Effects of vitamin E and C supplementation on lipid profile and atherogenic index of rats on diets enriched with high fat and high cholesterol

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Abstract

Composition of dietary fatty acid influences plasma lipids. This study was designed to investigate effects of vitamin E and C supplementation on lipid profile and atherogenic index of rats on diets enriched with high fat and high cholesterol. Total food intake was more in experimental group (high fat and high cholesterol treated groups) without supplemented with vitamin E and C as compared to control group. Highest body weight was observed in group consuming palm oil in both with and without vitamin supplementation and in sunflower and olive oil group the increase in body weight was almost same. Supplementation with vitamin E and C made significant (p≤0.05) differences in the lipid parameters; also HDL cholesterol were increased and total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol decreased in high fat and high cholesterol treated olive oil and sunflower oil group supplemented with vitamin E and C. So from the result it can be concluded addition of vitamin E and C lead to an improvement in the lipid profile and helped in lowering atherogenic index as decrease in the lipid fraction has been associated to the group with vitamin supplementation even when models were fed high fat and high cholesterol diet.

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**Introduction**

Composition of dietary fatty acid influences plasma lipids and their levels could be modified by the type and amount of fat in the diet (Michael et al., 1975; Pualvilai, Laoragpongse, 2004), which otherwise will lead to the development of atherosclerosis and ischemic heart disease (Erkkila et al., 2008). Atherosclerotic lesions appear to be related to elevated total plasma cholesterol (TC), low density lipoprotein cholesterol (LDL-C), decreased high density lipoprotein cholesterol (HDL-C), and excess fat consumption (Hwang, 2009). Different types of dietary lipids have been shown to affect lipid metabolism and serum lipid profile differently. A moderate decrease in plasma cholesterol levels was reported with low cholesterol diets (Toth, 2004). According to the world health organization (WHO), every year approximately 12 million people with most of them being from the developing world die from cardiovascular disease, (Knietowicz, 2002).

Antioxidant treatment with vitamins C and E are able of scavenging oxygen-derived free radicals in humans (Caballero, 2004), restore endothelial function in hyperlipidemic kids (Engler et al., 2009), lowers rate of ischemic heart diseases (Catalogol and Ozer, 2012) and prevent progression of hypertension (Jurasczek et al., 2012). Vitamin C can replace glucose in many chemical reactions because of its structural similarity to glucose and this is very useful in prevention of non enzymatic glycosylation of proteins and it mops up free radicals formed in the body (Lii et al., 1998) and vitamin E protects LDL-C particles which are susceptible to oxidation and finally lead to atherosclerosis events from oxidative attack (Afkhami- Ardekani et al., 2009).

Cholesterol produces different oxysterols because of its susceptibility to oxidation (Lu and Chiang, 2001) and consumption of saturated fatty acid (SFA) is strongly associated to the cholesterol concentrations. It is a general belief that vegetable oils decreases plasma cholesterol levels although they differ in their cholesterol lowering capacity. Several studies on experimental animal have shown that the fatty acids from different types of vegetable oils may have more or less equivalent hypocholesterolemic activity (Stein and Myers, 1975; Smith et al., 1993; Reaven et al., 1993). Palm oil contains approximately an equal amount of saturated and unsaturated fatty acids and palm olein is a liquid fraction obtained from the refining of palm oil, is rich in oleic acid (42.7%-43.9%), β carotene and vitamin E (tocopherol and tocotrienols) (Narang et al., 2004). Corn oil provides essential fatty acids (mostly linoleic acid, palmitic acid, oleic acid and linoleic acid) and its nutritional properties are excellent (Lita, 2001). Sunflower oil consists of high amount of n-6 EFA, linoleic acid and as a result, it is related with an improved blood lipid profile and thus a reduced risk of CVD. The useful properties of olive oil are mainly because of high content of monounsaturated fatty acids (predominantly oleic acid). The fatty acid composition of olive oil is around 14% saturates, 73% monounsaturates and 8% polyunsaturates (mainly in the form of LA), and it also contains tocopherol (vitamin E) carotenoids, phenolic compounds and lutein (Foster et al., 2009). Shad et al., 2003, showed that consumption of olive, corn and rapeseed oils is beneficial to health. There is lack of study showing the effect of vitamin E and C supplementation on lipid profile on diets enriched with high fat and high cholesterol and different vegetable oils. Therefore this study was designed to find the effects of vitamin E and C supplementation on lipid profile and atherogenic index of rats on diets enriched with high fat and high cholesterol.

