Protective effect of Vitamin D on Cardiac Mitochondrial Apoptotic pathway in Rats fed with high fat diet

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ABSTRACT

Background: Both diet quality and vitamin D status are important factors associated with many cardiovascular risk factors. Cardiac mitochondrial pathway of apoptosis has been associated with those factors. The current work aimed to assess the effect of chronic consumption of high fat diet (HFD) on the cardiac markers of mitochondrial pathway of apoptosis and whether vitamin D supplementation with high fat diet (HFD) would alter the expression of these markers.

Materials and methods: A total of 25 rats were grouped as follows: Control Group: received the standard diet (10% fat) for 6 months (n= 5). High fat diet (HFD) fed group: received high fat diet (HFD) (45% fat) for 6 months (n=10), High fat diet (HFD) fed group with vitamin D: received HFD for 6 months with vitamin D 400 IU/kg/day oral gavage (n=10). Body weight and body mass index (BMI) were assessed at the start, at the interval of every 45 days and at the culmination of the experiment. After the experiment, heart specimens were prepared as per the protocol for the quantitative real-time polymerase chain reaction (qRT-PCR) based analysis.

Results: The BAX (BCL2 Associated X, Apoptosis Regulator) gene was found to be expressed highly in the group of high fat diet (HFD) fed rats for 6 months, and BCL2 up-regulation and BAX down regulation was observed in rats fed high fat diet (HFD) containing vitamin D.

Conclusion: The findings of the present study confirm the negative influence of high fat diet (HFD) intake on cardiomyocytes, as well as the beneficial effects of supplementation with vitamin D on mitochondrial pathway of apoptosis.

Keywords: Apoptosis; Heart; Vitamin D; High fat diet.

INTRODUCTION

Obesity is becoming a global epidemic in both children and adult (World Health Organization, 2015). It described as an extreme quantity of body fat or adipose tissues in relative to slim body build (World Health Organization, 2015). It is related with many diseases such as heart and circulatory disorder, metabolic syndrome, increase blood pressure, stroke and coronary artery disease (Fitchett D, 2015; Chorin E, Hassidim A, Hartal M, Havakuk O, et al., 2015). Obesity may affect the heart through its influence on known risk factors such as dyslipidemias, hypertension, glucose intolerance, and inflammatory markers (Hubert HB, Feinleib M, McNamara PM, Castelli WP, 1983), these abnormalities could relate to genetic alterations (Swinburn B and Egger G, 2002). Reports account a persistent rise in the incidence of obesity in the previous 3 years in Saudi Arabia. From previous 1980 till middle-1990, general incidence of obesity was predictable by twenty percent (Kang JH and Kang JY 2015; Dyson J, Jaques B, Chattopadyhay D, Lochan R; 2014).

Memish et al. proved that the deteriorating tendency in incidence of increase in weight alarmed the young adult, in contrast among the old adult where the tendency was increase. Irrespective this repartition, the important reduction of fatness in our kingdom is a suitable reveal of the efficiency of native healthiness trials and strategy to regulator the obesity, and the responsiveness operations assumed by the Saudi ministry of health, over the previous period, which concentrated on behavioral and dietary alterations (Memish ZA, Bcheraoui C, Tuffaha M, Robinson M, 2014). An increase in the body mass index more than thirty was observed to be related with vitamin D deficiency. Several studies and clinical analyses have exposed a close association with vitamin D deficiency and heart circulatory system disorder (Earthman CP, Beekman LM, Masodkarand K, Siblay SD, 2012). Deficiency of Vitamin D has been indicated to be extremely widespread among wide-ranging population with several chronic illnesses involving heart and circulatory disorder which may be a prominent cause of death (Assalin HA, Rafacho B, Santos P, Ardisson L, 2013).

While Saudi Arabia has sunshine weather throughout the year, the population do not display themselves to daylight as reported by Ardawi et al. (2012). Numerous reports revealed that a vitamin D deficiency give rise to cardiac alterations. Yet, the underline mechanism need to be further clarified. The impact of the extent of VD deficient on cardiac muscle is undetermined.

