Disodium Ascorbyl Phytostanyl Phosphates (FM-VP4) reduces plasma cholesterol concentration, body weight and abdominal fat gain within a dietary-induced obese mouse model

Norbert A. Looije[1], Verica Risovic[1], David J. Stewart[2], Daniel Debeyer[2], James Kutney[2] and Kishor M. Wasan[1*]

[1]Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver BC, Canada & [2]Forbes Medi-Tech Inc, Vancouver B.C., Canada

Received March 19, 2005,Revised June 15, 2005, Accepted August 15, 2005, Published August 24, 2005,

ABSTRACT

Purpose: The purpose of this study was to determine if Disodium Ascorbyl Phytostanol Phosphates (FM-VP4) alters animal body weight and plasma lipid levels in a dietary-induced obese mouse model. **Methods:** Twenty-four C57BL6 mice (28 days old) were housed individually and fed a standard mouse diet for 2 weeks upon arrival. After 2 weeks the animals were weighed and divided in 4 groups of similar average weight, and the groups received a low fat (10% kcal from fat) and high fat (45% kcal from fat) diet with or without FM-VP4 (2% w/w) for 12 continuous weeks. Food, water and caloric intake and body weight were recorded on a daily basis throughout the duration of the study. Following the 12^{th} week of the study all animals were humanely sacrificed and blood and abdominal fat pads were harvested for further analysis. Plasma cholesterol, triglyceride, AST/ALT and creatinine levels were measured using enzymatic kits. **Results:** There is a significant difference in weight gain between the low-fat diet and the low-fat diet + 2% w/w FM-VP4 treatment groups ($P<0.05$), as well as between the high-fat diet and the high-fat diet + 2% w/w FM-VP4 treatment groups ($P<0.05$). However, the reduction of weight gain of the high-fat diet + 2% FM-VP4 treatment group compared to the high-fat group was 51%, while the reduction in weight gain between the low-fat diet + 2% w/w FM-VP4 treatment group and the low-fat diet group was 17% over the duration of the study. No significant differences in food and water intakes, serum creatinine and AST/ALT levels were observed between the four groups. No significant differences in caloric intake between the low-fat diet and the low-fat diet + 2% w/w FM-VP4. However, a significant difference in caloric intake between high-fat diet and the high-fat diet + 2% w/w FM-VP4 treatment groups was observed. In addition, significant reductions in plasma cholesterol levels and abdominal fat pad weight between diet alone and diet + FM-VP4 treatment groups were observed. **Conclusions:** These findings suggest that FM-VP4 may have potential weight-loss and cholesterol lowering activity in both High Fat and Low Fat Diets treated groups.

INTRODUCTION

The obesity epidemic has been recognized by the World Health Organization as one of the top 10 global health problems (1). Worldwide, more than one billion adults are overweight and over 300 million are obese (1). Most countries are experiencing dramatic increases in obesity.

Obesity is a condition associated with the accumulation of excessive body fat resulting from chronic imbalance of energy whereby the intake of energy exceeds expenditure. The excess body fat predisposes an obese individual to chronic diseases, such as coronary heart disease, type 2 diabetes, diseases of the gall bladder and cancer (2, 3). The high incidence of obesity, its multifactorial nature and the scarcity of adequate therapeutics have fuelled an increase in anti-obesity drug-related research. Although a number of pharmacological approaches have been investigated in recent years, few therapeutically effective products have been developed (2).

Our laboratory has been investigating the lipid lowering and anti-atherosclerotic effects of a novel cholesterol absorption inhibitor, FM-VP4 (disodium ascorbyl phytostanyl phosphates, FM-VP4, Figure 1), in several animal models (4-9). In several of these studies, we noted that FM-VP4 administration caused a decrease in body weight without any observable liver or kidney toxicity or changes in

Corresponding Author: Dr. Kishor M. Wasan, Faculty of Pharmaceutical Sciences, University of British Columbia, 2146 East Mall Avenue, Vancouver, BC, Canada, V6T 1Z3
kwasan@interchange.ubc.ca

food or water intake (7, 8). Although several studies have described the effects of plant sterols/stanols on significantly decreasing total plasma and lipoprotein cholesterol levels between 10-20% at doses between 1-5 g/day (10-24), there is no evidence for any weight loss properties. Therefore, our observation of non-toxic weight loss appears to be specific for FM-VP4. However, to date, weight loss studies with FM-VP4 have not been conducted in a dietary-induced obese animal model. Therefore, the purpose of this study was to determine if disodium ascorbyl phytostanol phosphates (FM-VP4) alters animal body weight and plasma lipid levels in a dietary-induced obese mouse model receiving a low-(10% kcal from fat) and high-fat diet (45% kcal from fat).

