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Effect of dietary magnesium on glucose tolerance and plasma lipid during oral contraceptive administration in female rats

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Summary
Studies that associated oestrogen-progestogen oral contraceptive (OC) use with altered glucose and lipid metabolisms in women did not account for possible influence in dietary magnesium. The use of OC and glucose and lipid metabolism seems to remain a broad public health concern since over 100 million women use OC world wide for a prolonged period of time. The study, therefore, sought to investigate in a female rat model whether or not glucose intolerance and dyslipidaemia associated with OC are influenced by dietary magnesium status. Control and OC-treated rats were maintained on control diet, whereas OC+ Mg- treated rats were on high magnesium diet. OC-treated and OC+Mg treated rats also received a combination of OC steroids, ethinyl oestradiol and norgestrel (orally). When compared with the controls, OC treatment led to significant reduced glucose tolerance and plasma HDL-cholesterol and significant increases in plasma LDL-cholesterol and atherogenic indices in OC- treated rats. Treatment with OC did not result in significant attenuation in these parameters in OC+Mg- treated rats when compared with the controls. In conclusion, these results suggest that impaired glucose tolerance and dyslipidaemia associated with OC use may be prevented by increased dietary magnesium.

Keywords: *Dietary magnesium, dyslipidaemia, glucose tolerance, oral contraceptive.*

Résumé
Les études qui associent l'usage de l'oestrogen-progestogene contraceptif orale avec le glucose altéré et le métabolisme des lipides chez les femmes n'expliquaient pas l'influence possible du régime de magnésium. L'effet de l'OC, du glucose et du métabolisme des lipides demeure un problème de santé publique majeur, puisque plus de 100 millions de femmes utilisent l'OC dans le monde entier pour une période de temps prolongé. L'étude cherche donc à examiner si chez les rats femelles il y a ou pas intolérance du glucose et dyslipidemie associé à l'OC sont influencés par un état de régime en magnésium .les rats étaient divisés en groupes, de contrôle (CR), OC traitée, OC+Mg traité (n=8 par groupe). Les rats du control et le OC traité étaient maintenus à un régime contrôlé, alors que les rats traités de OC+Mg étaient sous un régime en magnésium élevé. Les rats traités d'OC et ceux traitée d'OC+Mg recevaient aussi une combinaison de stéroïde OC (ethinyloestradiol et norgestrel, P.O). comparée au control, le traitement à l'OC conduit à une réduction importante en tolérance du glucose et du plasma HDL cholestérol et une augmentation importante dans le plasma LDL-cholesterol et les indices atherogeniques chez les rats traités d'OC. Le traitement avec l OC ne conduisait pas à une altération considérable en tolérance du glucose, le plasma HDL cholestérol, LDL-cholesterol et les indices atherogeniques chez les rats traités vde OC+Mg comparée à ceux du control. En conclusion, ces résultats suggèrent que la tolérance en glucose détérioré et la dyslipidemie associé à l'utilisation de l'OC peut être amélioré par un régime riche en magnésium.

Introduction

Combined oestrogen-progestogen oral contraceptive (OC) use has been documented to produce deterioration in carbohydrate tolerance ranging from mild impairment to overt diabetes mellitus [1,2]. Studies in humans have reported that daily administration of high dose of synthetic estrogens, ethinyl oestradiol in combination with progestogen caused impaired glucose tolerance following an oral glucose load [1]. Studies on laboratory animals have shown that estrogens treatment improves glucose tolerance by increasing insulin sensitivity of glucose uptake whereas progestogens, especially 19-nortestosterone derivatives such as norgestrel, have the potential to counteract the effect of oestrogen by factors that are yet to be fully understood [2].

The dyslipidaemia associated with insulin resistance syndrome is characterized by elevated triglyceride, low high-density lipoprotein

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(HDL) cholesterol with normal or elevated low-density lipoprotein (LDL) cholesterol levels [3]. Studies have established the importance of low HDL cholesterol as well as high LDL cholesterol as strong independent predictors of the development of atherosclerotic cardiovascular diseases (CVD) [3,4]. Hence total cholesterol/ HDL cholesterol ratio and LDL cholesterol/HDL cholesterol ratio are considered as strong atherogenic indices [4,5].