METHOD

Wistar rats (male) weighing in the range of 150-200 g were housed in standard laboratory conditions with temperature of 23 ± 3°C and a natural light conditions with 12 hours light/dark cycle along with free access to water ad libitum. In accordance with the guidelines approved by the institutional ethical committee, all the rats were reared under the laboratory conditions that avoided them from experiencing unnecessary pain and discomfort. The experimental rats were randomly grouped/assigned to receive either the standard diet (D12450H), which are regarded as normal control rats or high-fat diet (D12451) considered as high-fat diet group of rats. Diet composition is detailed in Table 1.

EXPERIMENTAL PROTOCOL

25 wistar rats were included in current study and randomly distributed into the following groups:

- Control group (C): rats received the standard diet (n=5 rats).
- High fat diet fed group (HFD): The group of rats were fed with a high fat diet (HFD) for a total of six

months (n=10 rats)

- High fat diet fed group + vitamin D group (HFD+VD): The group of rats fed with a high fat diet (HFD) daily with oral gavage supplemented with vitamin D at the rate of 400 IU/kg (n=10 rats).

Table 1: DIO series diets (research diet center).

(DIO) Formulas[12]			D12451 Match 17% Sucrose[12]	
Product #	**D12451**		**D12450H**	
	gm%	**kcal%**	**gm%**	**kcal%**
Protein	24	20	19.2	20.0
Carbohydrate	41	35	67.3	70.0
Fat	24	45	4.3	10.0
Total		100		100.0
kcal/gm	4.73		3.85	
Ingredient	gm	Kcal	**gm**	**Kcal**
Casein, 30 Mesh	200	800	200	800
L-Cystine	3	12	3	12
Corn Starch	72.8	291	452.2	1808.8
Maltodextrin 10	100	400	75	300
Sucrose	172.8	691	172.8	800
Cellulose, BW200	50	0	50	0
Soybean Oil	25	225	25	225
Lard	177.5	1598	20	180
Mineral Mix S10026	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H_2O	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
FD&C Red Dye #40	0.05	0	0.04	0
FD&C Blue Dye #1			0.01	0
Total	858.15	4057	1055.05	4057

Vitamin D was given by means of gavaging technique at a dose of 400 IU/kg/day (Vieth R, 2004). The study continued for sixth months, body weight and oral –anus (OA) length were measured every 45 days to assess body mass index (BMI). Towards the end of the experimental schedule, the experimental rats were sacrificed under the effect of diethyl ether and the heart specimens were kept in RNA later for Quantitative Real-time polymerase chain reaction (qRT-PCR based analysis).

RNA extraction

Following the manufacturer instructions and using the column purification technology by RNeasy Mini Kit (Qiagen), the total RNA was isolated from the cells.

Reverse transcription

Using cDNA reverse transcription kit (ImProm-II™ ReverseTranscription System cat no. A3800) and following the manufacturer procedure, the reverse transcription of the separated RNA into the complementary deoxyribonucleic acid (cDNA) was carried out.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the KAPA SYBR® FAST qPCR Kit Master Mix (2X) Universal with the Cat no. KR0389. The below mentioned rat primers were used for the PCR: rat Bcl2 forward primer AGTGGGATACTGGAGATG: rat Bcl2 reverse primer CTGGCTGTCTCTGAAGAC: rat BAX forward primer CTGGACAACAACATGGAGC: rat Bax reverse primer CAGACGGCAACTTCAACTG.

Statistical Analysis

The data obtained were subjected to statistical analysis of using SPSS for windows package version 23. (SPSS Inc., Chicago, IL, USA). The analysed data are reported in terms of mean ± SD for gene expression. In order to make comparison of the response, between the control and the experimental groups, one way analysis of variance (ANOVA) test was applied. The Rq values obtained for each gene was compared using one-tailed student's t-test. The differences were considered statistically significant, if the *p* value was found to be either less than or equal to 0.05.

RESULTS

The results obtained from the present study are presented in Table 2, and also illustrated in Figure 1, 2 and 3.

1-Differences in the body weight, oral –anus (OA) length and the body mass index (BMI):

At the end of the experimental period of six months, the body weight, the length of the body (oral –anus (OA)), as well as the body mass index (BMI) were found not significantly different in the experimental group when compared to their respective controls (Figure 1).

