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R. Komba and R. A. Max

Department of Veterinary Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture

Email: robertmax@sua.ac.tz

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Department of Veterinary Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture

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SUMMARY

Combined oral contraceptives (COC) consisting of estradiol and progestin are used by women worldwide for family planning. However, COC usage has been linked adverse effects like anaemia and impaired energy metabolism. This study explored the benefits of vitamin B6 supplementation on glucose tolerance, haemoglobin, and body weight in mice. Adult female mice in their reproductive age were randomly divided into three groups (G1 - G3). G1 received a basic di*et al*one, G2 received the basic diet with 0.062 mg of COC, while G3 received the basic diet with 0.062 mg of COC and 0.028 g of vitamin B6 daily for 56 consecutive days. Glucose tolerance tests and haemoglobin levels were measured weekly whereas body weight was recorded before, and at the end of the study. The results show that COC reduced glucose tolerance but was moderated by Vitamin B6 supplementation at day 7, 28 and 42. The effect of COC and vitamin B6 was not apparent at day 14 and 56 respectively. Furthermore, neither COC nor vitamin B6 had a significant effect on haemoglobin levels although the vitamin reversed weight loss observed in COC treated mice. The study show vitamin B6 had an improvement trend in modulating adverse effects of COC on glucose tolerance and weight loss but COC and vitamin B6 supplementation did not have any significant effect on haemoglobin levels.

Keywords: Oestrogen, Progestin, Pyridoxine, Gestation, Blood, Diabetes, Haemoglobin.

INTRODUCTION

The study's motivation stemmed from the fact that millions of women worldwide rely on family planning methods, including birth control, for economic, humanitarian, and political reasons (Maitre, 2013; Mosher *et al*., 2004). Family planning encompasses various methods, such as hormonal therapies, barrier methods, condoms, intrauterine devices (IUDs), as well as natural methods like abstinence (Everett *et al*., 2000).

Hormonal methods, which employ estrogen and/or progestin, primarily target the endocrine system to prevent pregnancy. They are commonly administered through pills, patches, or injections. Combined oral contraceptives, containing both estrogen and progestin, work by inhibiting ovulation, thickening cervical mucus, and preventing sperm from entering the uterus (Cooper *et al*., 2022; Rivera *et al*., 1999). However, the use of combined oral contraceptives has been associated with side effects, including breast tenderness, headaches, changes in body weight, mood swings, and abnormal menstruation.

Additionally, these contraceptives can have negative impacts on glucose metabolism, insulin levels, blood clotting, and various health conditions such as cardiac and renal disease, diabetes, hypertension, cancer, asthma, and sickle cell anaemia (Basciani and Porcaro, 2022; Hall and Trussel, 2012).

The severity of these effects varies depending on the specific type and combination of contraceptives used (Roach *et al*., 2015). The estrogenic component of contraceptives has been found to negatively affect insulin sensitivity and carbohydrate metabolism (Sitruk and Nath, 2013).

Furthermore, therapies based on combined oral contraceptives have been linked to lower levels of essential vitamins and minerals in the body, including vitamins B, C, and E, zinc, magnesium, and selenium (Veninga, 1984). The biologically active form of vitamin B6 (pyridoxine), the pyridoxal 5′-phosphate (PLP), acts as coenzyme in about 150 distinct enzymatic activities that regulate the metabolism of glucose, lipids, amino acids, DNA, and neurotransmitters (Mascolo and Vernì, 2020). Given the plethora of reactions in which vitamin B6 is involved, its deficiency has been implicated in several clinically relevant diseases including diabetes and even cancer (Kamani *et al*., 2022; Contestabile *et al*., 2020).

Therefore, the study aimed to explore the potential benefits of vitamin B6 supplementation in mitigating the adverse effects of a specific combined oral contraceptive, monophasic Ethinylestradiol (EE) and Levonorgestrel (LNG), with a focus on glucose impairment, haemoglobin levels, and body weight in female mice. Findings are expected to contribute to the existing knowledge on mitigating the adverse effects of these contraceptives.

MATERIALS AND METHODS

Permission to carry out this study was granted by Sokoine University of Agricultre. A total of fifty-one Albino mice, all in their reproductive age (6 – 12 weeks) and weighing between 26 – 28 grams were obtained from Sokoine University of Agriculture. The mice were randomly divided into three groups, with each group consisting of 17 mice, and were housed in separate cages for 2 weeks for acclimatization before the experiment.