Magnesium is the fourth most abundant cation in the organisms, which is an important cofactor in over 300 enzymatic reactions critical for carbohydrate metabolism [6]. Hypomagnesaemia has been associated with impaired glucose tolerance and insulin sensitivity [7,8,9]. Glucose intolerance has been suggested to be a factor associated with the development and progression of atherosclerotic CVD and hypertension [3,10]. Therefore, low dietary magnesium may be an important link between insulin resistance, hypertension and accelerated atherosclerotic CVD.

It has been shown that OC use may result in decrease plasma magnesium level [11,12]. Nevertheless, studies that associated OC treatment with altered glucose [1,2] and lipid [1,13,14] metabolisms in women, did not account for possible variations in dietary. The use of OC on glucose and lipid metabolisms seems to remain a broad public health concern since over 100 million woman use OC world wide for a prolonged period of time. The study, therefore, sought to investigate in a female rat model whether or not glucose tolerance and dyslipidaemia associated with OC are influenced by dietary magnesium status.

Materials and methods

Animals and treatments

Female Sprague-Dawley rats weighing between 120-150g were obtained from the animal house of the college of Health Science, University of Ilorin (Ilorin, Nigeria). The animals were housed in a group of two in a well-ventilated room maintained at 26±2°C, on a 12-hour light-dark cycle. Procedures in involving animals and their care were performed in accordance with the NIH guideline for the care and use of laboratory animals. Rats were pair-fed with appropriate diet and tap water in addition to olive oil (vehicle) or OC treatment for six weeks. They were assigned to one of the four experimental groups of comparable body weight, food intake and fasting blood glucose levels (table1). Control group were maintained on rat chow, which contained 0.24% (w/v) magnesium in addition to daily administration of 0.2mL olive oil (p.o). OC-treated group was maintained on chow containing 0.2% (w/v) magnesium, in addition to daily administration of 0.2ml of olive oil containing a combination of 1.0ìg ethinyl oestradiol and 10.0ìg norgestrel (Wyeth-Ayerst, Inc, Montreat, Canada; p.o). OC-Mg group was maintained on chow containing 0.04% (w/v) magnesium and also received OC treatment as in OC-treated group. Extra magnesium was supplied as the chloride salt, and otherwise the chows were identical.

Oral glucose challenge

An oral glucose tolerance test was performed two days prior to the end of the experiment. The animals had 12-hour overnight fasting. Glucose (2g/kg body wt) was given for 30% solution (p.o.). Blood samples were collected before glucose load and sequentially for 30, 60 and 90min after glucose load and were immediately analysed for glucose colourimetrically. Glucose tolerance was expressed as a function of the area under the tolerance curve (K_G) [15,16]

Biochemical assay

At the end of the experimental period, the animals were sacrificed by cervical decapitation and blood was collected in heparinized centrifuge tubes, and centrifuged at 3000rpm for 10mi. Plasma magnesium concentration were determined by colourimetry using assay kit (PPC Pharm tec Gmbtt, Essen, Germany). Fasting plasma levels of total cholesterol (TC), triglyceride (TG), and HDL cholesterol (HDL-C) were determined by standard enzymatic method coupled with spectrophotometry using assay kit (Randox Lab. Ltd., Co. Antrim, UK). LDL cholesterol was estimated with the use of Friedewald's formula [17].

Statistical analysis

Data are expressed as means±SEM of 8 rats per group. Statistical analyses were done by ANOVA, followed by Duncan's multiple range test for pairwise comparison. $P<0.05$ was considered to be significant. All analyses were done using Statistical package for Social Sciences (SPSS Inc., Chicogo, IL. USA).

