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Effect of Increased Dietary Calcium on Body Weight, Food and Water Intake in Oral Contraceptive Treated Female Rats

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Summary: The effects of high calcium diet on body weight in OC treated rats are unknown. This study therefore investigated the effect of increasing dietary calcium from 0.9% to 2.5% on body weight, food ingestion, water intake, heart weight index and renal weight index in female Sprague-Dawley rats treated with a combination of OC steroids (ethinyloestradiol + norgestrel). The rats were assigned into three groups of average of 11 rats each; control, OC-treated and OC + Calcium – treated groups and administered orally for 10 weeks. Food and water intake, body weight, cardiac weight index, left ventricular weight index, renal weight index and serum calcium level were determined. The result shows that OC treated rats had significantly lower serum calcium concentration, body weight gain, food, water and calcium intake than those of the control rats. The OC + Calcium – treated rat had significantly higher serum calcium concentration, food, water and calcium intake but significantly lower body weight than those of the OC – treated rats. OC + Calcium – treated rats had significantly higher water intake, calcium intake and significantly lower body weight and food intake when compared with the control rats. Cardiac weight index and renal weight index was comparable in all groups. In conclusion, combined OC-induced reduction in weight gain might be associated with inhibition of the feeding center and consequent inhibition of the thirst center. Co-administration of dietary calcium augmented the reduction in weight gain seen in OC-treated rats probably by further suppression of the feeding and thirst centers.

Keywords: Calcium, oral contraceptives, food intake, feeding centre, body weight.

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INTRODUCTION

Prolonged administration of synthetic steroids commonly used as oral contraceptives (OC) has been associated with an increased risk of stroke, myocardial infarction, renal disease, venous thromboembolic disease, hypertension and obesity (Lim *et al*, 1984; Manton, K.G., 1998; WHO, 1998). Among women, obesity is a primary cause of clinical and psychological problem, which is associated with some other health problems such as myocardial infarction and hypertension (Manton, K.G., 1998; NCEP, 1993).

Despite the extensive use contraceptive steroids worldwide, little is known about the effects of these steroids on the regulation of body weight. The present knowledge of the effect of OC steroids use on the regulation of body weight is inconsistent (Brill *et al*, 1994; Durand *et al*, 1997; Rosenberg, 1998). However, it is a common perception among women that OC use causes weight gain. Studies have demonstrated that steroid hormones can affect the skeletal system and affect overall body mass and composition (Clark and Torttelim, 1982). Previous studies have reported that administration of oestradiol reduced body weight gain, while administration of

progesterone increased body weight gain (Schwartz and Wade, 1981; Gray and Wade, 1981). Food and water intake have also been shown to be altered by circulating endogenous steroid hormones, especially oestradiol (Ganesan, 1994). A series of studies has documented that the use of combined OC is associated with a decrease in food and water consumption (Schwartz and Wade, 1981; Kisley *et al*, 1999; Wallen *et al*, 2002; Akhigbe *et al*, 2008)

Apart from the significant role of dietary calcium in the maintenance of skeletal integrity, it has been shown that calcium supplementation plays important role in preventing chronic disease risk (Zemel, 2001). Recent findings indicated that OC may result in decreased plasma calcium level (Ribeiro-Alves *et al*, 2003) and increased plasma levels of vitamin D (Harris and Dawson-Hughes, 1998). There has been growing interest in the role of dietary calcium in the prevention of obesity (Zemel, 2001) since women using oral contraceptive are assumed to gain weight (Jorde and Bonaa 2000; Zemel, 2001). High dietary calcium or dairy – rich diets not only reduces the risk of hypertension and cardiovascular disease, but may play an important role in the prevention and treatment of obesity as well, by suppressing circulating concentrations of vitamin D and inhibiting lipogenesis (Zemel, 2001).

However, no study has documented the effect of combined OC and calcium diet on body weight, and food and water intake. Thus, this study aimed at documenting the weekly changes in body weight gain, and food and water intake following high dietary calcium in OC-treated female rats.