MATERIALS AND METHODS

Chemicals

Disodium ascorbyl phytostanyl phosphates (FM-VP4; Lot number: 81699 BRI FM-VP4-06; Figure 1) was prepared by the chemistry group of Forbes Medi-Tech Inc. Research Laboratories. FM-VP4 is a semi-synthetic esterified phytostanol derivative, produced as the disodium salt. The two major components of FM-VP4 are disodium ascorbyl campestanyl phosphate and disodium ascorbyl sitostanyl phosphate. The powdered active ingredient was stored at 4^{o}C and to date has been demonstrated to be stable for up to 12 months under these conditions. The low and high fat diets were purchased from Research Diets Inc. (New Jersey, USA; See Table 1 for the complete composition of each diet) (25).

Dietary-Induced Obese Mouse Model

Four-week-old male C57BL6 mice were purchased from Charles River Laboratories, Quebec, Canada. These animals normally exhibit significant weight gain following the consumption of a high fat-diet (45% kcal from fat) over a 12-week period (25).

Experimental design

Twenty-four C57BL6 mice (4 weeks old) were obtained from Charles River Laboratories (Montreal, Quebec, Canada). Upon arrival the animals were acclimatized by being housed individually and fed a standard mouse diet for 2 weeks. Housing consisted of a 12 h light/dark cycle at a constant temperature (21^{0}C ± 2) and humidity. After 2 weeks the animals were weighed and divided in 4 groups (n=6 for first 9 weeks, n=5 for weeks 9-12; one mouse was randomly sacrificed at week 9 for genotyping) of similar average weight, and the groups received a low fat-diet (10 % kcal from fat); high fat-diet (45% kcal from fat); low fat-diet + 2% (w/w) FM-VP4 or a high fat-diet + 2% (w/w) FM- VF4 respectively for 12 consecutive weeks (Table 1). Allocation of treatment to each group was randomly determined before the start of the study. Homogeneity of groups was validated on the criteria of body weight, plasma cholesterol and plasma triglyceride on the day of randomization.

Food, water and caloric intakes (calculated based on the amount of food consumed daily and the nutritional information provided in table 1) intakes and body weight were recorded for all animals on a daily basis. Following the 12^{th} week of the study animals were humanely sacrificed using a CO_2 chamber and blood and abdominal fat pads were harvested for further analysis. Plasma cholesterol, triglyceride, serum creatinine and AST/ALT levels were determined using enzymatic kits (Boehringer Mannheim, Germany) as previously described (7,8)

Diet Preparation and Animal Care

Diet preparation was carried out at Research Diets (Table 1) and FM-VP4 was incorporated into the diet as previously published (5,7,8). The Animal Care Committee of the University of British Columbia approved the study. The concentration of FM-VP4 in food was confirmed at the beginning of study. 15-20 grams of the control food was collected in glass containers, labelled accordingly and stored at 4^{0}C. Considering that the average daily food intake was estimated to be 3 g, mice were administered about 60 mg FM-VP4 each day, equivalent to 2% (w/w) of the diet.

Collection of blood and harvesting of abdominal fat pads

At the end of the study mice were sacrificed using CO_2 gas and blood was taken from the right ventricle. Blood cells were pelleted by centrifugation and plasma was harvested. Abdominal fat pads were removed from the abdomen of each mouse and weighed. The abdominal fat pads (this is the fat from the dorsal abdomen region of the mouse) were removed by a member of the animal

care unit at UBC who was blinded to the dose group of each individual mouse and used the dissection technique from the work of Henry *et al.* (26).

Statistical Analysis

Results were expressed as mean ± SD (standard deviation). Statistical analysis were conducted using an analysis of variance (PCANOVA; Human Dynamic Systems) and assuming unequal variance (Newman Keuls post-hoc test) was used to assess the differences between the FM-VP4 treatment groups and the untreated control group for body weight, abdominal fat weight, plasma lipids, food, water and

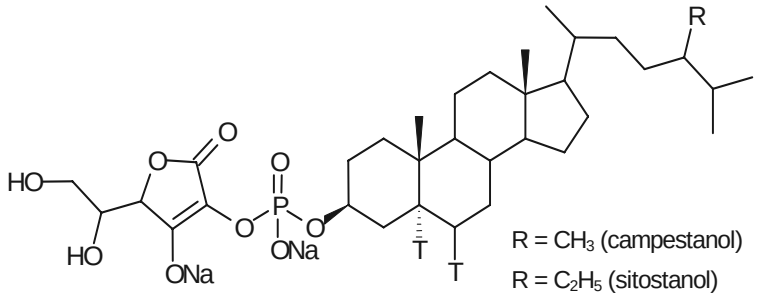

Figure 1. Chemical Structure of Disodium Ascorbyl Phytostanyl Phosphates (FM-VP4)

Table 1: The Composition of the Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 and High Fat + 2% (w/w) FM-VP4 diets and their caloric content

