

## Decreased weight gain and enhanced serum biochemical parameters in rats after vitamin D and Ca supplementation

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### ABSTRACT

**Introduction:** Obese individuals tend to have lower plasma concentrations of calcidiol and higher levels of plasma parathyroid hormone (PTH). Objective of this study was to evaluate the influence of vitamin D and Ca supplementation on weight gain and biochemical parameters in rats fed a high-fat high-calorie diet. **Methods:** Fifty-six male Sprague-Dawley rats were assigned randomly into 4 groups of 14 rats each, and receiving diets as follows: (1) high fat (HF) 40% total energy from fat; (2) high fat & vitamin D (HF-D) 2000 IU vit D/kg diet; (3) high fat & Ca (HF-Ca) 7 g Ca/kg of diet; and (4) high fat & vitamin D & Ca (HF-D & Ca) (2000 IU of vit D+7 g Ca/kg of diet). Measured variables included body weight gains, food intake, serum triglycerides, cholesterol, insulin, glucose, ALT, and AST at 5 weeks and 10 weeks of the trial. **Results:** Lowest amount of weight gain and feeding efficiency ratio were recorded for the (HF-D & Ca) group. Rats in the HF-D group had the lowest circulating cholesterol. No significant differences in food intake, blood glucose, insulin, triglycerides, ALT and AST were found among the treatment groups. **Conclusion:** This study showed that diet supplemented with vitamin D and Ca combined appeared to mitigate weight gain in weight-induced rats, while vitamin D supplementation alone lowered serum cholesterol concentrations. Further studies are recommended to confirm these results.

**Keywords:** Obesity, calcium, rats, vitamin D

### INTRODUCTION

Obesity is a major global health challenge with on the rise prevalence regardless of gender, age, socioeconomic status, or geographic location that is increasing considerably in both genders and across all ages. Vitamin D deficiency is a common problem that may lead to the development of several health problems

(Lamendola *et al.*, 2012). Many studies showed that obesity or overweight status is in close association with compromised vitamin D status (Tolassa *et al.*, 2016; Vanlint, 2013). The National Health and Nutrition Examination Survey (NHANES III) data reported that white women with normal BMI (18.5 to 25 kg/m<sup>2</sup>) had higher 25(OH) D serum levels

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compared to those with BMI  $\geq 30$  kg/m<sup>2</sup>. Increasingly more studies have shown the close association between vitamin D and obesity-induced physiological complications such as diabetes and cardiovascular disease (Anderson *et al.*, 2010).

Total body fat is associated positively with parathyroid hormone (PTH) and inversely with 25(OH) D levels, and also for its essential role in calcium homeostasis (Pacifico *et al.*, 2011). Evidence showed that supplementing Ca and/or vitamin D may contribute to an effective management of weight (Soars *et al.*, 2012). Other studies have showed that vitamin D in fat tissue lowers vitamin D bioavailability by slowing the release of vitamin D when levels are deprived (Roth *et al.*, 2011). Studies have demonstrated that increase in serum vitamin D<sub>3</sub> after sun exposure was 57% less in obese compared with non-obese subjects (Vanlint, 2013). This has led to the hypothesis that the decreased release of endogenously produced vitamin D into circulation is due to increased storage of the synthesised vitamin D in adipocytes of obese subjects (Vilarrasa *et al.*, 2007).

Increasing dietary Ca from 400 to 1000 mg/d for 1 year resulted in a 4.9 kg reduction in body fat (Zemel *et al.*, 2000). In contrast, feeding high Ca diet resulted in less weight gain among rats, compared to the control group fed less Ca (Thomas *et al.*, 2012). However, there is scarce information regarding the role of calcium and/or vitamin D supplements on the etiology of obesity and weight gain upon consumption of high fat. Our hypothesis suggests that adequate levels of Ca and vitamin D<sub>3</sub> supplements may reduce weight gain in rats consuming an extra 7 gm Ca/kg of diet and 2000 IU cholecalciferol/kg of diet daily. The objective of this experiment was to evaluate the influence of vitamin D, Ca and their combination on weight gain and selected biochemical parameters (fasting blood glucose, alanine transferase, aspartate

transferase, cholesterol, triglycerides and insulin) among rats fed a high-fat high-calorie diet.

