CHOLECALCIFEROL PREVENT OBESITY IN RATS UNDER HIGH FAT HIGH FRUCTOSE DIET

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ABSTRACT
Obesity is an accumulation of fat body condition due to calories and energy imbalance. Obesity often coexists with low vitamin D status. Low vitamin D status is known to establish abnormality in lipid profile and increasing weight. This study aims to look at the effect of the dose and duration of Cholecalciferol supplementation on lipid profiles in male rats induced by high fat high fructose diet. In this study, we conducted an experiment using a randomized pre and posttest control group design. This study analyzed 3 groups of male Sprague Dawley strain rats that were induced with a high fat high fructose diet to become diet-induced obesity rats. Each group either receives cholecalciferol in the amount of 2,500 IU/200gr/day in P1 group, 5,000IU/200gr/day in P2 group, and 10,000 IU/200gr/day in P3 group for 8 weeks. Lipid profile outcomes analyzed include levels of total cholesterol, triglycerides, High-density lipoprotein (HDL), and low-density lipoprotein (LDL). Cholecalciferol supplementation resulted in significant differences between all lipid parameters. Total cholesterol levels are lowest in the P3 group with a mean of 102.09±2.45, followed by P2 (115.80±3.98) and P1 group (138.33±4.82), and highest in the control group (196.52±4.36). Triglyceride levels are also lowest in the P3 group with a mean of 76.03±3.81, followed by the P2 (115.80±3.98) and P1 (138.33±4.82) groups, with the highest in the control group (134.46±5.84). HDL levels are instead highest in the P3 group with a mean of 80.93±8.29, followed by P2 (77.98±10.38) and P1 (66.54±7.25) group, while lowest in the control group (25.45±2.32). LDL levels are lowest on the P3 group with a mean of 33.71±2.49, followed by P2 (38.72±1.41), and P1 (47.62±2.76) group, while highest in the control group with a mean of 82.96±1.88. Cholecalciferol supplementation was found to provide a beneficial effect across all lipid profile outcomes in this study such as total cholesterol, triglyceride, HDL, and LDL in high fat high fructose induced male Sprague Dawley rats.

Keywords: cholecalciferol; lipid profile; obesity

INTRODUCTION
Obesity is the excessive or abnormal accumulation of fat tissues in the body caused by an imbalance of energy intake and expenditure (Purnell, 2018). Obesity may increase many health related risks such as metabolic syndromes, diabetes mellitus, cardiovascular diseases, non-alcoholic fatty liver diseases, cancer, sleep apnea, and various reproduction disorder (Ahmad and Iman, 2015). The prevalence of obesity can be divided into various groups of the population, such as toddlers, school-aged children, adolescents, adults, and the elderly (Oktavia, 2019). In the US, the prevalence of obesity reached 42.4% in the year 2018, while in Europe, 59% of the population suffers from obesity (Boutari and Mantzoros, 2022). In Asia in 2019, almost half of children aged up to 5 years suffering obesity or overweight (Lin and Li, 2021).

The causes of imbalanced energy intake and expenditure may be multifactorial, which involves biological, psychosocial, and behavioral factors. Primary causes can be divided into genetic or monogenic causes, while secondary causes may include nervous, endocrine, psychological, or drug-induced causes (Apovian, 2016). It has been reported an increase in the prevalence of obesity also corresponds an increase in vitamin D deficiency (Avtanski et al., 2018). Vitamin D deficiency
was found to an increase the risk of various diseases such as changes in lipid profiles such as dyslipidemia (Noeman & Powell, 2014). Inversely, obesity may also increase the risk of vitamin D deficiency (Jang et al., 2019). Low vitamin D levels can lower HDL levels increase triglyceride levels, increase free fatty acids, and increase triglyceride deposition levels in hepatocyte and liver parenchyma (Tavakoli et al., 2016). Correcting vitamin D levels have also been proposed as a therapy for hypercholesterolemia (Qin et al., 2015).

There have been various reported benefits of vitamin D supplementation for obesity conditions, such as Dibaba et al, which found that vitamin D supplementation corrects lipid profiles but not benefit to HDL levels (Dibaba, 2019). Cardeiro et al reported that vitamin D supplementation also corrects lipid profiles in rats (Cordeiro et al., 2022). Vitamin D supplementation also benefits in reducing hip circumference, waist circumference, and immune benefits by reducing type-1 proinflammatory cells and increasing type 2 proinflammatory cells (Khosravi et al., 2018; Saraci et al., 2021). Jorde et al (2010), however did not find a positive effect of vitamin D on serum lipids in a randomized controlled study involving 438 overweight or obese subjects. Although there are various studies regarding vitamin D supplementation on obesity, there are still conflicting results. In this study, the author is interested in finding the correlation between cholecalciferol supplementation on lipid profiles on obesity model rats.