MATERIALS AND METHODS

Animals and treatments:
Experiments were performed with inbred female Sprague-Dawley rats, initially weighting 110-120g and age between 10-12 weeks. They were divided into three groups (11 rats per group). The animals were housed in wire-bottomed, stainless steel cages in a well ventilated room maintained at 25^0C, on a 12-hour light – dark cycle. Group 1 (vehicle treated) which serve as the control, received 0.2ml of olive oil per 100g body weight by oral gavage and fed on rats chow containing 0.9% calcium. Group 2 (OC – treated) received 0.2ml of olive oil 1.0µg ethinyl estradiol and 10 µg norgestrel (Wyeth-Ayerst, Inc. Canada) per 100g body weight by oral gavage as in previous studies (Ciavalti *et al*, 1989) and fed on rats chow containing 0.9% calcium. Group 3 (OC + calcium treated group) received similar treatment as in group 2 but fed on rat chow containing 2.5% calcium. The 2.5% calcium diet was prepared by addition of 1.6g calcium to 98.4g of powdered rat chow containing 0.9% calcium and then mixed thoroughly (Olatunji *et al*, 2008). Extra calcium was supplied as the carbonate salt (May and Baker, Ltd. Dagenham, England) and otherwise the chows were identical. All animals had free access to water and appropriate diet for 10 weeks.

All experiments were carried out in accordance with the American Physiological Society "Guiding principles for Research Involving Animals.

Measurement

Food and water intake: Food and water intake were monitored daily throughout the experimental period of 10 weeks between the hours of 0800 and 1100h. Two rats were kept in a cage and the total food and water consumed by the rats per day were measured and divided by the members of rats (2) to obtain the average daily consumption per rat. The daily calcium intake for each rat was calculated from the daily food consumption while water intake was measure with a feeder containing known volume of water, attached to the cage. After each day, the volume of water remaining in the feeder was deducted from the initial volume to obtain the average daily consumption of water per rat. (Akhigbe *et al*, 2008)

Cardiac weight index, left ventricular weight index and renal weight index determinations. The heart and kidneys were excised, cleaned and weighed. The atrial and the right ventricle were then removed and the remaining tissue (left ventricle plus septum) weighed. The heart weight index (Heart Weight/Body Weight), the left ventricular weight index (left ventricular weight/Body Weight) and the renal weight index (Kidney Weight/Body Weight) were calculated by dividing the heart weight, the left ventricular weight and the kidneys weight by the body weight (Jolma *et al.*, 2000).

Determination of serum calcium level

Blood (2ml) was collected from the anaesthetized rats by cardiac puncture and allowed to stand at room temperature for 10-15 minutes after which it was centrifuged at 3,000g for 10 minutes. Serum was extracted for calcium concentration determination. Serum calcium concentration was estimated according to Merck Diagnostic (E. Merck, Darmstadt, Germany) using the methyl thymol blue method as reported by Olatunji *et al (*2008)

Statistical Analysis

Results are expressed as means to SEM of measurements. Comparison was done using one-way

analysis of variance (ANOVA) followed by Bonferroni's test post hoc for pair wise comparisons. $P<0.05$ was considered statistically significant. All statistics were performed using the statistical program for social sciences (version 9; SPSS Inc, Chicago, U.S.A).

RESULTS

Body weight

Fig. 1 illustrates the changes in the body weights of the rats in the different groups over the 10 weeks period of experiment. The body weight of OC – treated rats became significantly lower ($p<0.05$) than the control rats as from the 56th day and the decrease persisted till the end of treatment period.

On the other hand, the body weight of OC + calcium – treated rats became significantly lower ($p<0.05$) on the 21st day of treatment than those of the control rats and the decreases in body weight persisted throughout the experimental period. The decreases in body weight observed in OC + calcium – treated rats were significant greater ($p<0.05$) than those of the OC – treated between the 63rd and 70th days of the treatment.

Food intake

The pattern of food intake of the rats in each group is shown in fig. 2. OC – treated and OC + calcium – treated rats were consuming significantly lower food ($p<0.05$) relatively to the control rats on 7th and 28th days, though there was a transient return of food intake of OC – treated and OC + calcium – treated rats to the pre-treatment levels about the 36th day. The food consumption of OC – treated and OC + calcium – treated rats continued to decrease after 7th week until the end of the experimental period. However, the decrease in food intake in OC + calcium – treated rats was significantly attenuated when compared with the OC – treated rats.

Table 1:
Effect Of Administration Of Oral Contraceptives On Calcium Intake And Plasma Level Of Calcium In Control, Oral Contraceptive Treated (Oc) And Oral Contraceptive + Calcium (Oc + Calcium) Treated Rats.