## MATERIALS AND METHODS

### Design

Fifty-six rats were equally randomised into four treatment groups of 14 rats each group provided with high-fat high-calorie diet and supplemented with vitamin D and Ca as follows; (1) high fat (HF) group (40% of total energy from fat) without any supplements which is more like a control group; (2) high fat vitamin D (HF-D) group (40% of total energy from fat and 2000 IU vitamin D/kg diet); (3) high fat Ca (HF-Ca) group (40% of total energy from fat and 7g Ca/kg diet); and (4) high fat vitamin D and Ca (HF-D & Ca) group (40% of total energy from fat, 2000 IU vitamin D/kg and 7g Ca/kg of diet). The doses of Ca and vitamin D<sub>3</sub> were obtained in this study after an investigation of the literature and scientific resources about tolerable upper intake levels of vitamins and minerals in rats.

Calcium carbonate and vitamin D<sub>3</sub> in powder form were used and they were obtained from Jovet Company (Amman, Jordan).

### Animals

Fifty-six male Sprague-Dawley (SD) rats (weighing 215±16.2 g and aged 120±1.5 days) were purchased from the Animal House at Jordan University of Science and Technology (JUST) after receiving the approval of ACUC (Animal Care and Use Committee) at JUST. Rats were individually housed in shoebox cages to properly measure individual feed intake and weight gains throughout the trial period (10 weeks). Identification numbers (ID) were assigned to each rat and the researcher chose even numbers of IDs to be given HF and HF-D while odd numbers of IDs were assigned to HF-Ca and HF-D/Ca treatments for randomisation purposes. Surrounding

climatic conditions were stabilised at thermoneutrality (23°C air temperature, 50-60% relative humidity), while light was cycled every 12 hours, with darkness period from 1900 to 0700 hrs.

### **Diet composition and preparation**

Accentuated BW gain-inducing diet was adopted from Dyets Inc.<sup>®</sup> (Pennsylvania, USA). Diet components were individually weighed and then mixed for 20 minutes until homogenised. Diets were freshly prepared in batches of 12 kg each and stored in sterile bags and refrigerated until offered to animals (within a week period). The caloric content of the diet was 4120 calories/kg of the diet. Diet composition was as follows: 35.5, 17.8, 1.8, 3.6, 17.8, 0, 8.9, 3.6, 1.8, 0.4, 7.1, and 1.8% for casein, sucrose, coconut oil, DL/methionine, cellulose, corn oil, mineral mix, vitamin mix, choline bitartrate, cholic acid, corn starch and cholesterol, respectively.

### **Measurements**

Weight of the rats were measured and recorded weekly. Daily feed intake was assessed by the difference between food offered *ad libitum*- and feed refusals. Blood triglycerides, cholesterol, glucose, insulin and liver enzymes (ALT and AST) were assessed on week 5 and 10 of the study period.

On the 5<sup>th</sup> week of the experiment, six rats from each treatment group were sacrificed in order to collect blood samples (via cardiopuncture, upon deep ketamine/xylazine anaesthesia), into vacutainer tubes. The same procedure was done with the remaining rats at week 10 of the trial.

Blood samples were analysed using Beckman Coulter (Access 2) with commercially available reagents (Roche Diagnostic). One blood sample was drawn from each rat via cardiopuncture into 10 vacutainer tubes, and stored for 30 minutes at room temperature before centrifugation (4000-RPM for 5 minutes). Sera were then separated and

transferred into endocrine automated analysers (Modular-E170-Roche-Germany) and used for measurements of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides and insulin using electro chemiluminescence immunoassay "ECLIA".

### **Statistical analyses**

All data were analysed using SPSS software version 19.0 for Windows (IBM Corp., USA). One-way ANOVA was used for normally-distributed variables, and *Post-hoc* ANOVA (Least Square Difference) was conducted to determine the difference between variables. *P*-value of  $\leq 0.05$  was considered the cut-off level for statistical significance.

## **RESULTS**

### **At week 5 (Table 1)**

No significant differences were observed in the initial and final body weights at week 5 among the treatment groups (Table 1). However, rats in HF-D & Ca groups had lower ( $p < 0.05$ ) body weight gain when compared to rats in the other treatment groups. The lowest weight gain was observed in rats consuming diets supplemented with Ca and vitamin D (HF-D & Ca).