Mice in Group 1 (G1) were designated as the control group and were fed with basic diet consisting of maize bran throughout the study. Mice in Group 2 (G2) were administered with a daily oral dose of 0.062 mg of COC (Familia®, Jai Pharma Ltd, India; containing 0.03 mg of EE and 0.15 mg of LNG per pill) per mouse in addition to the basal diet. Mice in Group 3 (G3) received a daily oral dose of 0.062 mg of COC per mouse along with 0.028 mg of vitamin B6 (Easy Swallow Tablets®, 21st Century, USA; containing 100 mg per tablet) per mouse, in conjunction with the basal diet. These treatments were administered daily for 56 days. The amount of COC and vitamin B6 was determined based on the average body weight of the experimental mice.

Oral glucose tolerance test (OGTT)

The OGTT test is a method used to evaluate the body's ability to process a higher amount of sugar. Prior to conducting the test, the mice were fasted from midnight for a period of 6 hours. After six hours, the first blood sample was taken, and then two hours after administering a bolus of glucose solution orally, the second blood sample was collected.

To obtain the tail vein blood sample, the tail of the mice was prepared as follows: the tail was sterilized using cotton swabs soaked in 70% ethanol (Neosafe®, UK). Then, the tail was gently rubbed to improve blood circulation before sampling. Approximately 10 µL of blood was collected, and 5 µL of the sample was used for the oral glucose tolerance test (OGTT). The OGTT was performed using a haemoglucometer (Easytouch® GHb, Taiwan) and glucose strips (Easytouch® Glucose strips, Taiwan). A glucose strip was inserted into the haemoglucometer, and 5 µL of blood was added to the strip's tip. After allowing the blood to settle for 9 seconds, the reading was recorded in mg/dL. The oral glucose tolerance test was repeated on days 7, 14, 28, 42, and 56 respectively.

Blood haemoglobin (Hb) and body weight measurement

A separate aliquot of the remaining 5µL of blood was utilized to measure the haemoglobin level. The measurement of haemoglobin level was conducted using a haemoglucometer as described by the

manufacturer. Briefly, 5 μL of blood was added to the strip's tip and left to settle for 6 seconds before the reading was recorded in g/dL**.** Body weight of all mice for the respective groups was measured at the beginning of the experiment (day zero) and after 56 days.

Statistical analysis

Data were checked for normality using QQ-plot and Shapiro-Wilk normality test before statistical analysis. The analysis was done using a non-parametric Wilcoxon pairwise group comparison since the data were not normally distributed. Graphical data were generated using R software (R Core Team, 2021) with -ggplot2 (Wickham 2016), and ggpubr (Kassambara 2023) packages. A significance level of $P < 0.05$ was considered statistically significant with a confidence level of 95%

RESULTS

The ability of mice to clear blood glucose on day 0 was similar among the treatment groups although G3 had a slightly higher blood glucose level before and after administration of the glucose bolus. On day 7, the ability to clear blood glucose in G2 which are COC fed mice decreased and demonstrated higher blood glucose compared to control (G1) ($p=0.0015$). Supplementation with vitamin B6 along with COC had a better blood glucose clearance compared to COC fed mice without vitamin B6 (G2) ($p=0.015$) and mice fed with basic diet (G1) ($p= 0.036$). Improvements of glucose clearance in mice supplemented with vitamin B6 was also observed on 28 and 42 mice. However, there was no significant difference in blood glucose clearance between groups at days 14 and 56 (Figure 1 and 2) and exposure to COC and vitamin B6 to mice did not have effect on mice's blood haemoglobin levels throughout the study period as there was no significant difference between groups G1 to G3 (Figure 3). Furthermore, at the beginning of the experiment, the average body weights of mice in all groups were comparable (Figure 4A). Following the different treatments, the median body weight gains on day 56 for G2 decreased compared to control (G1), but was normalized when mice were concurrently given COC and vitamin B6 (G3) (Figure 4B).