**METHOD**

**Study Design**

Thirty male Sprague-Dawley rats aged 6-8 weeks were randomly allocated into 5 groups comprising 6 rats each. The groups are divided into normal control (KN), negative control (K-), P1, P2, and P3 groups. The rats were acclimated in the laboratory for 7 days for conditioning to the study environment. The rats had free access to water and the cages are routinely cleaned twice daily. The cages are made of hygienic polypropylene that houses 6 rats each. The cages had a 12-hour light-and-dark cycle. Obesity-model rats were achieved through high fat high fructose (HFHF) diet which were comprised of 32 grams of B2-2 food, 28 grams of duck egg yolk, 40 grams of cow meat, 12 grams of chicken liver, and 4 grams of butter. The diet also contains 10% fructose by dissolving 20 ml of high-fructose syrup into 100 ml of aquadest. Cholecalciferol supplementation is given in softgel form every 08.00 AM. The KN group received only BR-2 pellet and PAM ad libitum throughout the study. K- group received high-fat high-fructose diet (HFHF) for 28 days. P1 group received only HFHS for the first 28 days (day 8-36) and supplemented with cholecalciferol 2500 IU thereafter (days 38-87). P2 group received only HFHF for the first 28 days (days 8-36) and supplemented with cholecalciferol 5000 IU thereafter (days 38-87). P3 group received only HFHF for the first 28 days (day 8-36) and supplemented with cholecalciferol 10000 IU thereafter (days 38-87).

**Serum measurement**

Blood samples for analysis were taken twice throughout the study, which are on day-37 and day-88. The samples were acquired through the retro-orbitalis vein. Vitamin D levels were measured with ABclonal ELISA kit, while lipid profiles were measured with Microlab 300 spectrophotometer in 500 (480-520) nm wavelength. The lipid profiles measured were total cholesterol, triglycerides, HDL, and LDL levels.
Statistical analysis
Statistically significant differences between the three groups of rats were determined with one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test; \(p < 0.05\) was considered statistically significant.

RESULTS AND DISCUSSION
Obesity-induced rats using HFHF shows worse levels of all lipid profile parameters which consisted of total cholesterol, triglycerides, HDL, and LDL levels compared to rats with normal feeding. The mean values of total cholesterol levels in normal control group rats were 86.8767 ± 2.6318 mmol/L, while total cholesterol levels in rats fed with HFHF in the negative control group were 196.52 ± 4.36 mmol/L. Mean total cholesterol levels of rats fed with HFHS and supplemented with 2500, 5000, and 10000 IU cholecalciferol were 138.33 ± 4.82 mmol/L, 115.80 ± 3.92 mmol/L, and 102.09 ± 2.45 mmol/L respectively. Analysis of ANOVA shows that there a significant difference between values of total cholesterol levels of 5 intervention groups \((p = 0.000)\).

![Figure 1. Mean values of Total Cholesterol Levels in the different experimental groups, \(p = 0.000\); (KN: Normal Control group; K(-): Negative control group; P1: Intervention group using 2500 IU Cholecalciferol; P2: Intervention group using 5000 IU cholecalciferol; P3: Intervention group using 10000 IU cholecalciferol)](image)

Triglyceride levels of rats in normal control group were 67.67 ± 4.63 mmol/L, while mean triglyceride levels in rats fed with HFHF in the negative control group were 134.46 ± 5.84 mmol/L. Mean triglyceride levels of rats fed with HFHF and supplemented with 2500, 5000, and 10000 IU cholecalciferol were 103.25 ± 2.14 mmol/L, 85.89 ± 3.02 mmol/L, and 76.03 ± 3.81 mmol/L respectively. Analysis of ANOVA shows that there is significant difference between values of triglyceride levels of 5 intervention groups \((p = 0.000)\).
HDL levels of rats in the normal control group were 81.92 ± 2.64 mmol/L, while mean HDL levels in rats fed with HFHF in the negative control group were 25.34 ± 2.32 mmol/L. Mean HDL levels of rats fed with HFHF and supplemented with 1000, 5000, and 10000 IU cholecalciferol were 66.54 ± 7.25 mmol/L, 77.98 ± 10.38 mmol/L, and 80.93 ± 8.29 mmol/L respectively. Analysis of ANOVA shows that there is a significant difference between values of HDL levels of 5 intervention groups ($p = 0.000$).