No differences were shown among the different treatment groups with regard to serum concentrations of glucose, ALT, AST, triglycerides or insulin.

### **At week 10 (Table 2)**

Amounts of food consumed by all the studied groups did not differ significantly. However, rats fed high vitamin D and high Ca (HF-D & Ca) gained significantly lowest among of body weight. No differences were detected among different dietary treatments with regard to serum concentrations of glucose, ALT, AST, triglycerides or insulin.

## **DISCUSSION**

Sergeev & Song (2014) reported that

**Table 1.** Initial and final body weights (BW), food intakes, and feed efficiency ratio (FER) and biochemical tests of rats at 5 weeks

Parameter	Diets*			
	HF	HF-D	HF-Ca	HF-D & Ca
Initial BW	208.5±20.5	216.2±21.5	220.4±20.4	216.92±2.5
Final BW	306.5±32.0	318.5±21.4	311.2±17.2	297.7±19.2
Weight gain	98.1±25.1 <sup>a</sup>	102.3±16.0 <sup>a</sup>	90.8±18.6 <sup>ab</sup>	80.8±24.1 <sup>b</sup>
Food intake (g)	564.0±43.8 <sup>a</sup>	597.3±45.6 <sup>a</sup>	593.5±42.2 <sup>a</sup>	578.3±48.5 <sup>a</sup>
FER**	0.2±0.04 <sup>a</sup>	0.2±0.1 <sup>a</sup>	0.2±0.03 <sup>ab</sup>	0.14±0.04 <sup>b</sup>
Glucose (mmol/L)	16.6±1.3 <sup>a</sup>	17.5±1.6 <sup>a</sup>	16.5±0.9 <sup>a</sup>	16.5±1.3 <sup>a</sup>
ALT (U/L)	71.9±4 <sup>a</sup>	68.1±4.2 <sup>a</sup>	65.5±3.2 <sup>a</sup>	66.0±3.8 <sup>a</sup>
AST (U/L)	197.0±23.5 <sup>a</sup>	171.7±16.4 <sup>a</sup>	180.9±26.2 <sup>a</sup>	171.4±18.1 <sup>a</sup>
Cholesterol (mmol/L)	2.1±0.1 <sup>a</sup>	1.8±0.1 <sup>b</sup>	2.0±0.1 <sup>ab</sup>	2.0±0.1 <sup>ab</sup>
Triglyceride (mmol/L)	1.3±0.13 <sup>a</sup>	1.2±0.1 <sup>a</sup>	1.3±0.1 <sup>a</sup>	1.3±0.1 <sup>a</sup>
Insulin (u IU/ ML)	0.1±0.02 <sup>a</sup>	0.158±0.05 <sup>a</sup>	0.14±0.06 <sup>a</sup>	0.14±0.06 <sup>a</sup>

\*Diets; HF: high fat diet group, HF-D: high fat diet plus vitamin D, HF-Ca: high fat diet plus Ca, HF-D/Ca: high fat diet plus vitamin D and Ca

\*\*FER = body weight gain for experimental period/food intake for the experimental period. Values represent means±SD

Values with different letters (<sup>a</sup> and <sup>b</sup>) within a row are significantly different by LSD test ( $p < 0.05$ )

**Table 2.** Initial and final body weights (BW), weight gain, food intakes, and feed efficiency ratio (FER) and biochemical tests of rats at 10 weeks

Parameter	Diet*			
	HF*	HF-D	HF-Ca	HF-D & Ca
Initial BW (g)	208.5±20.5	216.2±21.5	220.4±20.4	216.9±19.5
Final BW (g)	364.29±47.38	359.3±36.7	362.14± 30.8	347.50±26.6
Weight gain (g)	152.9±34.7 <sup>a</sup>	149.3±20.3 <sup>a</sup>	143±18.7 <sup>a</sup>	128±23.4 <sup>b</sup>
Total food intake (g)	980±94.8 <sup>b</sup>	1023±71.3 <sup>b</sup>	1055±58.9 <sup>b</sup>	1005±46.1 <sup>b</sup>
FER**	0.15±0.02 <sup>a</sup>	0.15±0.01 <sup>a</sup>	0.14±0.01 <sup>ab</sup>	0.13±0.02 <sup>b</sup>
Glucose (mmol)	16.06±1.48 <sup>b</sup>	16.43±2.04 <sup>b</sup>	17.00±1.51 <sup>b</sup>	16.67±1.78 <sup>b</sup>
ALT (U/L)	63.82±2.78 <sup>a</sup>	64.2±5.71 <sup>a</sup>	65.74±3.91 <sup>a</sup>	76.18±3.36 <sup>a</sup>
AST (U/L)	198.54±43.2	176.33±28.3	151.53±12.8	156.50±20.2
Cholesterol (mmol)	2.02±0.06 <sup>ab</sup>	1.78±0.09 <sup>b</sup>	2.01±0.15 <sup>ab</sup>	2.04±0.17 <sup>ab</sup>
Triglyceride (mmol)	1.54±0.26	1.23±0.17	1.19±0.17	1.20±0.17
Insulin	0.08±0.03	0.17±0.04	0.19±0.10	0.21±0.12