Variables	***Cr***	***Oc Treated***	***Oc + Ca Treated***
Calcium Intake(G)	0.16 ± 1.1	0.12 ± 1.3*	0.19 ± 0.9**
Plasma Calcium(Mmol/L)	2.25 ± 0.04	2.02 ± 0.08*	2.19 ± 0.05*

Values Are Expressed As Means±S.E.M Of 11 Rats Per Group. Means In Rows Showing Common Superscript Letters * Are Not Significantly Different; $P<0.05$.

Table 2:
Effect Of Administration of Oral Contraceptive on Organ Weight and Organ To Body Weight Ratio In Control, Oral Contraceptive Treated (Oc) And Oral Contraceptive + Calcium (Oc + Calcium) Treated Rats.

Variables	Cr	Oc Treated	Oc + Ca Treated
Heart Weight(G)	0.55 ± 0.01*	0.49 ± 0.03*	0.51 ± 0.02*
Heart Weight/Body Weight	0.26 ± 1.0*	0.3 ± 1.0*	0.28 ± 0.9*
Kidney Weight(G)	1.22 ± 0.06*	1.18 ± 0.04*	1.25 ± 0.02*
Kidney Weight*/Body Weight*	0.64 ± 0.3*	0.65 ± 0.1*	0.68 ± 0.4*

Values Are Expressed As Means±S.E.M Of 11 Rats Per Group. Means In Rows Showing Common Superscript Letters * Are Not Significantly Different; $P<0.05$.

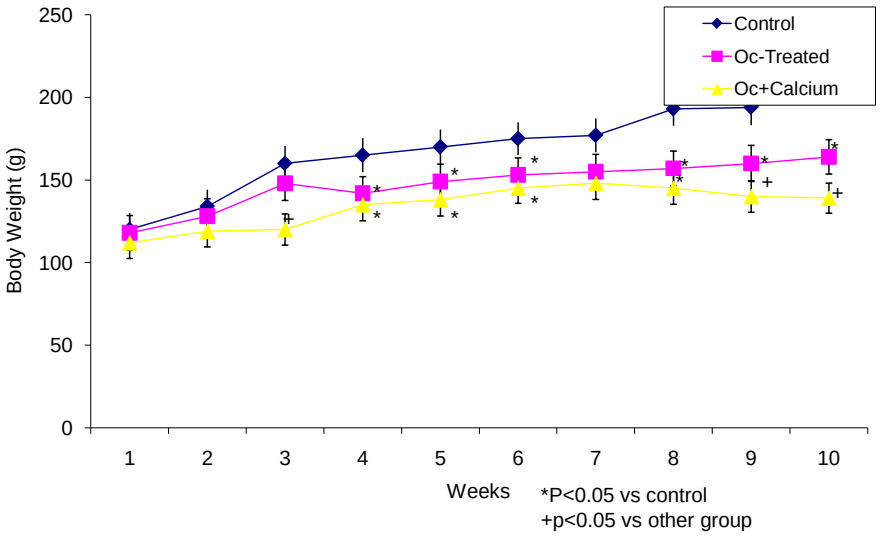

Fig. 1:
Body weight change in control, oral contraceptive(OC)-treated and oral contraceptive + calcium (OC+Calcium)-treated rats. Values are expressed as means±S.E.M of 11 rats per group. Groups showing different superscript letters $^{*,+}$ are significantly different; p<0.05.

