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Research Article

BENEFICIAL EFFECT OF VITAMIN D ON HIGH-FAT DIET-INDUCED OBESITY IN WISTAR RATS

ASHIMA VERMA, AMIT GOYAL*, RAMNEET KAUR, ANJOO KAMBOJ, UPENDRA K JAIN

Department of Pharmacology, Chandigarh College of Pharmacy, Mohali - 140 110, Punjab, India. Email: goyal141186@yahoo.com

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ABSTRACT

Objectives: The aim of the present study was to investigate the antiobesity effects of vitamin D (VD) on high-fat diet (HFD)-induced obesity in Wistar rats.

Methods: In the present study, male Wistar rats were selected after 4 weeks feeding of HFD and then treated with different doses of VD (2.5-10 mcg/kg/day, p.o.o.d.) for 6 weeks along with HFD. Orlistat (30 mg/kg/day, p.o.o.d.), which is a lipase inhibitor a standard drug for obesity was used as a standard control in the present study. The effects of these treatments on body weight parameters, feed intake (Kcal), weight and size of fat pads, levels of serum glucose, triglycerides (TGs), total cholesterol (TC), high-density lipoproteins (HDL), and low-density lipoprotein (LDL) were analyzed.

Results: Treatment with VD (2.5-10 mcg/kg/day, p.o.o.d.) produced significant dose-dependent decrease ($p<0.05$) in body weight parameters, feed intake (Kcal), weight and size of fat pads, levels of serum glucose, TGs, TC, and LDL as compared to HFD group. Moreover, the level of serum HDLs was increased as compared to HFD group.

Conclusions: VD treatment ameliorated established obesity and associated biochemical consequences. The results suggest that administration of VD can inhibit the development of obesity and associated metabolic consequences in HFD-induced obesity.

Keywords: Obesity, Vitamin D, High-fat diet.

INTRODUCTION

Obesity is an important health problem that threatens health and quality of life worldwide. The prevalence and severity of obesity have increased markedly in recent decades making it a global public health concern. Obesity is no more viewed as a cosmetic issue, but it becomes a potential risk factor in the development of hypertension, Type-2 diabetes, cardiovascular diseases, infertility, etc., [1]. Currently, only a few Food and Drug Administration-approved antiobesity drugs such as Orlistat, lorcaserin, phentermine-topiramate, and naltrexone-bupropion are available in the market, but they have considerable side effects [2]. On the other hand, bariatric surgery as an alternative is associated with high risk and expensive. No such sure shot remedy is available for obesity epidemic. Earlier reports show that vitamin D (VD) deficiency can account for the secular trends in the prevalence of obesity and for individual differences in its onset and severity. It may be possible to reverse the increasing prevalence of obesity by improving VD status. VD is a fat-soluble vitamin with hormonal functions. It helps calcium and phosphorus homeostasis and bone metabolism [3]. Obesity is associated with VD insufficiency [4]. VD plays a very important role in the normal regulation of metabolism. VD have role in obesity via modulating the Toll-like receptors (TLRs) pathways [5]. In obesity, there is upregulated the expression of TLR1-9 and TLR11-13 in adipose tissue [6]. VD has been shown to downregulate intracellular TLR-2, TLR-4, and TLR-9 expression in human monocytes [7]. VD has beneficial role in obesity by modulating the action of renin-angiotensin system (RAS) [8,9], apolipoprotein E (APOE) [10,11], poly (ADP-ribose) polymerase (PARP) [1,12-14], matrix metalloproteinases (MMPs) [1,15-17], mitogen-activated protein kinase (MAPK) [18,19], inflammatory process [15,20,21], reactive oxygen species (ROS) [22,23], and nitric oxide synthase (NOS) [24,25], and these have role in physiological and pathophysiological processes of obesity. Moreover, the role of VD in experimental obesity has not been studied yet. Therefore, the current study has been designed to investigate the ameliorative effect of VD treatment in dietary obesity and associated pathologies in male Wistar rats.