\*Diets; HF: high fat diet group ( $n=7$ ), HF-D: high fat diet plus vitamin D ( $n=7$ ), HF-Ca: high fat diet plus Ca ( $n=7$ ), HF-D/Ca: high fat diet plus vitamin D and Ca ( $n=6$ ).

\*\*FER = body weight gain for experimental period/food intake for the experimental period. Values represent means±SD ( $n=7$ ).

Values with different letters (<sup>a</sup> and <sup>b</sup>) within a row are significantly different by LSD test ( $p < 0.05$ ).

mice fed fatty diets supplemented with vitamin D (1000 IU/kg of the diet) and Ca (1.2%/kg of the diet) had the lowest fat weight gain and showed improvement in adiposity markers, compared to vitamin D or Ca. Findings of human studies support the possible role of a combined supplementation of calcium and vitamin D on obesity prevention (Vilarrasa *et al.*, 2007; Roth *et al.*, 2011).

Ca and 1,25(OH) D work in a way to control metabolism of lipids in adipose cells by stimulating the oxidation of fatty acid and suppressing the lipogenic process (Mahdieh *et al.*, 2018). Furthermore, Ca has a role in decreasing the absorption of fatty acids through the formation of insoluble Ca and fatty acid soap in the intestine that could increase faecal fat excretion, leading to a decrease in the digestibility of fat (Zhu *et al.*, 2013). Another finding of our study was the cholesterol-lowering effect of vitamin D supplementations to high fat diets. Low vitamin D<sub>3</sub> levels may impair insulin action as well as glucose metabolism and various metabolic processes in adipose and lean tissue (Roth *et al.*, 2011). Asemi *et al.* (2013) noticed a significant reduction in serum total cholesterol concentrations with daily 4000 IU vitamin D given to obese patients for 12 weeks. In cross-sectional studies, serum vitamin D levels were positively correlated with HDL cholesterol (Jorde & Grimnes, 2011). Moreover, the indirect vitamin D immune-modulatory and cytokine suppressive effects can decrease cholesterol synthesis and absorption (Hart *et al.*, 2011). The vitamin D effect in decreasing cholesterol absorption might also be linked with lipid lowering therapy as dietary absorption which can lead to less circulating cholesterol levels rather than reducing endogenous production. It is also believable that vitamin D effects on lipids might increase with underlying

abnormal lipid metabolism or metabolic disorders such as hypercholesterolemia or diabetes (Al-Daghri *et al.*, 2012).

In contrast, some investigations found that vitamin D supplementation had no significant effects on serum lipid profile (Wang *et al.*, 2012). Furthermore, in a cross-sectional study, it was found that 25(OH) D levels of >30 ng/ml compared to <20 ng/ml were markedly associated with a healthier lipid profile. On the other hand, and in the same population, it showed no effect on lipid when raising 25(OH) D levels on the short run (Ponda *et al.*, 2012).

## CONCLUSION

This study showed that rats fed diet with 2000 IU vitamin D/kg and 7 g Ca/kg of diet appears to mitigate weight gain despite being provided high fat (40% of total energy) for 10 weeks. Further studies are needed to investigate the potential effects of vitamin D in obesity prevention.

