginine/NO pathway. However, further long-term studies are necessary to validate this hypothesis.

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