METHODS

Drugs and chemicals

Casein (Modern Diary, New Karnal, India) and cholesterol (Thomas Baker, Mumbai, India) were used to prepare high-fat diet (HFD). VD was purchased from Fermenta Biotech Limited, Mandi, Himachal Pradesh, India. The standard control of study, i.e. Orlistat was purchased from Macleods Pharmaceuticals Ltd., Mumbai 400059, Batch no. TT901, Mfd Date 01/2015, Exp Date 12/2017. The estimation kits for serum glucose, cholesterol, triglycerides (TGs), and high-density lipoprotein (HDL) were obtained from Reckon Diagnostics [P] Ltd., Vadodara, India. All other chemicals used in the present study were of analytical quality. All drug solutions were freshly prepared before use.

HFD-induced obesity

Experimental obesity was induced by feeding HFD (containing: Powdered normal chow (NC), 365 g; lard, 310 g; casein, 250 g; cholesterol, 10 g; vitamin mix and mineral mix, 60 g; dl-methionine, 03 g; yeast powder, 01 g; NaCl, 01 g were added to make 1.0 kg of diet) to rats for a period of 10-week [26]. The HFD contained 5.33 Kcal/g while the NC contains 3.80 Kcal/g. This diet provides 68% energy as carbohydrate, 20% as protein, and 12% as fat to produce obesity in rats, whereas NC provides 65% of energy as carbohydrate, 20% as protein, and 4% as fat [27].

Animal treatment

Male Wistar rats of 7-8 weeks of age were procured from the Animal Facility of the Institute. The animals were housed in standard polypropylene cages (two rats/cage) and maintained under controlled room temperature (25±2°C) with 12:12 h light and dark cycle. The Guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study. Animals were randomized on the basis of their body weight and divided into various groups (n=6). The Group 1: Normal control group rats were fed on NC for 10 weeks; Group 2: VD

per se group rats were administered with VD at a dose of 10 mcg/kg for 6 weeks along with NC; Group 3: High-fat control group rats were fed on HFD for 10 weeks; Group 4: Standard control group rats were administered with Orlistat at a dose of 30 mg/kg to the 4 weeks HFD treated rats and treatment continued up to 10th week along with HFD. The Group 5: Low VD, Group 6: Medium VD, and Group 7: High VD were administered with VD at a dose of 2.5, 5, and 10 mcg/kg, respectively, to the 4 weeks HFD treated rats, and treatment continued up to 10th week along with HFD. VD and Orlistat were orally administered to the rats by oral gavage except normal control; all the groups were continually fed the HFD during the experiment. All the animals had free access to water, and the animals were inspected daily. Food intake and body weight were measured twice weekly. At the end of the stipulated period, blood for various biochemical parameters was obtained by retroorbital puncture under light ether anesthesia, and the animals were sacrificed by cervical dislocation. The blood was collected into tubes, serum separated and analyzed on the same day. The epididymal, mesenteric, and retroperitoneal white adipose tissue (WAT) were dissected, cleaned of, weighed, and stored in 10% buffered formalin solution. Lee index [28], i.e., (body wt. in g) 1/3/(ano-nasal length in cm) and body mass index (BMI) [29], i.e., (body wt. in g)/(height in cm^2) an index of obesity were calculated at the end of the experiment.

Histological analysis and morphometry

Epididymal adipose tissue was fixed in 10% formalin and then embedded with paraffin. Tissue sections (10 μm) were cut and mounted on microscope slides. After being air-dried, they were stained with hematoxylin and eosin and photographed at ×100 magnification. At least two fields per slice and six slices per fat mass were analyzed for the purpose of quantifying adipocyte size.

Measurements

Serum glucose, TG, total cholesterol (TC), and HDL cholesterol concentrations were measured using commercially available kits.

Statistical analysis

All values are expressed as mean±standard deviation. The significance of the differences between the means of various groups was established by one-way ANOVA with a Tukey's post hoc test using the GraphPad Prism 4 Software. The $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of various pharmacological interventions on anthropometric parameters

In HFD model, a significant increase ($p<0.05$) in body weight, BMI, Lee index, feed consumption (in kilocalories) (Kcal), and decrease in feed consumption (in g) were observed in rats fed on HFD as compared to the normal rats fed on standard diet. Orlistat (30 mg/kg) which was standard control in the present study decreases all the anthropometric parameters of obesity. However, oral supplementation with VD in low, medium, and high doses (2.5, 5, and 10 mcg/kg) produced significant decrease ($p<0.05$) in body weight, BMI, Lee index, and feed intake (Kcal) as compared to HFD control group, and the result was very near to the standard control group, i.e., HFD+Orlistat. There was no significant *per se* effect of VD (Table 1).