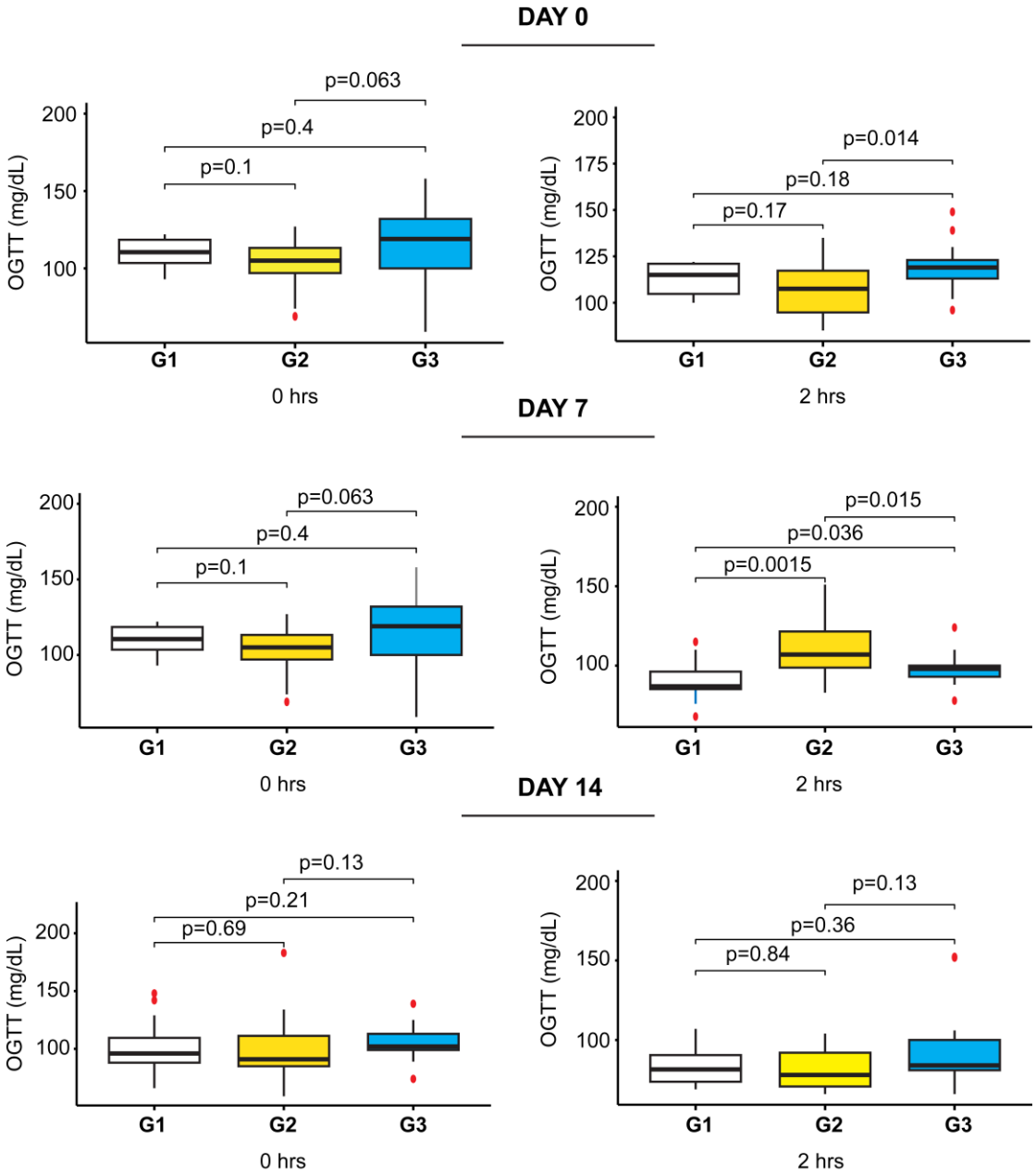

Figure 1. Box plots show the results of the glucose tolerance test at day 0 (before COC and/or vitamin B6 treatment), and at day 7 and 14 after treatment. Blood glucose levels in the three different treatment groups of mice are presented. G1: Control (white box), G2: Treated with COC (yellow box), G3: Treated with COC and vitamin B6 (blue box). Glucose measurements were taken twice, at 0 h (before oral glucose bolus) and 2 h after glucose bolus. High blood glucose was recorded on G2 compared to G1, but stabilized in G3 at day 7, 2 hours after glucose bolus. No differences across all groups at day 14. Red dots represent outliers.

