Original Article:

BENEFICIAL EFFECT OF VITAMIN D SUPPLEMENTATION ON WEIGHT AND BMI OF MICE TAKING HIGH-FAT DIET

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ABSTRACT:

Objective: Obesity is a serious health issue that is rising worldwide. Underdeveloped and developed countries both are becoming the culprits of this pandemic. Decreased levels of vitamin D and obesity are correlated. This study was planned to see whether vitamin D supplementation has any effect on weight and body mass index (BMI) of mice taking the high-fat diet.

Methods: This was a randomized control trial, conducted at the Physiology Department of Akhtar Saeed Medical and Dental College, Lahore from October 2017 to December 2017. Ninety (90) male mice were randomly divided into 3 groups. Each group had 30 mice. The total duration of the study was 6 weeks. Group A was the normal diet control group, Group B was the high-fat diet control group, Group C was high-fat diet and vitamin D taking test group. The weight of every mouse was recorded twice a week for 6 weeks by the electronic weighing machine. Initial and final nasoanal length of every mouse was taken and initial and final BMI was calculated. The difference in the nasoanal length and BMI was calculated and data was analyzed using SPSS version 20.

Results: Mean weight of group B mice increased significantly as compared to group A mice($p=0.005$). Mean weight of group C mice reduced significantly as compared to group B mice($p=0.028$). Mean weight of group A and C mice was not significantly different from each other ($p=0.822$).Mean BMI of group C mice reduced significantly as compared to group B mice ($p=0.002$). BMI of group A and B mice was not significantly different from each other ($p=0.330$). Difference of BMI between group C and A was also statistically insignificant ($p=0.111$).

Conclusion: Vitamin D prevents weight gain and increase in BMI of mice taking high fat diet.

Key Words: Obesity, Body Mass Index, Vitamin D

INTRODUCTION:

Obesity has emerged as an epidemic of the 21st century according to the WHO report.[1] WHO fact sheet reveals that about 300 million adults are obese worldwide.[2] Obesity is related to a number of disorders like diabetes, hypertension, various malignancies, chronic kidney disease, infertility and musculoskeletal problems.[3]

Vitamin D in diet or formed in the skin in the presence of sunlight is inactive. For it to be activated, it needs two hydroxylations, first hydroxylation in the liver and the second one in the kidney to form 1, 25 $(OH)_2D_3$ also called calcitriol.[4]

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It controls the transcription of many genes. Vitamin D is now being evaluated for its multiple roles. It has anti-inflammatory action, modulates our immune system and has anti-proliferative action too.[5]

Obesity is an alarming risk factor for various diseases like carcinogenesis, diabetes, hypertension, etc. Adipose tissue stores vitamin D and decreases its bioavailability. Vitamin D prevents the conversion of pre-adipocytes to mature adipocytes by regulating the gene expression of various transcription factors. However, these effects are different in different species. Adipose tissue is not merely a storehouse of fat, it also secretes many proteins and peptides which cause inflammation and produce comlications related to obesity. Obesity causes hypertrophy of the adipose tissue and thus its blood supply is compromised leading to hypoxia. This hypoxia initiates

inflammation and recruitment of macrophages to adipose tissues. The secretion of adiponectin by adipocytes is reduced and secretion of various inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), etc. Vitamin D inhibits chronic inflammation by decreasing production of inflammatory cytokines as evidenced by various mouse and human studies. Vitamin D inhibits nuclear-factor Kappa B (NF-KB) signaling pathway and mitogen-activated protein kinase (MAPK) signaling pathway, thus gene transcription is altered, and production of various pro-inflammatory substances is blocked.[6]

Previous studies, show that serum vitamin D levels are low in obese persons, and others show that low vitamin D levels are related to hyperlipidemia and obesity. Vitamin D levels have an inverse relationship with weight and body mass index (BMI).[7] However, further studies are required to have conclusive results.

As no cut off values for weight and BMI exist for underweight, normal and obese mice, we have compared the weight and BMI of the test group to that of control groups.[8]

MATERIAL AND METHODS:

Ninety healthy male mice were included in the study and they were randomly allocated into three groups, i.e, group A, B and C with 30 mice in each group. Group A was normal, control group given diet with normal constituents for 6 weeks duration. Group B was high-fat diet control group given a diet rich in fat constituents[9] for 6 weeks duration. Group C was test group, given high-fat diet and 100mg/kg/day oral dose of vitamin D for 6 weeks[10,11] (Table-1). The weight of every mouse was taken twice a week for 6 weeks by the electronic weighing machine. Initial and final naso-anal length of every mouse was taken and initial and final BMI was calculated. The difference in the naso-anal length and BMI was calculated and data was analyzed using SPSS version 20. The following formula was used to calculate BMI statistically.

BMI= weight in grams/ length in (cm)^2

p values ≤ 0.05 were considered statistically significant. p value > 0.05 is non significant.