Effect of various pharmacological interventions on serum biochemical parameters

There was a significant ($p<0.05$) increase in serum concentration of cholesterol, TGs, low- density lipoprotein (LDL), very LDL (VLDL), and decrease in HDL observed in HFD control group as compared to age-matched normal animals on standard diet and Orlistat (30 mg/kg) which was standard control in present study decreases all the biochemical parameters of obesity. Treatment of HFD rats with VD administration in low, medium, and high doses (2.5, 5, and 10 mcg/kg) produced a significant decrease ($p<0.05$) in serum level of glucose, TC, TG, VLDL, LDL, and significant increase in the level of HDL as compared to HFD control group, and the result was very near to the standard control group, i.e., HFD + Orlistat. There was no significant *per se* effect of VD (Table 2).

Effect of various pharmacological interventions on different fat depots

Administration of HFD for 10 weeks caused a significant ($p<0.05$) increase in body fat depots: Epididymal, retroperitoneal, mesenteric fat depots, and total fat (TF) depots compared to age-matched normal animals on NC diet and Orlistat (30 mg/kg) which was standard control in present study decreases all the body fat depots. Treatment with VD administration produced significant decrease ($p<0.05$) in body fat depots: Epididymal, retroperitoneal, mesenteric fat depots, and TF in comparison to HFD control, and the result was very near to the standard control group, i.e., HFD+Orlistat. There was no significant *per se* effect of VD (Table 3).

Effect of various pharmacological interventions on adipocyte size

Histological examination of epididymal WAT revealed that HFD fed rats had markedly increased adipocyte size (Fig. 1b) than did NC-fed rats (Fig. 1a). VD (Fig. 1d) or Orlistat (Fig. 1c) markedly suppressed epididymal adipocyte size compared to HFD fed rats.

DISCUSSION

In this study, we aimed to disrupt metabolic balance by HFD treatment and development of experimental obesity. Notably, metabolic disturbance results in elevation of plasma lipids [30] which is characterized by elevated TC, TG levels, LDL-cholesterol levels, and decreased serum HDL-C [31]. Further, feeding with HFD caused hyperglycemia in rats [32]. Therefore, the serum lipid levels (TC, LDL, VLDL, HDL, and TGs) and glucose levels were estimated in the present study as the marker of hyperlipidemia and hyperglycemia. In present study, HFD induction for 10 weeks leads to obesity and dyslipidemia as evidence by gain in body weight, increased feed intake (Kcal), BMI, Lee index, and increase in lipid levels [33]. In the present study, treatment with VD attenuates the effect of HFD treatment. The present study was undertaken to determine the effect of VD on HFD treated rats and effect was compared with the standard drug Orlistat used in obesity. We

Table 1: Effect of various pharmacological interventions on the body weight, BMI, Lee index, feed intake in gram, and feed intake in Kcal

Parameters	Initial body weight (g)	Final body weight (g)	BMI (g/cm^2)	Lee index (g/cm)	Feed intake (g)	Feed intake (Kcal)
Normal diet treatment						
Group 1: Normal control	203.3±18.62	265.8±14.29	0.86±0.08	366.63±14.20	25.17±3.41	95.66±12.9
Group 2: VD *per se*	200±11.14	230±13.04	0.77±0.04	354.63±8.43	21.17±2.00	80.43±7.61
HFD treatment						
Group 3: HFD control	200±14.14	351.6±19.15^a	1.14±0.08^a	402.8±12.24^a	20.38±1.06	108.64±6.29^a
Group 4: Standard control	200.8±14.97	240.83±13.93^b	0.80±0.03^b	360.2±8.31^b	15.75±0.58^b	83.95±3.12^b
Group 5: HFD+VD (low)	201.67±11.69	300.83±9.70^b	0.98±0.03^b	382.62±7.47^b	17.03±1.17^b	90.79±6.27^b
Group 6: HFD+VD (medium)	200±12.65	260±7.07^b	0.86±0.04^b	367.08±9.01^b	16.3±1.06^b	86.88±5.64^b
Group 7: HFD+VD (high)	201.67±13.29	246.67±11.69^b	0.82±0.05^b	361.04±10.14^b	14.13±0.51^b	75.33±2.71^b