LDL levels of rats in normal control group were 26.32 ± 2.51 mmol/L, while mean LDL levels in rats fed with HFHF in the negative control group were 82.96 ± 1.88 mmol/L. Mean LDL levels of rats fed with HFHF and supplemented with 2500, 5000, and 10000 IU cholecalciferol were 47.62 ± 2.76 mmol/L, 38.72 ± 1.41 mmol/L, and 33.71 ± 2.49 mmol/L respectively. Analysis of ANOVA shows that there is a significant difference between values of LDL levels of 5 intervention groups ($p = 0.000$).
Figure 4. Mean values of low-density lipoprotein levels in the different experimental groups $p = 0.000$; (KN: Normal Control group; K(-): Negative control group; P1: Intervention group using 2500 IU Cholecalciferol; P2: Intervention group using 5000 IU cholecalciferol; P3: Intervention group using 10000 IU cholecalciferol)

Table 1. Comparison and ANOVA analysis of lipid profiles levels before and after cholecalciferol supplementation

<table>
<thead>
<tr>
<th></th>
<th>Normal Control Group (HFHS)</th>
<th>Negative Control Group (HFHS)</th>
<th>P1 Group (HFHS + 2500 IU Cholecalciferol)</th>
<th>P2 Group (HFHS + 5000 IU Cholecalciferol)</th>
<th>P3 Group (HFHS + 10000 IU Cholecalciferol)</th>
<th>$P$ Value</th>
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</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>86.8767 ± 2.63181</td>
<td>196.52 ± 4.36</td>
<td>138.33 ± 4.82</td>
<td>115.80 ± 3.92</td>
<td>102.09 ± 2.45</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>67.67 ± 4.63</td>
<td>134.46 ± 5.84</td>
<td>103.25 ± 2.14</td>
<td>85.89 ± 3.02</td>
<td>76.03 ± 3.81</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL</td>
<td>81.92 ± 2.64</td>
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<td>0.000</td>
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Vitamin D has various effects on the physiological and pathological processes of the cardiovascular systems, in which vitamin D supplementation was found to alter levels of serum lipids such as total cholesterol, triglycerides, LDL, and HDL (Ahmad and Iman, 2015). It was observed in this study that cholecalciferol supplementation reduced the total cholesterol, triglycerides, LDL, and increases HDL values. This result is in accordance with previous studies, such as one by Lozano et al. (2015), which found that correction of vitamin D deficiency in type 2 diabetic patients decreases total cholesterol however the study did not find any significant changes in LDL, HDL, or tryglicerides. Another study found that vitamin D supplementation in healthy adolescent boys
has positive impacts on serum vitamin D, lipid profile, and inflammatory biomarker in which there is a decrease of triglyceride ($p = 0.001$) levels and an increase of HDL ($p = 0.021$) levels (Yarparvar et al., 2020). In healthy young adults, cholecalciferol supplementation reduced LDL levels significantly ($p = 0.030$) (Serrano et al., 2023). Several other studies however, did not find a significant difference of lipid profiles in subjects given vitamin D3 supplementation, such as by Jorde et al which found no positive effect of vitamin D on glucose tolerance, blood pressure, or serum lipids in a study involving 438 overweight or obese subjects (Jorde et al., 2010). Another study also did not find a beneficial effect of winter oral vitamin D3 supplementation on the blood pressure or serum cholesterol of elderly adults (Lozano and Romero, 2015).

Vitamin D has been proposed to regulate balance between cellular and plasma cholesterol, in which both also share an extensive common biosynthesis pathway. Cholesterol is a lipid that has many roles such as a precursor to bile acid, steroids, and oxysterols. Its dysregulation can result in elevated total blood cholesterol and an increase of LDL-C (Warren et al., 2021). The levels of vitamin D have been shown to affect the lipid profile, in which vitamin D deficient children may show higher triglyceride, and higher triglyceride/HDL ratio than the normal group (Kim & Jeong, 2019). There are also important links between vitamin D and obesity, in which it was found from a meta-analysis that there is an inverse association of vitamin D levels with body weight. The underlying pathogenic mechanism of low vitamin D levels in obesity includes volumetric dilution, sequestration into adipose tissue, limited sunlight exposure, and decreased vitamin D synthesis in the adipose tissue and liver (Karampela et al., 2021). Other study supports the hypothesis of volumetric dilution in obese patients. However, it has yet to be proven that low vitamin D as the cause of obesity (Vranić et al., 2019). Study results are still conflicting regarding the effect of vitamin D on obesity, as a review has concluded that vitamin D supplementation failed to improve obesity or adiposity. However, there has been support for the preventive effect of vitamin D adequacy on obesity, and in clinical practice it is necessary to keep 25(OH)D status within the normal range to avoid risks related to obesity and adiposity (Bennour et al., 2022). In conclusion, cholecalciferol supplementation can improve lipid profile parameters which are total cholesterol, triglyceride, LDL, and HDL levels. The ability to improve lipid profiles may also prevent the risk of other diseases such as metabolic syndromes and cardiovascular diseases.

REFERENCES


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