Diet Ingredient	**Low Fat**		**Low Fat + 2% FM-VP4**		**High Fat**		**High Fat + 2% FM-VP4**	
	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein, 80 Mesh	200	800	200	800	200	800	200	800
L-Cystein	3	12	3	12	3	12	3	12
Corn Starch	315	1260	315	1260	72.8	291	72.8	291
Maltodextrin 10	35	140	35	140	100	400	100	400
Sucrose	350	1400	350	1400	172.8	691.2	172.8	691.2
Cellulose, BW200	50	0	50	0	50	0	50	0
Soybean Oil	25	225	25	225	25	225	25	225
Lard	20	180	20	180	177.5	1598	177.5	1597
Mineral mix, S10026	10	0	10	0	10	0	10	0
DiCalcium Phosphate	13	0	13	0	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0	5.5	0	5.5	0
Potassium Citrate, $1H_2O$	16.5	0	16.5	0	16.5	0	16.5	0
Vitamin mix, V10001	10	40	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0	2	0
FM-VP4	0	0	20	0	0	0	20	0
FD&C Yellow Dye #5	0.05	0	0.025	0	0	0	0	0
FD&C Red Dye #40	0	0	0.025	0	0.05	0	0.05	0
FD&C Blue Dye #1	0	0	0	0	0	0	0	0
TOTAL	**1055**	**4057**	**1075**	**4057**	**858**	**4057**	**878**	**4057**

Diet	**Low Fat**		**Low Fat + 2% FM-VP4**		**High Fat**		**High Fat + 2% FM-VP4**	
	gm%	**kcal%**	**gm%**	**kcal%**	**gm%**	**kcal%**	**gm%**	**kcal%**
Protein	19.2	20	18.9	20	23.7	20	23.1	20
Carbohydrate	67.3	70	66	70	41.4	35.1	40.5	35.1
Fat	4.3	10	4.2	10	23.6	44.9	23.1	44.9
TOTAL Kcal/gm	**3.85**	**100**	**3.77**	**100**	**4.73**	**100**	**4.62**	**100**

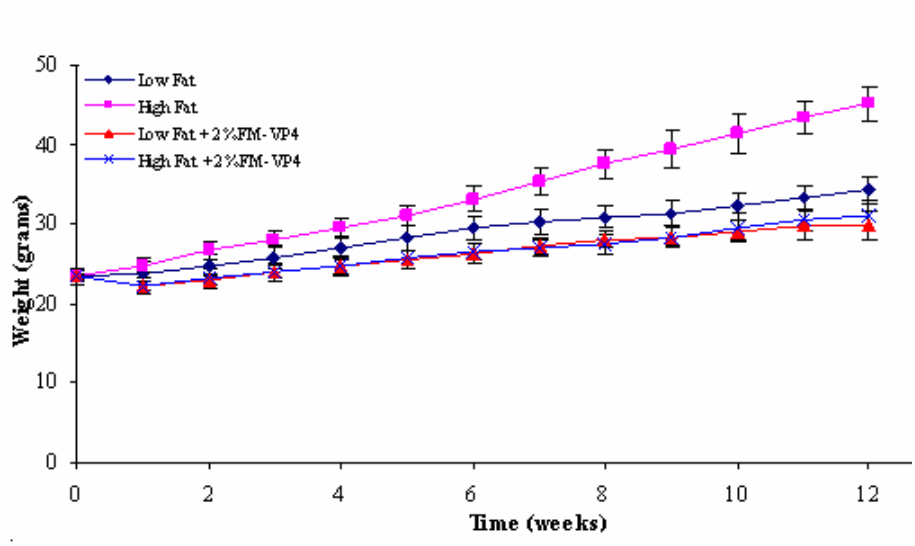

Figure 2: Average weekly weight of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet. Week 1 – 9, n=6; week 10 – 12, n=5.

caloric intake, AST/ALT and serum creatinine levels. A p-value of less than 0.05 indicated a significant difference between treated and untreated groups.

RESULTS

Total body and abdominal fat pad weight

The weight gain of the four groups is depicted in Figure 2. This figure clearly shows that weight gain of the High Fat group was higher throughout the entire duration of the experiment compared to all the other groups while all groups started at the same average weight, this finding is significant from week 2 until the end of the experiment ($p<0.05$). Even though there was a significant difference in weight gain between the Low Fat and the Low Fat + 2% (w/w) FM-VP4 groups ($P<0.05$), as well as between the High Fat and the High Fat + 2% (w/w) FM-VP4 groups ($P<0.05$), the difference in the weight gain between the High Fat and High Fat + 2% (w/w) FM-VP4 group was much more profound than between the Low Fat and the Low Fat + 2% (w/w) FM-VP4 group. The reduction of weight gain of the High Fat + 2% (w/w) FM-VP4 group compared to the High Fat group was 51%, while the reduction in weight gain between the Low Fat + 2% (w/w) FM-VP4 and the Low Fat group was 17%.