Table -1 Grouping of mice, type of diet and supplementation

Groups	**Normal diet (**10% of kcal% fat)	**High fat diet** (60% kcal% fat)	**Vitamin D** 100ng/kg/day
Duration	**6 weeks**	**6weeks**	**6 weeks**
Group A	Given	Nil	Nil
Group B	Nil	Given	Nil
Group C	Nil	Given	Given

RESULTS:

There was significant difference of weight between the groups A, B, and C (one way ANOVA-Table 2) Mean weight of group B mice increased significantly as compared to group A mice(p=0.005-Table 3). Mean weight of group C mice reduced significantly as compared to group B mice(p=0.028-Table 4). Mean weight of group A and C mice was not significantly different from each other (p=0.822-Table 5).

There was significant difference of BMI between group A, B and C (P=0.003-Table 2).Mean BMI of group C mice reduced significantly as compared to group B mice (p=0.002-Table 4). BMI of groups A and B mice was not significantly different from each other (p=0.330-Table 3). The difference of BMI between groups C and A was also statistically insignificant(p=0.111-Table 5).

Table-2 Comparison of weight and BMI between Groups A (normal diet), B (high-fat diet)and C (high fat diet + vitamin D) (one way ANOVA)

Parameters assessed	**Group A (n=30)**	**Group B (n=30)**	**Group C (n=30)**	**p-value**
Weight change (grams)	7.17± 6.15	11.30± 4.57	7.93± 3.93	0.004*
BMI change (grams/cm^2)	0.07± 0.06	0.09± 0.06	0.05± 0.03	0.003*

Table-3 Comparison of weight and BMI between groups A(normal diet) and B (high fat diet) (Post Hoc Tukey's test)

Parameters assessed	Group A	Group B	p-value
Weight change (grams)	7.17±6.15	11.30±4.57	0.005*
BMI change (grams/cm^2)	0.07±0.06	0.09±0.06	0.330

Table-4 Comparison of weight and BMI between groups B(high fat diet) and C(high fat diet+ vitamin D) (Post Hoc Tukey's test)

Parameters assessed	Group B	Group C	p-value
Weight change (grams)	11.30±4.57	7.93±3.93	0.028*
BMI change (g/cm^2)	0.092±0.06	0.05±0.03	0.002*

Table-5 Comparison of weight and BMI between groups C(high fat diet+ vitamin D) and A(normal diet) (Post Hoc Tukey's test)

Parameters assessed	Group C	Group A	p-value
Weight change (grams)	7.93±3.93	7.17±6.15	0.822
BMI change (g/cm^2)	0.05±0.03	0.07±0.06	0.111

DISCUSSION:

There are various anthropometric parameters that are used to asses obesity in mice like weight, BMI, thoracic circumference and visceral fat.[12] In this study, only weight and BMI were measured. Three groups A, B, and C with 30 mice in each were assessed. Weight of group B mice increased significantly as compared to group A mice, while BMI was not raised significantly in group B mice as mice weight and length both increased proportionately. In group C, weight and BMI both were reduced significantly as compared to those of group B. However, between test group C and control group A, no significant difference in weight and BMI was found. Thus high fat diet increases weight and BMI of mice, whereas if vitamin D is given along with a diet rich in fat, weight and body mass index(BMI) both are reduced. But we can't consider vitamin D as an agent to prevent obesity in humans without the support of a lot of observational and interventional human studies.

In one study, mice who were fed diet high in fat and sugar along with vitamin D for 10 weeks showed reduced serum triglycerides levels, less hepatic steatosis, and reduced products of lipid peroxidation as compared to the group of mice who were fed diet high in fat and sugar content.[13] In another study, 8 weeks old mice were given a normal diet and high fat diet till 24 weeks of age, then one group was given normal diet and vitamin D supplementation and the other group was given high fat diet with vitamin D supplementation. Serum levels of 25(OH) D3, weight and BMI were assessed. Weight and BMI of both groups showed no significant difference.[14] In one study, vitamin D supplementation for 6 weeks in women who were obese and overweight resulted in the reduction of body weight and BMI.[15] A recent study in humans shows that vitamin D potentiates weight reduction in individuals who were taking a weight-reducing diet. It reduces weight and BMI.[16] In another systematic research, three groups of individuals were assessed, one group of obese individuals who were not undergoing any weight loss therapy, the other group on the weight loss therapy, and the third group with individuals after bariatric surgery. All groups were given oral vitamin D for 3 months. Normal serum levels of vitamin D were achieved. Weight and body mass index (BMI) were not changed significantly.[17] Further randomized controlled trials of longer duration should be carried out for the evaluation of the relationship between vitamin D and obesity in human beings.

CONCLUSION:

Vitamin D prevents weight gain and increases in BMI in mice taking high fat diet.

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