

Is Hypercalcemic Diet A Possible Antidote To Oral Contraceptive-Induced Hypertension?

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Summary: Administration of oral contraceptive (OC) has been associated with body fluid retention and in high doses over a long period, promotes hypertension (Oelkers 1996). This present investigation tests the hypothesis that the dietary calcium supplementation increases salt and water excretion in OC (norgestrel/ethinylestradiol) treated 32 female albino rats randomly distributed into four (1-4) groups of 8 rats each: Control, OC-treated, OC-treated+ Calcium diet fed and Calcium diet fed only respectively. OC was administered to the appropriate groups by gavage. Experimental diet contained 2.5% calcium supplement. Plasma and urinary $[Na^+]$ $[K^+]$ were evaluated after 8 weeks of experimentation by flame photometry and plasma $[Ca^{2+}]$ by colorimetric method. OC-treatment induced a significant fall in urinary $[Na^+]$. Water excretion was significantly reduced in these animals (control, 3.1 ± 0.56 Vs OC-treated rats, 1.47 ± 0.16 ; $p < 0.05$). OC-treated rats had significantly higher ($p < 0.05$) plasma $[K^+]$ compared to control rats. Calcium supplementation induced increases in plasma $[Na^+]$, $[K^+]$ and augmented urinary Na^+ excretion (OC-treated + Ca^{2+} diet Vs OC-treated only, $p < 0.05$). Compared with the control rats, high Ca^{2+} diet fed rats exhibited significant increases in plasma $[Na^+]$ and $[K^+]$ accompanied by significant decreases in urinary H_2O excretion ($p < 0.05$). These results strongly suggest that high dietary Ca^{2+} supplementation increases salt and water excretion in OC-treated rats and potentially moderates fluid retention and blood pressure in these animals, and may be of clinical significance in OC-induced abnormal fluid retention and perhaps OC-induced hypertension.

Keywords: *Hypercalcemic-diet, Oral contraceptive, Plasma electrolytes, Hypertension, Female-albino-rats*

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INTRODUCTION

Sexual dimorphism has been observed in the incidence of cardiovascular disease. For example hypertension; arterial pressure in males exceeds that in similarly aged women until the age of menopause, after which, arterial pressure in women increase rapidly and eventually equals or exceeds that in males (Kolehen et al 1982). This has been attributed to the interaction between circulating ovarian hormones and blood pressure control in women (Fang et al 2001). A similar sexual dimorphism is present in some of the most common rat models of hypertension (e.g. spontaneously hypertensive rats (SHR), Dahl-NaCl hypertensive rats) (Otsuka et al 1997, Cahoun et al 1994).

Several studies suggest that women who eat large amounts of plant estrogen (phytoestrogen) experience a slower rise in the incidence of postmenopausal hypertension (Fang et al 2001). Hormone replacement therapy is known to have many cardiovascular benefits for postmenopausal women, however long term use of oral contraceptive (OC) and other hormone replacement therapies have been implicated in increasing the risk of developing hypertension. Such hormonal use can lead to significant body fluid retention (Stachenfeld et al 2002) and in very high doses hypertension (Oelkers 1996). The mechanism underlying this effect is still unclear, although a number of recent studies have demonstrated a downward resetting of osmotic threshold for thirst and vasopressin release

(Stachenfeld et al 1999). The renin-angiotensin system also has been implicated as a possible mechanism (Stachenfeld et al 2002).

Lastly, accumulated evidence suggests that calcium supplementation inversely correlates with blood pressure in clinical and experimental studies (Hatton et al 1994, Cutler and Brittain 1990). Calcium supplementation has also been shown to decrease blood pressure in human hypertension (Saito et al 1989), including those hypertensive disorders of pregnancy and salt sensitive individuals (Belizan et al 1991). Plausible antihypertensive mechanisms have been suggested as vasorelaxation (Jolma et al 2002), increased diuresis and natriuresis (Butler et al 1995; Oparil 1991) among many others. Conversely, high calcium diet has been demonstrated to prevent and reduce high blood pressure, in part by its effect on renal body fluid homeostasis. Finally increasing evidences now show that OC usage especially over a long term can induce intracellular salt/water expansion and possibly hypertension. This is due partly to the influence of OC on renin-angiotensin-system and other body fluid regulating mechanisms (Oelker, 1996; Oparil et al 1975; Stachenfeld et al 1998).