Both groups, which have been fed the FM-VP4 enriched diets, followed a very similar weight pattern throughout the entire experiment despite the difference in caloric content of the diet, and there was no significant difference in average weight between the two groups throughout the entire experiment.

The weight of the abdominal fat pads of the treatment groups is depicted in Figure 3. The weight of the abdominal fat pads of the High Fat group was increased by 115% compared to the Low Fat group as a result of the high fat content of their diet. The weight of the abdominal fat pads of the High Fat + 2% (w/w) FM-VP4 group has decreased by 70% compared to the High Fat group and by 38% compared to the Low Fat group. The Low Fat + 2% (w/w) FM-VP4 group compared to the High Fat + 2% (w/w) FM-VP4 group did not significantly differ in weight of the fat pads, otherwise all differences between groups were statistically significant ($P<0.05$).

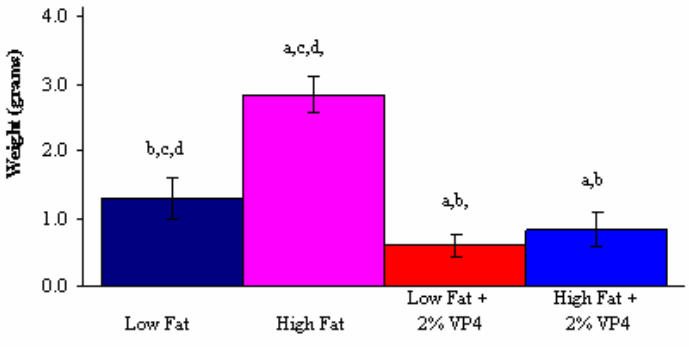

Figure 3: Weight of the abdominal fat pads in grams of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet. a. $P<0.05$ vs. Low Fat; b. $P<0.05$ vs. High Fat; c. $P<0.05$ vs. Low Fat + 2% FM-VP4; d. $P<0.05$ vs. High Fat + 2% FM-VP4.

Food, caloric and water intake

The patterns of food intake and of caloric intake of the four treatment groups are depicted in Tables 2 and 3. Food and caloric intake were steady throughout the experiment for all treatment groups. But even though it is not as evident from the data of the food intake, the caloric intake of the High Fat group was slightly higher compared to all other treatment groups throughout the duration of the study (Table 3). In weeks 6 through 10 this difference in caloric intake is even significant ($p<0.05$) compared to the High Fat + 2% (w/w) FM-VP4 group that has a similar caloric content per gram diet (Table 1). This was an unexpected result since food with the same caloric content is expected to be eaten in approximately the same amount by the animals.

Table 2: Average weekly food intake in grams of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet.

Group / Week	Low Fat	High Fat	Low Fat + 2% FM-VP4	High Fat + 2% FM-VP4
1	3.3 ± 0.5	3.5 ± 0.7	2.5 ± 0.8	2.6 ± 1.0
2	3.1 ± 0.6	3.0 ± 0.4	3.2 ± 0.4	3.1 ± 0.5
3	3.1 ± 0.3	2.8 ± 0.5	3.2 ± 0.2	2.9 ± 0.3
4	3.2 ± 0.3[d]	3.0 ± 0.4	3.2 ± 0.2[d]	3.7 ± 0.3[a,c]
5	3.3 ± 0.4 [d]	3.0 ± 0.3	3.2 ± 0.3 [d]	2.7 ± 0.2[a,c]
6	3.3 ± 0.4	3.2 ± 0.4	3.2 ± 0.3	2.8 ± 0.2
7	3.2 ± 0.4 [d]	3.3 ± 0.3 [d]	3.3 ± 0.2 [d]	2.7 ± 0.2[a,b,c]
8	3.0 ± 0.5	3.3 ± 0.4 [d]	3.3 ± 0.2 [d]	2.7 ± 0.2[b,c]
9	3.1 ± 0.4 [d]	3.2 ± 0.3 [d]	3.2 ± 0.2 [d]	2.7 ± 0.2[a,b,c]
10	3.3 ± 0.5	3.5 ± 0.3	3.2 ± 0.3	2.9 ± 0.2
11	3.4 ± 0.5	3.3 ± 0.5	3.2 ± 0.5	3.0 ± 0.5
12	3.2 ± 0.5	3.1 ± 0.3	3.2 ± 0.3	2.8 ± 0.3

a. P<0.05 vs. Low Fat; b. P<0.05 vs. High Fat; c. P<0.05 vs. Low Fat + 2% FM-VP4; d. P<0.05 vs. High Fat + 2% FM-VP4. Average weekly food intake is given in grams.

Table 3: Average weekly caloric intake in kilocalories of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet.