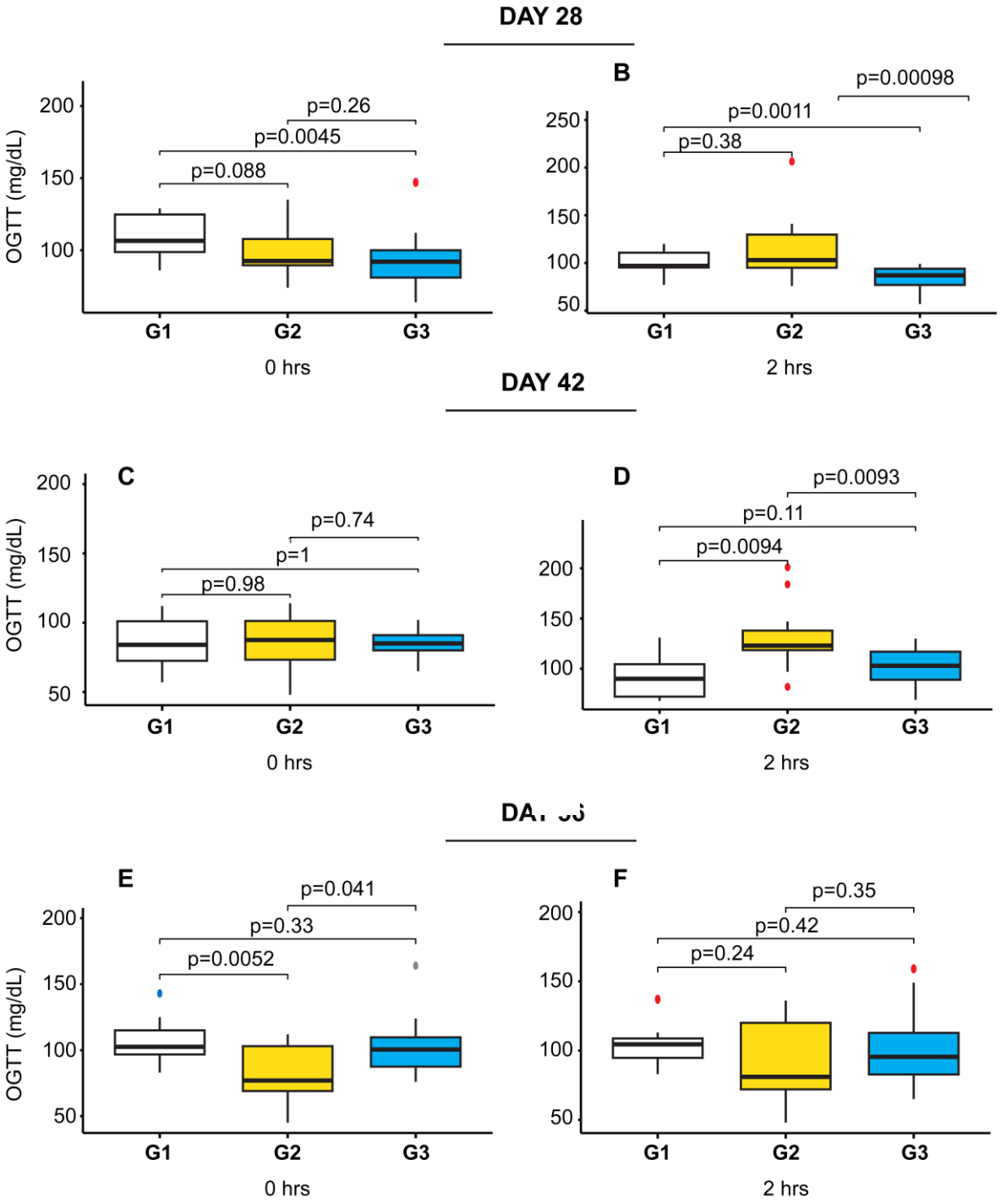

Figure 2. Vitamin B6 treated mice show improved glucose tolerance at day 28 and 42 but not at day 56. G1: control (white box), G2: treated with COC (yellow box), G3: treated with COC and vitamin B6 (blue box). Vitamin B6 had no effect on glucose tolerance beyond 42 days. Red dots represent outliers