It is against this background that the present investigation was construed. This study specifically tests the hypothesis that calcium supplementation counteracts, the fall in electrolyte excretion, the accompanying fluid retention and potential hypertension elicited by long term OC use in female albino rat model. In other words, is hypercalcemic diet an antidote to OC- induced hypertension? Attention was also paid to plasma level of certain electrolytes.

MATERIALS AND METHODS

Thirty two female albino rats (*Rattus norvegicus*) of wistar strain were obtained from the department of Physiology, University of Ibadan, Ibadan Nigeria. The rats of age 6-8 weeks weighed between 110-140g. Granulated rat chow used in feeding the animals was obtained from Animal Care Feeds, Yoruba Road, Ilorin, Nigeria. Calcium carbonate was obtained from Griffin and George Limited, London, England. Oral contraceptive tablets of norgestrel with ethinyl/estradiol (0.3mg: 0.03mg) contained in Duo fem were obtained from local Pharmacy. Other reagents were of analytical grade. The experimental diet was normal rat chow which had been further supplemented with 2.5% calcium for every 100g meal.

Animal handling: All animals were allowed one week period of acclimation in well ventilated battery

cages housed in the Department of Human Anatomy, University of Ilorin, Ilorin, Nigeria. They were given unrestricted access to granulated normal rat chow and tap water. A 12h L/D cycle was maintained. Following the acclimation period, the animals were randomly divided into 4 groups (1-4) of 8 rats each. Group 1 which served as the control, continued on the normal rat chow and was administered tap water. Groups 2-4 served as experimental groups. The detailed experimental design and treatment protocol are summarized in table 1.

Drug and Dosing: Two tablets of norgestrel with estradiol contained in Duo fem were dissolved in a total volume of 10ml tap water; of which 30% was administered orally to the animals that received OC. The OC was administered using 1ml insulin syringe. Each rat received a 0.3ml dose of the OC prepared every morning between the hours of 0700-0900 for a period of 8 weeks. The choice of dose simulated that usually administered to a human female of child bearing age of an average bodyweight (Bwt) of 60 ± 5 kg, adjusted for the rats Bwt. Following the 8 experimental weeks, a 24h renal clearance was conducted. Urine samples were collected and measured with insulin syringe, and subsequently stored at 10°C until analyzed.

Blood Sample Collection and Analysis: The rats were anesthetized with ether and dissected through a mid abdominal incision reaching the thorax to expose the heart, from whence blood samples were collected directly into heparinized sample bottles. All samples were subsequently spun at 3500rpm for 10minutes. Hemolysis free plasma samples were recovered and stored at 10°C until plasma electrolyte concentrations could be determined in triplicates.

Both plasma and urine electrolytes were analyzed at the Chemical Pathology Laboratory of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. The plasma and urine $[\text{Na}^+]$ and $[\text{K}^+]$ were measured by flame photometry using Corning 400 series instrumentation laboratory. The plasma $[\text{Ca}^{2+}]$ was determined by standard colorimetric method. The method was essentially based on the formation of violet complex by Ca^{2+} upon reaction with cresolphthalein in an alkaline medium. Color change was monitored spectrophotometrically at a wavelength of 570nm. A mean of three determinations were reported.

Statistical Analysis: All values of variables are reported as means \pm standard error of the mean (SEM). Univariate analysis of variance (ANOVA) model was performed to test differences in the

dependent variables due to OC treatment and Ca²⁺ supplementation, with appropriate post hoc test of significance (Namboodiri et al 1975). A p<0.05 was considered significant.

RESULTS:

The effects of high Ca²⁺ diet on the plasma [Na⁺], [K⁺] and [Ca²⁺] in the presence or absence of OC treatment are summarized in Table 2. Plasma [Na⁺] were similar in the control and OC treated animals. Ca²⁺ supplement significantly elevated

plasma [Na⁺] in the animals fed Ca²⁺ alone and those treated with OC and fed the Ca²⁺ diet when compared to either control rats and those treated with OC alone (p < 0.05). As evident in Table 2, compared to control rats, OC treatment significantly increased the plasma [K⁺] (p < 0.05). The plasma [K⁺] in both the Ca²⁺ only and OC + Ca²⁺ diet groups were significantly (p < 0.05) lower than those of the rats administered OC alone. Ca²⁺ supplementation normalized the elevated plasma [K⁺] towards the control value. Generally, Ca²⁺ supplementation had no significant effect on plasma [Ca²⁺] (Table 2).