Group / Week	Low Fat	High Fat	Low Fat + 2% FM-VP4	High Fat + 2% FM-VP4
1	11.8 ± 2.1	16.5 ± 3.2[c]	9.8 ± 3.0[b]	12.6 ± 4.6
2	12.1 ± 1.3	14.4 ± 2.0	11.9 ± 1.5	14.2 ± 2.5
3	12.2 ± 1.3	13.2 ± 2.4	12.0 ± 0.7	13.5 ± 1.5
4	12.2 ± 1.3	14.1 ± 2.1	12.0 ± 0.9	12.7 ± 1.5
5	12.6 ± 1.5	14.2 ± 1.3[c]	12.0 ± 1.3[b]	12.5 ± 1.0
6	12.6 ± 1.6[b]	15.1±1.9[a,c,d]	12.0 ± 1.0[b]	12.8 ± 1.0[b]
7	12.3 ± 1.4[b]	15.4±1.5[a,c,d]	12.5 ± 0.8[b]	12.6 ± 0.9[b]
8	11.7 ± 1.8[b]	15.3±2.0[a,c,d]	12.4 ± 0.9[b]	12.6 ± 1.1[b]
9	11.9 ± 1.5[b]	15.2±1.6[a,c,d]	12.0 ± 0.9[b]	12.7 ± 1.0[b]
10	12.8 ± 1.9[b]	16.4±1.3[a,c,d]	12.3 ± 1.2[b]	13.4 ± 1.0[b]
11	13.2 ± 2.0	15.8 ± 2.5	12.2 ± 1.7	13.8 ± 2.3
12	12.3 ± 2.0	14.6 ± 1.6	12.0 ± 1.1	13.0 ± 1.4

a. P<0.05 vs. Low Fat; b. P<0.05 vs. High Fat; c. P<0.05 vs. Low Fat + 2% FM-VP4; d. P<0.05 vs. High Fat + 2% FM-VP4. Average weekly caloric intake is given in kilocalories.

Table 4: Average weekly water intake in milliliters of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet.

Group / Week	Low Fat	High Fat	Low Fat + 2% FM-VP4	High Fat + 2% FM-VP4
1	5.4 ± 1.3	5.5 ± 1.2	4.8 ± 1.5	4.8 ± 1.9
2	6.2 ± 1.5	5.8 ± 1.8	5.6 ± 1.5	6.3 ± 1.4
3	6.5 ± 1.4	6.2 ± 1.3	7.4 ± 1.5	7.4 ± 1.7
4	5.9 ± 1.3	6.3 ± 1.5	7.1 ± 1.2	6.5 ± 1.1
5	6.6 ± 1.7	6.1 ± 1.2	7.0 ± 1.3	6.9 ± 1.2
6	5.2 ± 0.5	4.7 ± 0.6[c]	5.9 ± 0.6[b]	5.3 ± 0.4
7	4.5 ± 0.2	4.5 ± 0.4	4.5 ± 0.5	4.6 ± 0.2
8	5.0 ± 0.6	4.6 ± 0.3	5.0 ± 0.5	5.0 ± 0.3
9	5.1 ± 0.4	4.9 ± 0.8	4.7 ± 0.6	4.9 ± 0.9
12	4.5 ± 0.1	4.8 ± 0.5	4.5 ± 0.2	4.6 ± 0.4

a. P<0.05 vs. Low Fat; b. P<0.05 vs. High Fat; c. P<0.05 vs. Low Fat + 2% FM-VP4; d. P<0.05 vs. High Fat + 2% FM-VP4. Average weekly water intake is given in milliliters. Weeks 10 and 11 were not determined.

Water intake was measured on a daily basis for the first 34 days while it was measured twice a week for the remainder of the experiment. The weekly averaged data and statistical significance of comparison of each group is given in Table 4. Water intake of the groups did vary on a week to week basis but the differences in water intake between the treatment groups were not significant during the entire experiment. Water intake was not measured in weeks 10 and 11, but since there was no difference in the water intake between groups for all the other weeks(weeks 1-9 and week 12) it might be assumed that the water intake in these 2 weeks were not significantly different.

Plasma total cholesterol and triglyceride levels

The effects of FM-VP4 on plasma total cholesterol and triglyceride levels are shown in Figures 4 and 5 respectively. The animals of the High Fat group displayed a significant increase in total plasma cholesterol of the High Fat + 2% (w/w) FM-cholesterol as compared to the animals of the Low Fat group as would be expected (p<0.05). The total VP4 group was significantly lower compared to the High Fat group by 32.2% (p<0.05), while the total plasma cholesterol concentration of the Low Fat + 2% (w/w) FM-VP4 group was significantly lower compared to the Low Fat group by 45.1% (p<0.05). These results are in accordance with previously obtained results suggesting that FM-VP4 decreases plasma cholesterol levels following chronic oral administration (5,7,8).