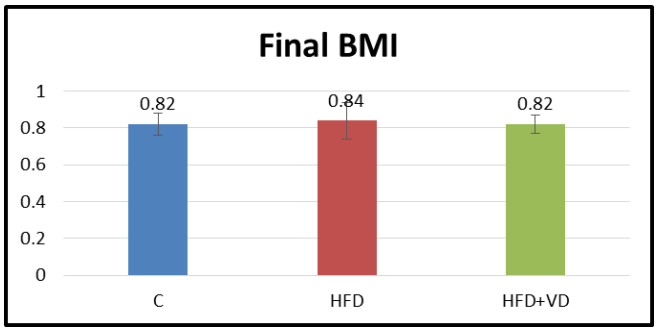

Figure 1: The body mass index at the end of 6 months experimental period. C: control rats, HFD: rats fed with high fat diet, HFD +VD: rats fed with high fat diet treated with vitamin D.

2-Quantitative Real-time polymerase chain reaction (qRT-PCR):

BAX and BCL2 polypeptides

The mitochondrial-dependent apoptotic pathway was analyzed by means of measuring the expression level of the anti-apoptotic BCL2 and pro-apoptotic Bax proteins. At the end of experimental period of 6 months, BCL2 was found to be down-regulated while pro-apoptotic BAX was found to be significantly upregulated by high fat diet (HFD) consumption. Vitamin D supplementation with the high fat diet (HFD) increased BCL2 expression, although

did not reach significance, while vitamin D significantly decreased Bax expression when compared to high fat diet group (Table 2, Figures 2 and 3).

Table 2: Comparative expression level of target genes in control and each of the experimental group in comparison to reference gene, RE = $2^{[\Delta CT]} = 2^{[CT\ reference\ gene\ -CT\ target\ gene]}$

Groups	Genes (Relative Expression)	
	Bax	Bcl2
C	0.278 ± 1.32	18.892 ± 1.73
HFD	220.494 ± 1.76	2.945 ± 0.30
HFD +VD	1.556 ± 0.92	5.093 ± 1.65

C: Control rats, HFD: Rats Fed With High Fat Diet, HFD +VD: rats fed with high fat diet treated with vitamin D. Values are expressed in the form of mean ± SEM.

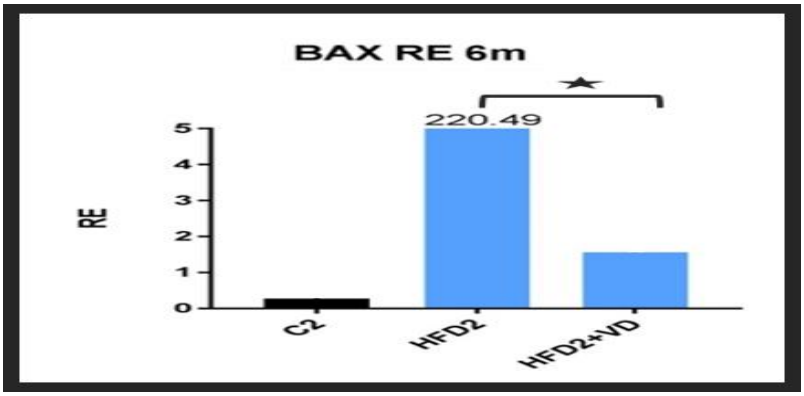

Figure 2: Expression of Bax gene in all comparisons at 6 months. C: control rats, HFD: rats fed with high fat diet, HFD +VD: rats fed with high fat diet treated with vitamin D.*P < 0.05.