All values are expressed as mean±SD; ^{a}p<0.05 versus normal control, ^{b}p<0.05 versus HFD. BMI: Body mass index, VD: Vitamin D, HFD: High-fat diet, SD: Standard deviation

Table 2: Effect of various pharmacological interventions on the serum glucose and lipid profile

Parameters	Serum glucose (mg/dl)	Serum TC (mg/dl)	Serum TG (mg/dl)	Serum HDL (mg/dl)	Serum VLDL (mg/dl)	Serum LDL (mg/dl)
Normal diet treatment						
Group 1: Normal control	95.32±4.03	58.26±1.93	75.33±1.95	32.13±1.87	14.99±0.52	11.14±3.08
Group 2: VD *per se*	101.04±2.27	57.80±2.55	67.33±1.74	33.90±1.33	13.46±0.35	10.44±1.08
HFD treatment						
Group 3: HFD control	160.77±1.65[a]	139.06±1.40[a]	145.39±1.81[a]	22.89±0.79[a]	29.08±0.36[a]	87.08±1.18[a]
Group 4: Standard control	102.67±1.56[b]	67.09±1.39[b]	78.59±1.06[b]	32.16±1.22[b]	15.71 0.21[b]	19.22±0.69[b]
Group 5: HFD+VD (low)	135.08±3.04[b]	100.08±1.44[b]	127.59±1.49[b]	25.36±1.33[b]	25.54±0.29[b]	49.18±0.58[b]
Group 6: HFD+VD (medium)	120.86±1.51[b]	80.8±1.11[b]	116.34±1.59[b]	27.16±0.79[b]	23.27±0.32[b]	30.37±0.68[b]
Group 7: HFD+VD (high)	100.71±1.13[b]	60.33±0.98[b]	85.15±2.15[b]	32.14±1.03[b]	17.03±0.43[b]	11.16±0.69[b]

All values are expressed as mean±SD; [a]p<0.05 versus normal control, [b]p<0.05 versus HFD, TC: Total cholesterol, TG: Triglycerides, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, HFD: High-fat diet, SD: Standard deviation

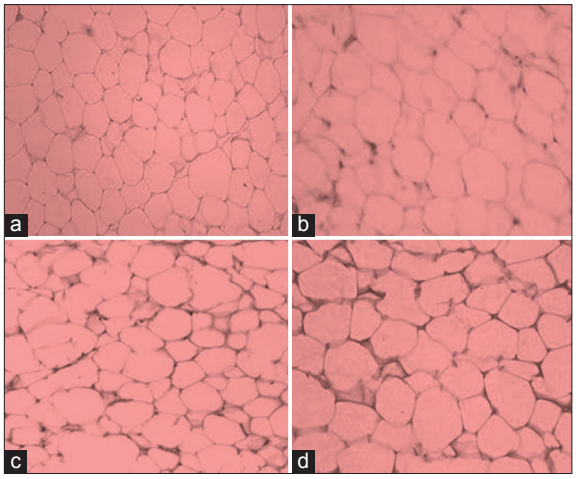

Fig. 1: Effect of various pharmacological interventions on adipocyte size (a) normal control, (b) high-fat diet (HFD), (c) HFD+Orlistat, (d) HFD+vitamin D (high)

observed that VD showed positive affects in the alteration of various parameters of obesity.

Obesity is a risk factor for high blood pressure, heart disease, stroke, gallbladder disease, breast cancer, prostate cancer, colon cancer, and Type-2 diabetes [1]. Obesity occurs when the balance between energy input (food intake) and energy expenditure (exercise and activity) is disrupted, i.e., more food is consumed than utilized, leading to excess fat stores being laid down. There are many environmental factors that predispose individuals to gain weight, e.g. freely available high-calorie food and sedentary lifestyle [34]. Obesity is defined as a BMI of 30 or more, where a person's BMI is defined as their weight in kg divided by the square of their height in meters. Overweight is defined as a BMI between 25 and 29.9. HFD has been used to develop experimental obesity characterized with dyslipidemia and insulin resistance in rodents [31]. HFD-fed rats exhibited significant increase in body weight, plasma glucose, insulin, TGs, and TC level as compared to normal powdered diet-fed control rats [26].