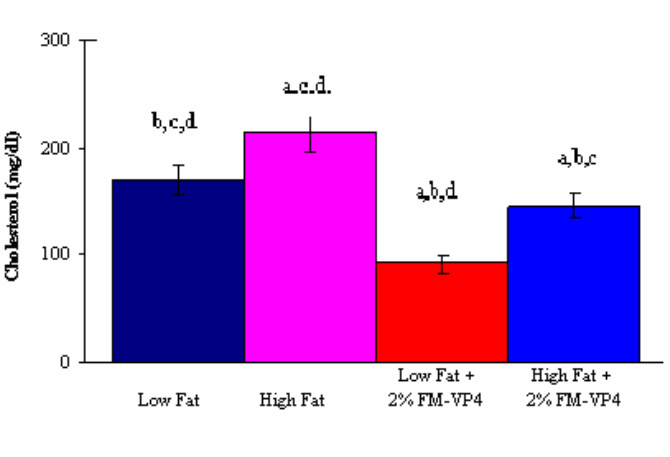

Figure 4: Total plasma cholesterol levels in mg/dl of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet. a. P<0.05 vs. Low Fat; b. P<0.05 vs. High Fat; c. P<0.05 vs. Low Fat + 2% FM-VP4; d. P<0.05 vs. High Fat + 2% FM-VP4.

The plasma triglyceride levels were not significantly different between High Fat versus High Fat + 2% (w/w) FM-VP4 treatment groups (Figure 5). However, significant lowering of the triglyceride levels between the Low Fat + 2% (w/w) FM-VP4 group compared to the High Fat and the Low Fat groups respectively (p<0.05) was observed.

Table 4: Average weekly water intake in milliliters of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet.

Group / Week	Low Fat	High Fat	Low Fat + 2% FM-VP4	High Fat + 2% FM-VP4
1	5.4 ± 1.3	5.5 ± 1.2	4.8 ± 1.5	4.8 ± 1.9
2	6.2 ± 1.5	5.8 ± 1.8	5.6 ± 1.5	6.3 ± 1.4
3	6.5 ± 1.4	6.2 ± 1.3	7.4 ± 1.5	7.4 ± 1.7
4	5.9 ± 1.3	6.3 ± 1.5	7.1 ± 1.2	6.5 ± 1.1
5	6.6 ± 1.7	6.1 ± 1.2	7.0 ± 1.3	6.9 ± 1.2
6	5.2 ± 0.5	4.7 ± 0.6[c]	5.9 ± 0.6[b]	5.3 ± 0.4
7	4.5 ± 0.2	4.5 ± 0.4	4.5 ± 0.5	4.6 ± 0.2
8	5.0 ± 0.6	4.6 ± 0.3	5.0 ± 0.5	5.0 ± 0.3
9	5.1 ± 0.4	4.9 ± 0.8	4.7 ± 0.6	4.9 ± 0.9
12	4.5 ± 0.1	4.8 ± 0.5	4.5 ± 0.2	4.6 ± 0.4

a. P<0.05 vs. Low Fat; b. P<0.05 vs. High Fat; c. P<0.05 vs. Low Fat + 2% FM-VP4; d. P<0.05 vs. High Fat + 2% FM-VP4. Average weekly water intake is given in milliliters. Weeks 10 and 11 were not determined.

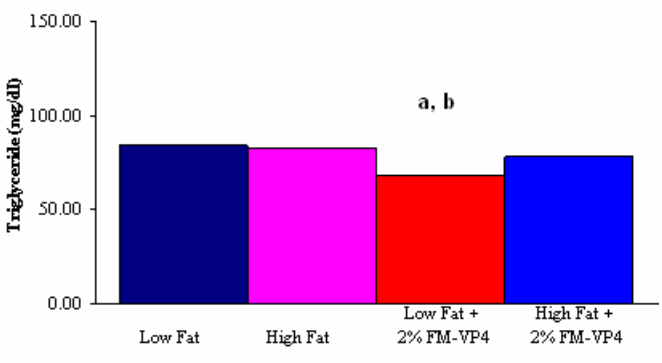

Figure 5: Total plasma triglyceride levels in mg/dl of male C57Bl6 mice on either Low Fat; High Fat; Low Fat + 2% (w/w) FM-VP4 or High Fat + 2% (w/w) FM-VP4 diet. a. P<0.05 vs. Low Fat; b. P<0.05 vs. High Fat.

Plasma creatinine and AST/ALT levels

No significant differences in plasma creatinine, AST and ALT levels were observed for all four groups in this study (data not shown).