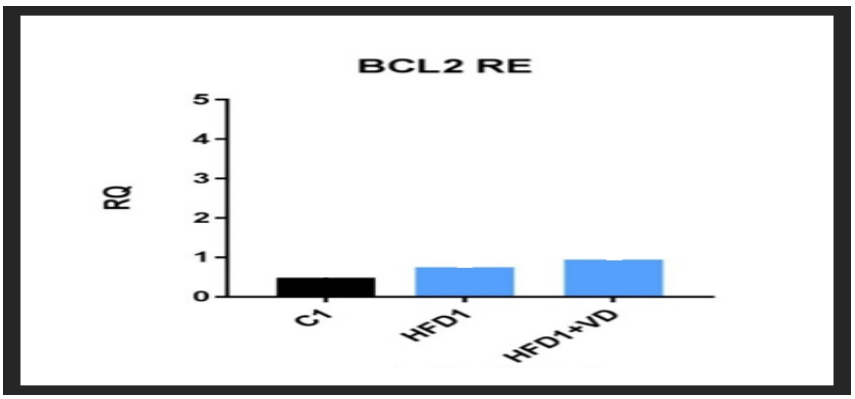

Figure 3: Expression of Bcl2 gene in all comparisons at 6 months. C: control rats, HFD: rats fed with high fat diet, HFD +VD: rats fed with high fat diet treated with vitamin D.

DISCUSSION

There has been a substantial increase in the prevalence of nutrition associated diseases, such as obesity, diabetes and cardiovascular diseases over the past few decades. The nutritional quality of the diet could be one of the most important factors/causes associated with hypertriglyceridemia, hyperglycemia, and hypertension, decreased level of high-density lipoprotein-cholesterol and even in the increased abdominal circumference (Ramalho et al., 2017). Previously studied were conducted with high fat diet (HFD) in experimental animals, particularly in mice and rats. Some of these animal studies have associated high fat diet (HFD) intake with hyperphagia, weight gain and increased adiposity (Woods SC et al., 2003), while other studies associated high fat diet (HFD) consumption to hyperinsulinemia, insulin resistance without developing obesity (Ramalho et al., 2017).

The present experimental study using laboratory animals was comprised of control group (fed with standard diet containing 10% fat), on experimental group of rats termed HFD-fed group (fed with diet containing 45% fat), and another experimental group of rats termed HFD-fed group (fed with HFD supplemented with vitamin D). At the end of the experiment of 6 months, no significant difference was observed between all the three groups concerning body weight and BMI. These finding are in conformation with a previous study by Ramalho et al., 2017, where the authors have observed that feeding rat with HFD (containing 45% fat) for a total period of 15 weeks didn't significantly differ in their body weight when compared to the control group.

In the present study, it was observed that the BAX gene was highly expressed in cardiac tissue of the rats fed with high fat diet (HFD). This observation suggests that 6 months of high fat diet (HFD) ingestion lead to activation of cardiac mitochondrial-dependent apoptotic pathway in male Wistar rats. The results obtained from the current study also revealed that there was an up-regulation of BCL2 and down regulation of BAX in rats fed with high fat diet (HFD) supplemented with vitamin D, which indicates to the probable protective effect of vitamin D on the cardiac tissue by mitigating mitochondrial-dependent apoptosis. Our findings are compared with the report of Cheng et al. (2013), which showed induced-cardiomyocyte apoptosis in hamsters fed with high fat diet (2% cholesterol, 1% garlic oil) for 8 weeks with simultaneous increase in the levels of pro-apoptotic proteins BID and BAX, whereas the level of anti-apoptotic protein BCL2 was observed to be decreased. Furthermore, the findings of Zhu et al. (2007) showed elevation in the cardiac expression of BAX along with decreased expression of BCL2 in pigs fed with high fat diet for 12 weeks.

The genetic effects of high fat diet (HFD) intake on BAX expression extended beyond the cardiac tissue to the vascular smooth muscle cells where (Perales S, Alejandre MJ, Palomino-Morales R, Torres C, et al., 2009) in their in vitro study it was observed that addition of 25-hydroxycholesterol to cell culture led to upregulation of BAX expression in the group that received high fat diet (HFD). The cardio protective role of vitamin D in association with the reduction of the elevated levels of Bax and caspase-9 was shown in the study of Wang et al. (2013); the study showed that calcitriol inhibited apoptosis by modulating the proteins that play critical role in the mitochondrial-dependent apoptotic pathway, which matches with the observation of the present study.

CONCLUSION AND RECOMMENDATIONS

The results obtained from the current study confirm the negative influence of high fat diet intake on cardiomyocytes by enhancing myocardial independent pathways of apoptosis in wistar rats. The observations made in the present study extended the understanding of the cardiac beneficial effects of vitamin D supplementation of the high fat diet against chronic consumption of high fat diet (HFD) and thus highlighted an emerging probable protective role of vitamin D supplementation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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