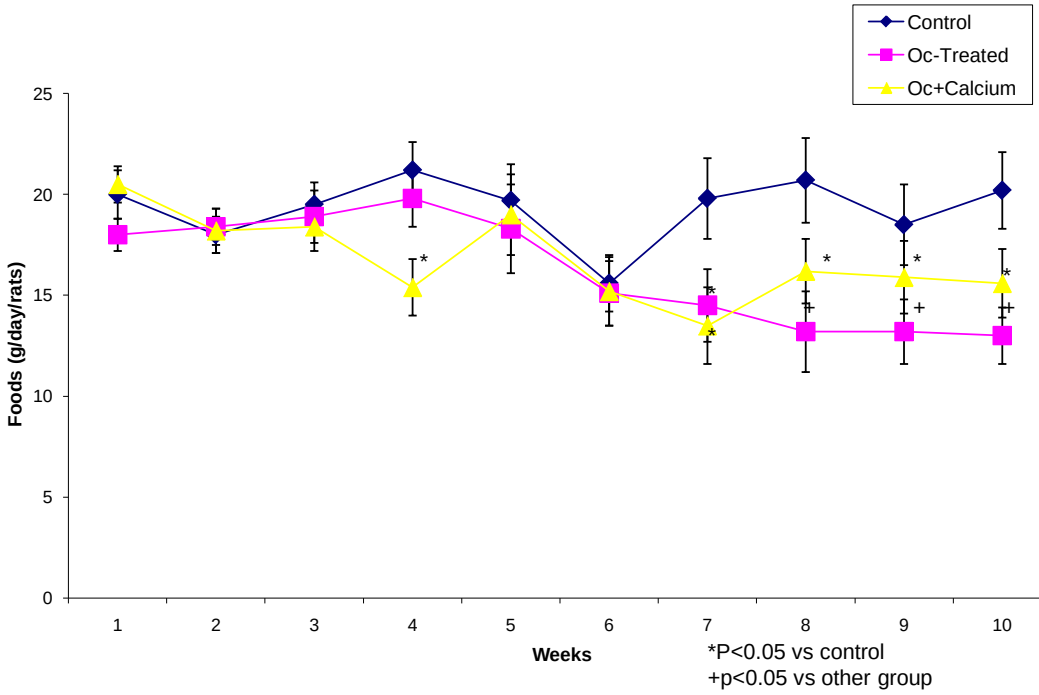

Fig. 2:
Food intake patterns of control, oral contraceptive-treated (OC) and oral contraceptive + calcium-treated (0C+Calcium) rats. Values are expressed as means±S.E.M of 11 rats per group. Groups showing different superscript letters $^{*,+}$ are significantly different; p<0.05.

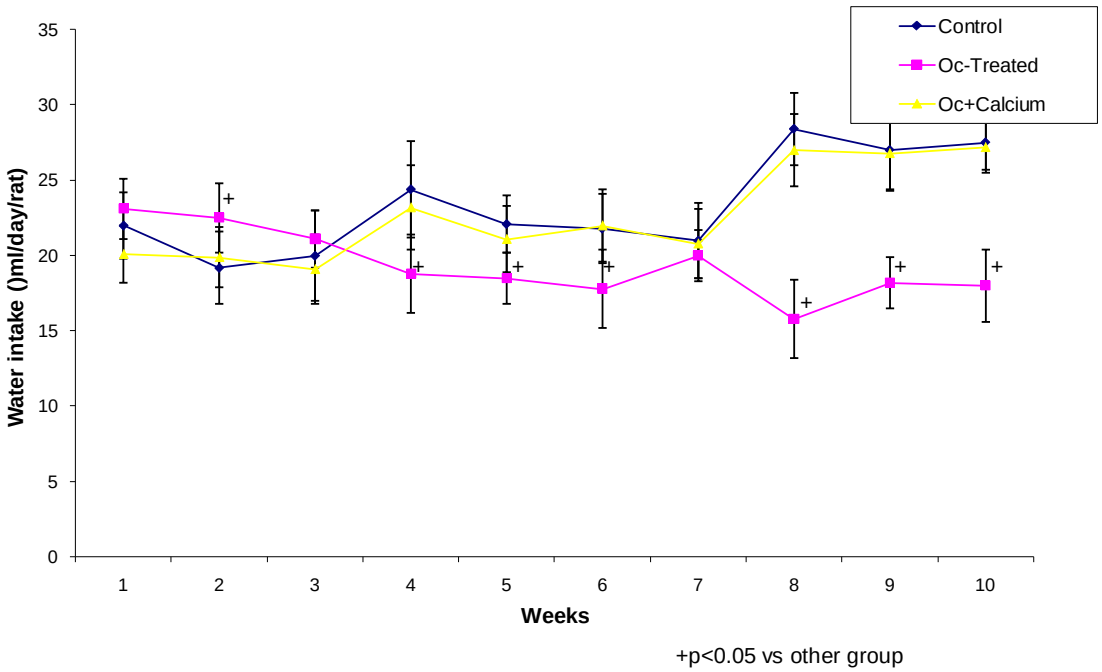

Fig.3:
Water intake change in control, oral contraceptive-treated (OC) and oral contraceptive + calcium-treated (OC+Calcium) rats. Values are expressed as means±S.E.M of 11 rats per group. Groups showing different superscript letters $^{*,+}$ are significantly different; $p<0.05$.