DISCUSSION

The purpose of this experiment was to elucidate if FM-VP4 reduced body weight gain in a dietary-induce obese animal model. The 2% (w/w) FM-VP4 dose chosen in this study was based on our previous findings reported in gerbils (7,8) and ApoE deficient mice (5). In those studies we have reported that FM-VP4 at 2% (w/w) significantly reduced body weight gain (gerbils only) (8) and plasma lipid levels (gerbils and mice) (5,8) without any side effects. In addition, FM-VP4 is well tolerated even at a high daily dose (100 mg/day) without producing diarrhoea or other gastrointestinal intolerance signs (8). Furthermore, since FM-VP4 comprises vitamin C (ascorbic acid) and phytostanyl moieties, covalently linked by a phosphodiester bridge, it is possible that the effects reported in these studies might be due to the ability of unesterified stanols to inhibit cholesterol absorption, or the combined effect of free ascorbate and unesterified stanols following cleavage of FM-VP4 into its component parts by digestive lipases. Thus, we have compared the effects of FM-VP4 with equivalent amounts of ascorbic acid or phytostanols given individually or together in the diet in the ApoE deficient mouse study and found minimal effects of these components of FM-VP4 (5). Therefore, only FM-VP4 was used in this study.

We observed that the largest effect could be seen between animals of the High Fat group compared with animals of the High Fat + 2% (w/w) FM-VP4 group, which suggests that FM-VP4 indeed has a weight gain reducing effect when it is administered to animals that have a high percentage of fat in their diet. In the 12 weeks this experiment lasted there is a reduction of weight gain of 51% between the High Fat and the High Fat + 2% (w/w) FM-VP4 groups, while the weight gain between the Low Fat and the Low Fat + 2% (w/w) FM-VP4 group was reduced by

17%. Interestingly the food and caloric intake of the animals in the High Fat group were slightly higher than the animals of all other groups including the High Fat + 2% (w/w) FM-VP4 group, suggesting that FM-VP4 might have an appetite suppressing effect since the caloric content of the two High Fat diets are comparable (Table 1). This is further supported by the observation that in all of the comparisons beyond week five of the study there were reductions in food intake in the High Fat + 2% (w/w) FM-VP4 group compared to the High Fat group. However, this finding was not observed in the Low Fat control and FM-VP4 treatment groups suggesting that FM-VP4 may only inhibit the gastrointestinal absorption of excessive fat. Further studies to explain these findings are required.

There was also a significant decrease in total plasma cholesterol levels and the weight of abdominal fat pads between the High Fat and High Fat + 2% (w/w) FM-VP4 groups (Figures 2 and 3). The observation that the caloric intake was lower in the High Fat + 2% (w/w) FM-VP4 group compared to the High Fat group could have impacted the difference in body weight gain (Figure 2), weight of the abdominal fat pads (Figure 3) and plasma cholesterol levels (Figure 4) in these animals. However, the finding that the High Fat + 2% (w/w) FM-VP4 group also showed a significant decrease in the values of the obesity parameters compared to the Low Fat group with an observed lower caloric intake, shows that the difference in weight gain can be attributed to the effect of FM-VP4.

The reduced weight gain in the FM-VP4 treatment groups did not seem detrimental to the animals. All the animals were healthy and active and showed no signs of any discomfort due to the treatment. The only group that showed signs of less activity throughout the last 3 weeks of the experiment was the High Fat group, possibly due to their high body weight. Interestingly the animals of the Low Fat + 2% (w/w) FM-VP4 and the High Fat + 2% (w/w) FM-VP4 groups had similar characteristics concerning weight gain, plasma cholesterol and weight of the abdominal fat pads. This observation suggests that FM-VP4 may inhibit the uptake of excessive amounts of cholesterol, but it does not hinder the uptake of essential amounts of cholesterol needed by the body to function properly. Future studies to investigate this are warranted.

In conclusion, FM-VP4 displayed potential weight-loss and cholesterol lowering properties in both High Fat and Low Fat Diets treated groups. These results warrant further investigation in a dose response study to see if FM-VP4 will show as potent anti-obesity effects in more clinically relevant doses.

ACKNOWLEDGEMENTS

Funding for this project was provided by a Collaborative Research Development Grant from the National Sciences and Engineering Research Council of Canada and Forbes Medi-Tech Inc. (#CRDPJ 305231-03 to KMW).