Results

There was no significant difference in body weight, food intake and fasting blood glucose among the group before and after the experimental period (Table1). There was a significant difference in plasma magnesium levels among the experimental groups (Table 2). In comparison to control rats, OC treatment led to significant decrease in glucose tolerance in OC-treated rats, whereas treatment with OC did not result

in altered glucose tolerance in OC+Mg-treated rats (Table 2 and fig. 1). OC treatment led to significant increases in plasma levels of LDL-cholesterol, atherogenic indices (TC/HDL cholesterol and LDL cholesterol/HDL cholesterol), and a decrease in plasma HDL cholesterol in OC-treated rats (table 3). On the other hand, OC treatment did not affect plasma lipid profile significantly in OC+Mg-treated rats (table 3).

Table1. Body weight, food intake and fasting blood glucose in experimental groups

	Control	OC-treated	OC+Mg-treated
Body weight, g			
Week 0	149.8±6.2[a]	148.8±5.7[a]	150.1±4.9[a]
Week 6	172.6±8.1[a]	176.1±6.2[a]	178.8±4.3[a]
Food intake, g			
Week 0	18.6±2.1[a]	18.2±3.5[a]	19.5±2.3[a]
Week 6	21.5±2.2[a]	20.2±2.6[a]	22.4±2.4[a]
Fasting blood glucose, mmol/L			
Week 0	4.1±0.2[a]	4.3±0.2[a]	4.1±0.2[a]
Week 6	4.2±0.1[a]	4.6±0.4[a]	4.3±0.2[a]

Values are expressed as mean ±S.E.M. of 8 rats per group. Means in rows not sharing common superscript letters are significantly different, $p<0.05$.

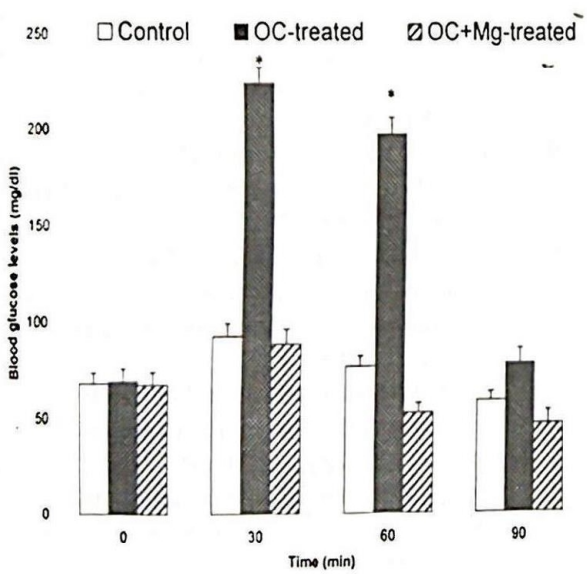

Fig. 1: Effect of increased magnesium intake during oral contraceptive treatment in female rats. Values are expressed as mean ± S.E.M. of 8 rats per group *$p<0.05$ significantly different from other groups

Table 2: Glucose tolerance index (K_G), plasma insulin and magnesium in experimental groups

	Control	OC-treated	OC+Mg-treated
K_G	20.1±0.3[a]	26.3±0.3[b]	20.4±0.4[a]
Magnesium (mmol/L)	0.86±0.04[a]	0.79±0.06[b]	0.88±0.07[a]

Values are expressed as mean ± S.E.M. of 8 rats per group. Means in rows not sharing common superscript letters are significantly different, $p<0.05$.

Table 3: Plasma lipid concentrations and antigenic indices (ratio) in experimental groups

	Control	OC-treated	OC+Mg-treated
Total cholesterol (mmol/L)	2.60±0.22[a]	2.88±0.43[a]	2.54±0.28[a]
HDL-cholesterol (mmol/L)	0.91±0.04[a]	0.61±0.05[b]	0.96±0.06[a]
LDL-cholesterol (mmol/L)	1.16±0.17[a]	1.88±0.14[b]	1.08±0.33[a]
Triglyceride (mmol/L)	1.12±0.12[a]	1.24±0.18[a]	1.13±0.22[a]
Total cholesterol/HDL-cholesterol	2.82±0.08[a]	4.63±0.07[b]	2.78±0.08[a]
LDL-cholesterol/HDL-cholesterol	1.27±0.08[a]	3.14±0.05[b]	1.21±0.06[a]

Values are expressed as mean ± S.E.M. of 8 rats per group. Means in rows not sharing common superscript letters are significantly different, $p<0.05$.