Water intake

Figure 3 shows the pattern of water intake of the three groups of rats. The water intake by OC – treated group decreased significantly ($p<0.05$) on the 28th day of treatment, though there was a transient normalization of water intake at the 47th day of OC treatment.

Water consumption decreased after the 56th day till the end of OC treatment. However, the patterns of water intake of the control and OC + calcium – treatment rats were comparable throughout the experimental period.

Calcium intake

The calcium intake of OC – treated rats was significantly lower ($p<0.05$) than that of the control rats. However, the OC + calcium – treated group consumed significantly more ($p<0.05$) calcium than those of the OC – treated and control groups.

Organ weight and organ weight ratio

There was no significant effect ($p<0.05$) of OC on the organ weight and organ weight ratio in the treated rats. However there was a reduction in the absolute heart weight and kidney weight, although the heart weight-body weight ratio and kidney weight ratio was not affected in treated rats when compared with the control group.

DISCUSSION

A decrease rate of body weight gain in oral contraceptive usage was consistently observed in two earlier studies (Durand *et al*, 1997, Kang *et al*, 2001). The present study also showed attenuated rate of weight gain in the rats treated with OC steroids. This is in consistent with earlier studies in the animal model (Fregly, 1973 and Fowler *et al*., 1985). Little is known about the effect of OC steroids on the regulation of body weight. The decrease in weight gain may be attributed to decrease food ingestion and water intake as observed in the present study which suggest that oestrogen component of the OC used may be accountable for the loss of body weight since previous studies have demonstrated that administration of oestrogen reduced body weight gain in ovarectomised and ovary-intact rats while administration of progesterone increased body weight.

In this study, changes in food and water intake in the same animals were monitored throughout the experimental period. The observations in this study are in consonance with those of other studies that found decreased food and water intake in female rats with oestrogen singly and in combination with progesterone (Kisley *et al*., 1999).

The fact that OC administration led to significant decreased in food ingestion and water intake four

weeks and two weeks respectively, before significant reduction in weight gain was noted, confirms that reduced weight gain could be primarily due to reduced food intake. Since water intake decreased significantly following OC treatment two weeks after the reduction in food ingestion implies that decreased water intake is due to feeding centre overriding thirst centre in the brain of OC-treated animals. This may be attributed to the ability of these steroids, especially oestrogen to exert their hypophagic and antidispsogenic effects by reducing the numbers of hypothalamic receptors available in the respective centres (Jonklass and Buggy, 1985 and Kisley *et al*., 1999).

The attenuation of food and water intake observed in OC – treated rats, is consistent with the report that administration of high circulating levels of ovarian is associated with reduction in food consumption as well as an attenuation of water intake elicited centrally via the activation of rennin – angiotensin system (Ganesan, 1994, kang *et al*, 2001, kisley *et al*, 1999). The fact that OC + calcium – treated rats consumed more food and water than OC – treated rats suggests that high dietary calcium could ameliorate the hypophagic and antidispsogenic effects of OC steriods.

In OC – treated rats, it was observed that there was a decrease in the serum level of calcium which indicates that oral contraceptives may affect the calcium level in the body. This might have been due primarily to the decrease in food intake resulting in calcium deficiency but interestingly; it was corrected by calcium supplementation.

Previous epidemiological and clinical studies have shown that the increased weight of the heart associated with left ventricular hypertrophy is independently associated with increased risk of premature cardiovascular morbidity and mortality (Julius and Gudbranson, 1992, Lim *et al*, 1987). In this study, cardiac weight index and renal weight index did not change between the OC treated and control rats which is in accordance with some of the reports in other models of hypertension (Arvola *et al*, 1993, Chasan-Taber et al, 1996), although contradictory findings has been reported (Clark and Torttelim, 1982).

Despite the absence of cardiac and renal hypertrophy in the OC treated group in high calcium diet, reduced absolute heart weight and kidney was observed in the present study, although the heart weight-body weight ratio and kidney weight ratio was not affected. Studies have shown that high dietary calcium attenuates weight gain in animals which has been attributed to reduced body fat content and inhibition of lipogenesis (Zemel, 2002). We observed that OC + calcium treated rats gained loss weight than the OC – treated group.

In conclusion, combined OC-induced reduction in weight gain might be associated with inhibition of the feeding center and consequent inhibition of the thirst center. Co-administration of dietary calcium augmented the reduction in weight gain seen in OC-treated rats probably by further suppression of the feeding and thirst centers.

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