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## Authors' contributions

Dr Hadil S Subih prepared the manuscript and the study design; Hiba Hamdan did the research in the animal house; Dr Hosam Al-Tamimi assisted in the study design and diet components; Dr Hiba Bawadi revised the manuscript and run the statistical analysis; Dr Sana Janakat revised the manuscript and assisted in study design

## References

Al-Daghri NM, Alkharfy KM, Al-Othman A, El-Kholie E, Moharram O, Alokail MS, Al-Saleh Y, Sabico S, Kumar S & Chrousos GP (2012). Vitamin D supplementation as an adjuvant therapy for patients with T2DM: an 18-month prospective interventional study. *Cardiovasc Diabetol.* 85(11), 1475-2840.

- Anderson JL, May HT, Horne BD, Bair TL, Hall NL, Carlquist JF, Lappé DL & Muhlestein JB (2010). Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general health care population. *Am J Cardio* 106: 963-968.
- Asemi Z, Hashemi T, Karamali M, Samimi M & Esmailzadeh A (2013). Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. *Am J Clin Nutr* 98: 1425-1432.
- Hart PH, Gorman S, Finlay-Jones JJ (2011). Modulation of the immune system by UV radiation: more than just the effects of vitamin D? *Nat Rev Immunol* 11:584-96.
- Jorde R & Grimnes G (2011). Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res* 50:303-312.
- Lamendola C, Ariel D, Feldman D & Reaven GM (2012). Relations between obesity, insulin resistance, and 25-hydroxyvitamin D. *Am J Clin Nutr* 95:1055-1059.
- Mahdih G, Bruce WH, Parvin M, Carol LW & Sakineh SB (2018). Vitamin D supplementation and body fat mass: a systematic review and meta-analysis. *Eur J Clin Nut* 32:687-92.
- Pacifico L, Anania C, Osborn JF, Ferraro F, Boncie E, Olivers E & Chiesa C (2011). Low 25 (OH) D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. *Eur J Endoc* 165: 603-611.
- Ponda MP, Dowd K, Finkelstein D, Holt PR & Breslow JL (2012). The short-term effects of vitamin D repletion on cholesterol A randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol* 32:2510-15.
- Roth C, Elfers C, Kratz M & Hoofnagle AN (2011). Vitamin D deficiency in obese children and its relationship to insulin resistance and adipokines. *J obes* doi:10.1155/2011/495101.
- Sergeev I & Song Q (2014). High vitamin D and calcium intakes reduce diet-induced obesity in mice by increasing adipose tissue apoptosis. *Mol Nutr Food Res* 58:1342-1348.
- Soares MJ, Murhadi L, Kurpad AV, Chan WL, She Ping-Delfos & Piers LS (2012). Mechanistic roles for calcium and vitamin D in the regulation of body weight. *Obes Rev* 13: 592-605.
- Thomas AP, Dunn TN, Drayton JB, Oort PJ & Adams SH (2012). A high calcium diet containing nonfat dry milk reduces weight gain and associated adipose tissue inflammation in diet-induced obese mice when compared to high calcium alone. *Nutr & Met* 9:3-14.
- Wakayo T, Whiting SJ & Belachew T (2016). Vitamin D Deficiency is Associated with Overweight and/or Obesity among Schoolchildren in Central Ethiopia: A Cross-Sectional Study. *Nutr* 8:190-205.
- Vanlint S (2013). Vitamin D and Obesity. *Nutr* 5:949-956.
- Vilarrasa N, Maravall J, Estepa A, Sanchez R, Masdevall C, Navarro MA, Alia P, Soler J & Gomez JM (2007). Low 25-hydroxy vitamin D concentrations in obese women: their clinical significance and relationship with anthropometric and body composition variables. *J Endoc Invest* 30:653-658.
- Wang H, Xia N, Yang Y & Peng DQ (2012). Influence of vitamin D supplementation on plasma lipid profiles: a meta-analysis of randomized controlled trials. *Lipids Health Dis*. doi:10.1186/1476-511X-11-42.
- Zemel MB, Shi H, Greer B, Dirienzo D & Zemel PC (2000). Regulation of adiposity by dietary calcium. *FASEB J* 14:1132-1138.
- Zhu W, Donglian C, Wang Y, Lin N, Hu Q, Qi Y, Ma SH & Amarasekara S (2013). Calcium plus vitamin D3 supplementation facilitated Fat loss in overweight and obese college students with very-low calcium consumption: a randomized controlled trial. *Nut J* 12:8.