Discussion

The results from the present study demonstrate that OC treatment produced impaired glucose tolerance and dyslipidaemia in experimental animals maintained on control dietary magnesium. The study also shows that increased magnesium intake prevented these effects. The results provide evidence that the use of OC might promote the development of impaired glucose tolerance and associated atherosclerotic CVD when users are maintained on inadequate magnesium intake. The importance of magnesium on glucose disposal has been suggested in non-diabetics and diabetics [6-9]. However, the influence of increased dietary magnesium intake on impaired glucose tolerance and associated lipid disorders during OC treatment has not been investigated. Although, a number of studies have reported that OC containing androgenic progestogens, especially 19-nortestosterone derivatives, such as norgestrel that was used in this study, in combination with ethinyl oestradiol caused glucose intolerance [1,2,18,19] and dyslipidaemia [1,13,14]. However, in these studies, dietary magnesium status was not taken into consideration.

The observation that impaired glucose tolerance induced by OC treatment in animals maintained on low magnesium intake was associated

with unaltered fasting blood glucose is consistent with previous findings in women taking OC [18,19]. One possible explanation for this observation could be attributed to the ability of progestogens with androgenic activities to cause resistance to insulin-mediated glucose disposal [1,8,19]. The findings that adequate or increased intake of magnesium prevented diminished glucose intolerance in these animals further suggest that adequate or increase dietary magnesium would be beneficial in preventing altered glucose metabolism. The beneficial effect might be due to improved insulin-mediated glucose uptake.

The finding in this study that low dietary magnesium resulted in increase in atherogenic indices during OC administration, and also that increased dietary magnesium prevented the dyslipidaemia by reducing LDL-cholesterol and increasing HDL-cholesterol in these animals corroborates findings in previous studies in non-diabetic individuals [20], magnesium depleted laboratory animals [21], and rats with insulin resistance [16]. This finding suggests that low dietary magnesium may have atherogenic effect among users of OC. The beneficial effect of oral magnesium could be due to its ability to increase insulin action [12,13,16] which in turn, could activates the enzyme (lecithin-cholesterol acyltransferase) that converts the LDL-cholesterol to HDL-cholesterol [21]. Studies in humans [4,5] and in rats [22] have shown that low HDL-cholesterol as well as high LDL-cholesterol is an independent predictor of the initiation of atherosclerosis by reducing endothelial biosynthesis or bioavailability of nitric oxide. On the other hand, high HDL-cholesterol has been associated with improved endothelial function, via an enhanced nitric oxide biosynthesis or bioavailability by decreasing the formation of superoxide radicals, and thus abrogating the adverse effect of LDL-cholesterol in vasculature [22,23].

It is noteworthy that incidence of atherosclerotic CVD is a principal challenge in patients with impaired glucose tolerance, accounting for clinical complications leading to increased mortality [3,10]. Low levels of plasma magnesium have been observed among users of OC [11,12]and OC use has been associated with increased atherosclerotic CVD [24,25], the findings in this study could imply that inadequate magnesium dietary intake might be of a great public health concern in OC users. Hence monitoring of dietary but not plasma level of magnesium during OC use may be desirable. Therefore, low magnesium intake may be a salient cardiovascular risk factor in OC users.

In conclusion, we have demonstrated that deteriorated glucose tolerance and plasma lipid profile associated with OC treatment may be dietary magnesium status-dependent, but not plasma magnesium level-dependent. The study suggests that increased magnesium dietary intake, may exert cardioprotective effect during OC use, presumably through improved glucose tolerance and plasma lipid profile.

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