Table 1
Details of Experimental Design⁺ and Treatment regimen

S/N	GROUP ⁿ	Parameters			
		Tap Water (H ₂ O)	OC	OC + Ca ²⁺ Diet	Ca ²⁺ Diet
1.	Control	++	--	--	--
2.	OC	--	++	--	--
3.	OC + Ca ²⁺ Diet	--	--	++	--
4.	Ca ²⁺ Diet	--	--	--	++

ⁿnumber of animals per group = 8; ⁺CRFx, completely randomized factorial design; ^{oc}Oral contraceptive; ^{Ca2+ Diet} normal rat chow supplemented with 2.5% Ca²⁺/100g meal.

Table 2
Effect of High Ca²⁺ Diet on Plasma [Na⁺], [K⁺] and [Ca²⁺] in the Presence or Absence of OC Treatment

S/N	GROUP ⁿ	Parameters		
		[Na ⁺]	[K ⁺]	[Ca ²⁺]
1.	Control	130.50 ± 0.96 [†]	4.2 ± 1.38	2.55 ± 0.04
2.	OC	134.13 ± 1.11 ^{ns}	7.36 ± 0.45 [*]	2.02 ± 0.08 ^{ns}
3.	OC + Ca ²⁺ Diet	145.40 ± 1.48 ^{*,#}	4.71 ± 0.15 [#]	2.19 ± 0.05 ^{ns}
4.	pCa ²⁺ Diet	141.70 ± 1.52	4.63 ± 0.09 [#]	2.19 ± 0.04 ^{ns}

ⁿnumber of animals per group = 8; [†] mean ± SEM; ^{oc}oral contraceptive; ^{Ca²⁺}high calcium diet; ^{ns} not significant versus control; ^{*}p<0.05 versus control; [#]p<0.05 versus OC treatment only

Table 3
Effect of High Ca²⁺ Diet on Urinary [Na⁺] and [K⁺], 24h Excretion rates and H₂O output in presence or Absence of OC treatment.

Group ⁿ	Parameters				
	Urine		24 Hrs Excretion (mMol/L/hr)		24 Hrs H ₂ O Output (mL)
	[Na ⁺]	[K ⁺]	Na ⁺	K ⁺	
Control	51.75 ± 18.32 [†]	57.48 ± 4.21	4.96 ± 1.41	6.50 ± 0.98	3.10 ± 0.56
OC	45.13 ± 9.32 [*]	72.59 ± 3.92 [*]	2.20 ± 0.03 [*]	5.11 ± 0.76 [*]	1.47 ± 0.16 [*]
OC + Ca ²⁺	187.10 ± 22.20 ^{*,#}	96.86 ± 4.27 ^{*,#}	16.45 ± 4.71 ^{*,#}	7.66 ± 1.05 ^{ns,#}	1.98 ± 0.38 ^{*,#}
Ca ²⁺	185.30 ± 26.40 ^{*,#}	103.30 ± 4.77 ^{*,#}	12.12 ± 2.46 ^{*,#}	7.21 ± 1.37 ^{ns,#}	1.76 ± 0.37 ^{*,#}

ⁿnumber of animals per group = 8; [†] mean ± SEM; ^{oc}oral contraceptive; ^{Ca²⁺}high calcium diet; ^{ns} not significant versus control; ^{*}p<0.05 versus control; [#]p<0.05 versus OC treatment only

Table 3 depicts the effects high Ca²⁺ diet on urinary [Na⁺] and [K⁺], as well as their 24h urinary excretion rates (mMOL/L/hr) and the 24h urine water output of the experimental animals. Compared to the control group, OC treatment significantly (p < 0.05) reduced urine [Na⁺] with an equally significant increase in [K⁺] (p<0.05). The 24h urinary excretion of these

ions (especially the Na⁺ ion) were significantly reduced (p < 0.05) with a corresponding significant depression of 24h urine water output (p < 0.05). The urine water output was more than halved in these OC treated animals. OC treatment thus enhanced fluid retention when compared to the control group. In contrast to both the control and OC treated animals,

the rats fed high Ca^{2+} diet alone had significantly ($p < 0.05$) higher urinary levels of Na^+ and K^+ . The 24h urinary excretion rates of these ions were equally significantly higher in these Ca^{2+} fed animals. The 24h urine water output of Ca^{2+} fed animals was significantly lesser than those of the controls but significantly higher than the OC treated animals ($p < 0.05$). Ca^{2+} treatment thus induced frank diuretic water loss in the treated animals, increasing and moving the 24h urine water loss closer to the control value.