REFERENCES

1. Marx J. Cellular warriors at the battle of the bulge. Science 299:846-849 (2003).
2. Fernandez-Lopez JA, Remesar X, Foz M, Alemany M. Pharmacological approaches for the treatment of obesity. Drugs 62(6):915-944, (2002).
3. Katzmarzyk PT. The Canadian obesity epidemic, 1985-1998. CMAJ, 166(8);1039-1040 (2002)
4. Ng AW, Lukic T, Pritchard PH, Wasan KM. Development of novel water-soluble phytostanol analogs: disodium ascorbyl phytostanyl phosphates (FM-VP4): preclinical pharmacology, pharmacokinetics and toxicology. Cardiovasc Drug Rev. 21: 151-168 (2003).
5. Lukic T, Wasan KM, Zamfir D, Moghadasian MH, Pritchard PH. Disodium ascorbyl phytostanyl phosphate reduces plasma cholesterol concentrations and atherosclerotic lesion formation in apolipoprotein E-deficient mice. Metabolism 52: 425-431 (2003).
6. Wasan KM, Zamfir C, Pritchard PH, Pederson RA. Influence of phytostanol phosphoryl ascorbate (FM-VP4) on insulin resistance, hyperglycemia, plasma lipid levels and gastrointestinal absorption of exogenous cholesterol in Zucker (fa/fa) fatty and lean rats. J Pharm Sci 92: 281-288 (2003).
7. Wasan KM, Najafi S, Peteherych KD, Pritchard PH: Effects of a novel hydrophilic phytostanol analog on plasma lipid concentrations in gerbils. *J Pharm Sci* 90:1795-1799, (2001).
8. Wasan KM, Najafi S, Wong J, Kwong M, Pritchard PH: Assessing plasma lipid levels, body weight and hepatic and renal toxicity following chronic oral administration of a water soluble phytostanol compound, FM-VP4, to gerbils. *J Pharm Pharmaceut Sci* 4:228-234, (2001).
9. Wasan KM, Peteherych KD, Najafi S, Zamfir C, Pritchard PH: Assessing the plasma pharmacokinetics, tissue distribution, excretion and effects on cholesterol pharmacokinetics of a novel hydrophilic compound, FM-VP4, following administration to rats. *J Pharm Pharmaceut Sci* 4:207-216, (2001).

10. Cullen P, G. Assmann. High-risk strategies for atherosclerosis. Clin. Chim. Acta. 286(1-2):31-45 (1999).
11. Hendriks HFJ, J.A. Weststrate, T. van Vliet, G.W. Meijer. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolemic and mildly hypercholesterolemic subjects. Eur. J. Clin. Nutr. 53:319-327 (1999).
12. Hallikainsen MA, M.I. Uusitupa. Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hyper-cholesterolemic subjects. Am. J. Clin. Nutr. 69:403-410 (1999).
13. Gylling, H, T.A. Miettinen. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism 48:575-580 (1999).
14. Jones PJH, F.Y. Ntanios, M. Raeini-Sarjaz, C.A. Vanstone. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic man. Am. J. Clin. Nutr. 69:1144-1150 (1999).
15. Weststrate JA, G. W. Meijer. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolemic and mildly hypercholesterolemic subjects. Eur. J. Clin. Nutr. 52:334-343 (1998).
16. Jones PJ, T. Howell, D.E. MacDougall, et al. Short-term administration of tall oil phytosterols improves plasma lipid profiles in subjects with different cholesterol levels. Metabolism 47:751-756 (1998).
17. Gylling H, R. Radhakrishnan, T.A. Miettinen. Reduction of serum choleserol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine: women and dietary sitostanol. Circulation 96:4266-4231 (1997).
18. Gylling H, T.A. Miettinen. Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterollemic non-insulin dependent diabetic men. J. Lipid Res. 37:1776-1785 (1996).
19. Miettinen TA, P. Puska, H. Gylling, et al. Reduction of serum cholesterol with sitostanol-ester margarine is a mildly hypercholesterolemic population. New England J. Med.333:1308-1312 (1995).
21. Gylling H, M.A. Siimes, T.A. Miettinen. Sitostanol ester margarine in dietary treatment of children with familial hypercholesterolemia. J. Lipid Res. 36:1807-1812 (1995).
22. Vanhanen HT, J. Kajander, H. Lehtovirta, T.A. Miettinen. Serum levels, absorption efficacy, fecal elimination and synthesis of cholesterol during increasing dosing of dietary sitostanol esters in hypercholesterolemic subjects. Clin. Sci. 87:61-67 (1994).
23. Gylling H, T.A. Miettinen. Serum cholesterol and cholesterol and lipoprotein metabolism in hypercholesterolemic NIDDM patients before and during sitostanol ester-margarine treatment. Diabetologia. 37:773-780 (1994).
24. Becker M, D. Staab, K. Von Bergmann. Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. J. Pediatr. 122:292-296 (1993).
25. Hildebrandt AL, Kelly-Sullivan DM, Black SC. Antiobesity effects of chronic cannabinoid CB_1 receptor antagonist treatment in diet-induced obese mice. European Journal of Pharmacology 462: 125-132 (2003).
26. Henry M, Ghibaudi L, Gao J, Hwa JJ.Energy metabolic profile of mice after chronic activation of central NPY Y1, Y2, or Y5 receptors. Obes Res. 2005 Jan;13(1):36-47.