**Materials and methods**

In this study, a total of 56 wistar albino rats, weighing approximately 100±5 g, were used. During the experiment, the rats received a standard laboratory diet with tap water ad libitum. Animals were housed at the animal laboratory in King Saud University. Rats were weighed individually and divided into 7 main groups each of 8 rats and housed in standard cages made up of stainless steel at an ambient temperature (22±2°C) and relative humidity (50±5%) in a quiet room with 12/12-h light/dark cycles. Rats were clinically observed and weighed at 21 day intervals.
The experimental period lasted for 6 weeks (excluding 1 week adaptation period). The composition of the diet of each group is shown in Table 1. At the end of experimental period, rats were fasted overnight before sacrificing. All animals were sacrificed using diethyl ether, without recovery from anaesthesia. Blood was collected, centrifuged (6000rpm for 20 min) and stored at -20°C until analysis. Part of the blood is collected on tubes coated with EDTA.

**total lipid and triglyceride**
Total lipid and triglyceride was determined by the method suggested by Zollner and Krisch, 1962 and Trinder, 1969 respectively.

**Total cholesterol**
Total cholesterol was estimated by the method described by Roeschlaub et al., 1974.

**HDL-C and VLDL-C**
HDL-C was measured according to the method described by Assman, 1979 and VLDL-C was measured by using the formula Triglyceride/5 = VLDL-C mg/100ml.

### Atherogenic Index
Atherogenic Index was determined by the formula suggested by Haglund et al., 1991

\[
AI = \frac{TC - HDL}{HDL}
\]

**Statistical analysis**
Each data in table was presented as average of three replicates. The data obtained to find the effect of high fat and high cholesterol (with and without vitamin E and C) on feed intake, body weight and plasma lipids was subjected to statistical analysis by conducting analysis of variance (ANOVA), using SPSS software package. The significant differences of their means were compared using Duncan test and p value was considered significant at 95%.

### Results and discussion
This study was designed to investigate effects of vitamin E and C supplementation on lipid profile and atherogenic index of rats on diets enriched with high fat and high cholesterol.

#### Table 1. Composition of diet.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Groups</th>
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<td>1</td>
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<tr>
<td>Starch</td>
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<tr>
<td>Casein</td>
<td>+</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
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<td>Corn oil</td>
<td>+</td>
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<tr>
<td>Dextrin</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Mineral mix</td>
<td>+</td>
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<tr>
<td>Vitamin mix</td>
<td>+</td>
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<tr>
<td>L-cystine</td>
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<tr>
<td>Choline bitartarate</td>
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<tr>
<td>Cholesterol</td>
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<td>Olive oil</td>
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<td>Sunflower</td>
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<td>Palm oil</td>
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<td>Vitamin E</td>
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<td>Vitamin C</td>
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**Total food intake**
Total food intake was more in experimental group (high fat and high cholesterol treated groups) as compared to control group (Fig.1). A significant (p≤0.05) difference was found between control and experimental group and also, a very slight significant (p≤0.05) difference was observed between olive and sunflower group compared to palm oil group in both
with and without vitamin supplementation group. More amount of fat may increases the palatability and hence in turn increases the consumption (Chautan et al., 1991).

**Body weight gain**

As compared to control group an increase in body weight has been found in all experimental groups. Highest body weight was observed in group consuming palm oil in both with and without vitamin supplementation and in sunflower and olive oil group the increase in body weight was almost same (Fig.1). Increase in weight has been found to be correlated with the percentage of fat in the diet. According to our data saturated fat play role in increasing body weight because saturated fat decreases activity of hormone sensitive lipolysis. This affects synthesis of fat in the body which deals directly with body weight (Awad et al., 1989).

**Blood lipids**

Supplementation with vitamin E and C made significant (p<0.05) differences in the lipid parameters; also HDL cholesterol were increased and total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol decreased in high fat and high cholesterol treated olive oil and sunflower oil group supplemented with vitamin E and C (Fig-2). Atherosclerotic lesions in man and in animals come out to be related to excess consumption of fat, elevated plasma total cholesterol and low density lipoprotein cholesterol and decreased high density lipoprotein cholesterol (Hwang, 2009).