Combined with OC treatment (OC+ Ca^{2+} diet group), both the urinary [Na^+] and [K^+] and 24h urinary (excretion) diuretic water loss were significantly increased ($p < 0.05$) by high Ca^{2+} diet vis-à-vis the other groups. The 24h urine water output rose significantly ($p < 0.05$) and was pushed even closer to the control value (Table 3). Kaliuresis was generally not statistically significantly affected by any of the treatments.

DISCUSSION

This present study was undertaken to evaluate the hypothesis that Ca^{2+} supplementation modulates the electrolyte excretion, body fluid dynamics and potentially the hypertension elicited by long term OC treatment in the rat model. The results from this study strongly suggest that chronic administration of OC (norgestrel/ethinylestradiol combination) impairs natriuresis and diuresis in rats suggestive of potential water retention by these studied animals. Na^+ is the major extracellular fluid (ECF) solute. Changes in total body [Na^+] therefore modify both the ECF volume, plasma space and possibly induce blood pressure changes.

Decrease in Na^+ excretion and the accompanying fluid retention observed in this study likely leads to chronic increase in ECF volume and ultimately increases the effective blood volume and potentially results in elevated blood pressure. Other endogenous factors such as humoral and neural which may cause increased vasotone may further facilitate this hypertensive tendency. Putatively, these factors by one way or the other increase peripheral vascular resistance or decrease vascular capacitance (Jolona et al 2000).

High dietary calcium supplementation by itself moderated water retention and in combination with OC regimen potently countered the observed OC effect. The result of this study is consistent with the findings of Oparil et al (1975) and Jirakulsomchok et al (1990) which had demonstrated pronounced natriuretic and diuretic effects of dietary calcium in volume loaded NaCl-sensitive spontaneously

hypertensive rats. Thus the use of OC is associated with an increase in salt and water loss in response to dietary calcium intake. Calcium supplementation also impacted the flux of other electrolytes such that the plasma [K^+] was markedly increased in OC treated animals and this was ameliorated by calcium supplementation. Effect of OC on Kaliuresis was modest relative to other electrolytes. These findings in this study are quite in agreement with those previous reports which had variously reported increases in urinary [Na^+] and urine volume in response to Ca^{2+} supplementation and even a positive correlation between urinary [Na^+] and Ca^{2+} excretion. However, evaluation of correlation of Na^+ excretion with Ca^{2+} supplementation was not undertaken in this present study.

Finally, the data presented in this study are not consistent with the reports that Ca^{2+} supplementation reduced plasma [Na^+] and thereby cause volume contraction (Zemel et al (1988); (Aryola et al 1993). In this present study similar to the findings of Jirakulsomchok et al (1990) Ca^{2+} supplementation rather increased plasma as well as urinary [Na^+] significantly with concomitant increases in excretion rate of these ions and water output. Furthermore, our results are also congruent with other results which had demonstrated that dietary calcium supplementation restores pressure natriuresis in experimental rat model (Butler et al 1995). However, our results do not exclude the possibility that the plasma Na^+ reductive effect of Ca^{2+} observed by those workers may be dependent on the amount of Ca^{2+} supplement. Reduction in total and fractional (extracellular) water as has been associated with Ca^{2+} induced natriuresis by Zemel et al (1988) may be suggestive of a possible modulating role or Ca^{2+} dose effect. In any case, the amount of Ca^{2+} supplement employed in this study had no significant effect on the overall body/blood Ca^{2+} status of the studied animals.

In conclusion therefore, the results of this study strongly suggest that chronic administration of norgestrel/ethinylestradiol markedly impairs natriuresis and diuresis in female albino rats. High dietary Ca^{2+} supplementation counteracted these impairments. Further investigations are however needed to establish the possible clinical significance of these findings and determine whether Ca^{2+} supplementation upon oral contraceptive therapy could indeed induce intravascular volume contraction. If this holds true in further experimental investigations then, such Ca^{2+} supplementation therapy could be of potential significance in oral contraceptive induced hypertension.

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