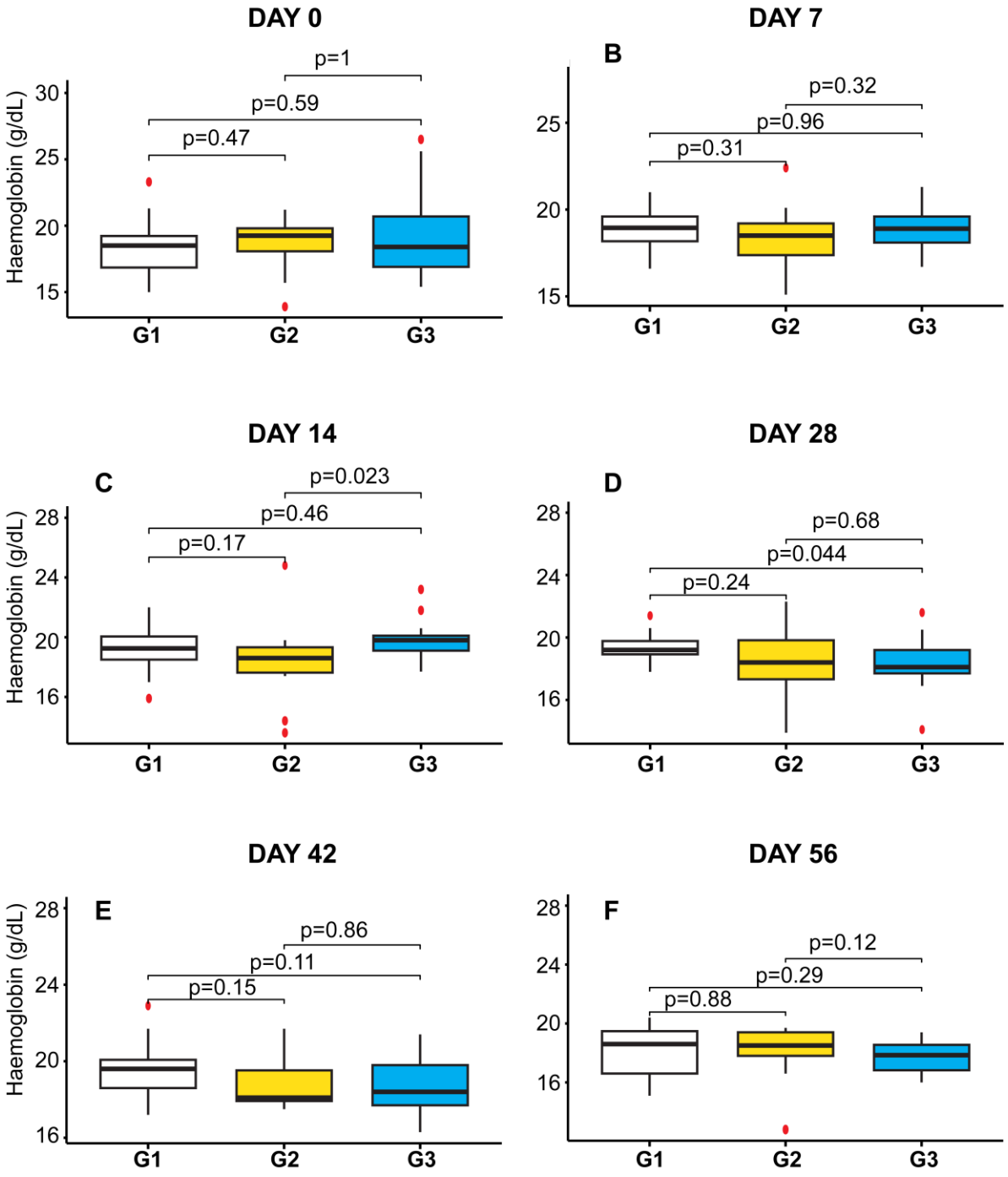

Figure 3. The haemoglobin levels of mice in the three treatment groups were not significantly affected by either COC or vitamin B6 supplementation. G1: control (white box), G2: treated with COC (yellow box), G3: treated with COC and vitamin B6 (blue box). Red dots represent outliers.

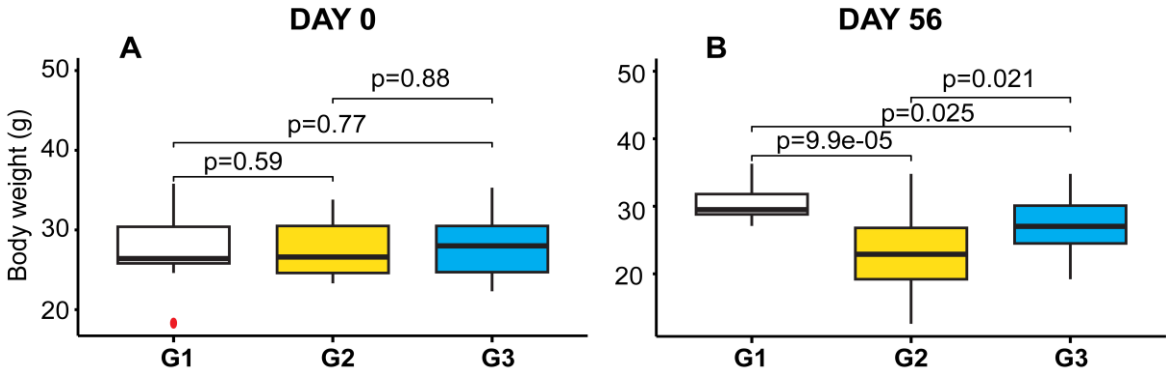

Figure 4 A: Median body weight across groups is relatively similar at the beginning of the experiment before COC and vitamin B6 treatment. **B**: The median body weight of G2 decreases after COC treatment, and stabilizes in mice fed concurrently with COC and vitamin B6 (G3).