VD is a fat-soluble vitamin with hormonal functions. VD helps calcium and phosphorus homeostasis and bone metabolism [3]. Exposure to sunlight, dietary intake, and supplementation with VD is the main source of VD in human obese subjects had significantly lower basal 25-hydroxyvitamin-D and higher parathyroid hormone concentrations [35-37]. It has been suggested that the metabolic clearance of VD may increase in obesity, possibly with enhanced uptake by adipose tissue [38]. BMI is inversely associated with the increase in the serum 25-OHD levels in response to VD supplementation [39]. The expression of VD metabolizing enzymes has been demonstrated in human adipose tissue. In present study, VD was given at the dose of 2.5 mcg/kg; 5 mcg/kg, and 10 mcg/kg i.p. to the 4 weeks HFD treated rats and treatment continued up to 10th week along with HFD. Various anthropometric parameters such as body weight, BMI, and Lee Index were assessed. Several biochemical parameters such as TC, VLDL, LDL, TGs, and glucose levels were also determined. In present investigation, it has been observed that administration of VD in low, medium, and high dose for 6 weeks to the HFD fed animals significantly decreased the markers of obesity as compared to HFD control group. The high dose of VD was found to be more effective as compared to its medium and low dose this result gives the evidence that VD prevent the progression of obesity from predisposed factors. VD decreases the body weight, BMI, Lee index, and feed intake (in Kcal) as compared to HFD treated rats. In the present study, due to the administration of VD level of serum LDL, TG, and TC got reduced and the level of HDL got enhanced. VD decreases weight of various adipose tissues: Epididymal, mesenteric, and retroperitoneal fat.

Hence, it has been observed that VD plays major beneficial role in obesity; also, this study has provided a rational pharmacological basis for the use of VD in obesity in man.

CONCLUSION

On the basis of above discussion, it may be concluded that VD attenuated HFD-induced increase in the body weight, visceral adipose pad weights, and Lee's index, serum TC, TG, and glucose levels. The antiobesity activity of VD appears to be mediated by modulating the action of RAS, APOE, PARP, MMP's, MAPK, inflammatory process, ROS, and NOS, and these have role in physiological and pathophysiological processes of obesity. The high dose of VD was found to be more effective as compared to its medium and low dose this result gives the evidence that VD prevent the progression of obesity from predisposed factors.

Hence, it has been observed that VD plays major beneficial role in obesity; also this study has provided a rational pharmacological basis for the use of VD in obesity in man.

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Table 3: Effect of various pharmacological interventions on the various fat pads

Parameters	MES	RET	EPI	TF
Normal diet treatment				
Group 1: Normal control	2.04±0.21	1.79±0.25	1.83±0.21	5.66±0.44
Group 2: VD *per se*	2.26±0.11	2.24±0.09	2.35±0.07	6.86±0.22
HFD treatment				
Group 3: HFD control	8.02±0.66[a]	7.62±0.76[a]	7.36±0.65[a]	23±1.98[a]
Group 4: Standard control	2.78±0.36[b]	2.59±0.31[b]	2.66±0.37[b]	8.02±0.94[b]
Group 5: HFD+VD (low)	4.06±0.54[b]	4.09±0.32[b]	4.01±0.28[b]	12.16±1.09[b]
Group 6: HFD+VD (medium)	3.51±0.64[b]	3.39±0.67[b]	3.3±0.64[b]	10.2±1.95[b]
Group 7: HFD+VD (high)	2.36±0.40[b]	2.45±0.48[b]	2.45±0.42[b]	7.26±1.25[b]

All values are expressed as mean±SD; [a]p<0.05 versus standard diet control, [b]p<0.05 versus HFD. MES: Mesenteric, RET: Retroperitoneal, EPI: Epididymal, TF: Total fat, HFD: High-fat diet, SD: Standard deviation

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