DISCUSSION

Combined oral contraceptives (COCs) reduced glucose tolerance although these findings were only observed at day 7, 28 and 42 and not on day 14 and 56. The cause of the variation is not known, but can potentially be related to physiological responses to maintain body homeostasis after exposure to COC. The reduction of glucose tolerance as a result of COC exposure is consistent with previous reports in human subjects, where COCs, were shown to affect glucose metabolism (Lecube *et al*., 2011; Tolino *et al*., 2009; Legro *et al*., 1998; Kjos *et al*., 1995; Dunaif *et al*., 1987). While most of these studies indicated reduced glucose tolerance following intake of COC (Legro *et al*., 1998; Kjos *et al*., 1995; Dunaif *et al*., 1987) a few other studies gave contrary results (Lecube *et al*., 2011; Tolino *et al*., 2009).

The mechanism of how COC affect glucose metabolism is not clearly known. Some reports suggest that the interaction between oestrogen hormone present in COCs, and insulin can either lead to positive or negative influences on insulin sensitivity and glucose metabolism (Dunaif *et al*., 1987). Consequently, women with a history of gestational diabetes or glucose intolerance are advised against the use of COCs due to their association with glucose intolerance and impaired insulin sensitivity (Ågren *et al*., 2011). In the present study, the concurrent supplementation of vitamin B6 to mice receiving daily doses of COC (G3) improved glucose intake although the results were not consistent throughout the study. It is also important to note that, the effect of vitamin B6 was dependent on COC. Mice that did not show differences in blood glucose tolerance after COC treatment compared to control group, the effect of vitamin B6 was also not apparent.

Vitamin B6 is a cofactor that serves as a coenzyme for glycogen phosphorylase, an enzyme responsible for the release of glucose from stored glycogen and amino acids through promotion of the release of insulin hormone (Spellacy *et al*., 1977).

Interestingly, although COC treatment affected glucose metabolism, neither COC nor vitamin B6 showed any significant effect on haemoglobin levels. This finding is contrary to results from a study conducted by Toryila *et al*. (2014), where significant decreases in haemoglobin levels were observed in Wistar rats receiving COCs. Interestingly, there have been reports of increased haemoglobin levels in human subjects after the administration of certain COC formulations (Tekle *et al*., 2022), suggesting conflicting findings regarding the impact of these drugs on haematological parameters. Egbunah *et al*. (2018) attributed changes in blood counts observed in women taking COCs during their reproductive years to an increase in the division and proliferation of hematopoietic stem cells. However, the changes that were observed in this study were marginal and inconsistent.

Nonetheless, mice receiving the COC exhibited the most significant decrease in weight gain compared to the other treatment groups. The effects of COCs on body weight in women of reproductive age have yielded conflicting results in numerous human subject studies. A review conducted by Warholm *et al*. (2012) found no evidence of COCs affecting the body weights of women under the age of 18. Similarly, other studies involving older subjects reported that COCs had no notable impact on body weight in humans (Lindh *et al*., 2011; Coney *et al*., 2001). It is possible that, COC feeding potentially reduced the ability of the respective mice to feed and consequently lose weight. Other explanation for the observed disparity in our study is the possibility of contraceptive interfering with carbohydrate metabolism and the uptake of glucose by tissues that can be reversed through vitamin B6 induced insulin release.

In summary, the present study shows a potential adverse effects of COC on glucose tolerance and body weight in female mice of reproductive age and the benefit of vitamin B6 supplementation as a remedial measure. However, the study has a number of limitations including long time lapse between glucose bolus and measurement.

Shorter time intervals for evaluation of glucose tolerance test could provide a more insight on the role of COC and vitamin B6 in glucose metabolism. Furthermore, lack of data on feed intake make it difficult to rule out whether the observed weight loss is or not related to reduced feed intake as a consequence of COC feeding.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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