**Values**

Values are means of three replicates. Mean values having different superscripts letters are significantly different (p≤0.05).

**Total lipids**

As compared to control group, results shows increase in total lipids for high fat and high cholesterol groups. Percentage increase in total lipids in groups without vitamin E and C supplementation was 31.8, 32.9 and 37.8 mg in olive oil, sunflower oil and palm oil respectively. A significant (p≤0.05) increase in total lipids was noticed in palm oil group whereas insignificant (p≥0.05) increase was found in olive oil and sunflower oil group without vitamin supplementation. From the result, it was noticed that increase in percentage of total lipids when adding vitamin was less as compared to without vitamin added group. Percentage increase in total lipids in vitamin E and C supplementation group was 12.6, 13.1 and 28.6 mg in olive oil, sunflower oil and palm oil respectively. This shows vitamin E and C decreases total lipids in plasma while consuming high fat and high cholesterol diet. This result is in agreement with the previous result (Fukushima et al., 1996); in which it was reported that dietary fats lead to increase in total lipids. This increase is correlated to the dominant fatty acids. Kleinveld et al., 1995 demonstrated that level of blood lipids does not change with the type of fats. Also, Willingham et al., 1993 mentioned that decrease in vitamin results in
hyperlipidemia in rabbit. Losonezy et al., 1996 mentioned that vitamin E enhances the effect of vitamin C which leads to decrease of relative risk of heart diseases.

![Graph showing effects of vitamin E and C supplementation on LDL/HDL, Total cholesterol/ HDL ratios and Atherogenic Index of rats on diets enriched with high fat and high cholesterol.](image)

**Fig. 3.** Effects of vitamin E and C supplementation on LDL-C/HDL-C, Total cholesterol/ HDL-C ratios and Atherogenic Index of rats on diets enriched with high fat and high cholesterol.

**Cholesterol**

It was noticed that high fat high cholesterol diet palm oil group without vitamin supplementation results in maximum increase in plasma cholesterol as compared to control. Lipid peroxidation is a major risk factor for atherosclerosis, and cholesterol rich diet results its increase by the induction of free radical production, followed by hypercholesterolemia (Attar, 2010).

Increase in cholesterol was significantly (p≤0.05) lower in other groups. This is due to difference in fatty acid composition of these oils. Palm oil contains 48-51%, olive and sunflower oil contains 13-14% and 10-11% saturated fat respectively. Percentage increase in cholesterol in without vitamins added groups were 40.12, 46.43 and 56.78 and the percentage increase in cholesterol in vitamin E and C added groups were 10.44, 6.06 and 36.65 in olive oil, sunflower oil and palm oil respectively. Olive oil and sunflower oil contains 73-77% and 19-20% MUFA and 9-10% and 65-69% PUFA respectively. Connor et al., 1969 reported, oils that contains unsaturated fatty acids has role in decreasing cholesterol production by inhibiting enzymes (OA reductase) that is responsible for cholesterol production. It also increases excretion of bile acid steroids (Goodnight et al., 1982). Evidences shows that dietary replacement of saturated fatty acids with unsaturated fatty acids decreases blood cholesterol and may provide favorable effects on vascular reactivity, inflammation and thrombosis (Kris Etherson et al., 1999; Kris Etherson et al., 2001).

In this study vitamin E and C supplementation significantly reduced lipid profile in high fat and high cholesterol treated group. This improvement in lipid profile is supported by the previous study (Kurowska et al., 2000). The reason behind hypocholesterolemic effect of vitamin C may be the property of degrading cholesterol by preventing LDL-cholesterol from oxidative damage (Owu et al., 2006).

**Triglycerides**

It was found that high fat and high cholesterol led to significant (p≤0.05) increase in the triglycerides in non vitamin treated group. On the other hand supplementation with vitamin E and C results in non significant (p≥0.05) difference in triglycerides in olive oil group and a significant (p≤0.05) increase in palm and sunflower oil groups. Ratnayake and Gilani, 2004 reported that the concentration of triglycerides in blood plasma increases by increasing fat intakes. High doses of vitamin E and C have been shown to decrease triglycerides, blood glucose and plasma cholesterol in type 2 diabetes mellitus (Chen et al., 2006). Increased plasma triglycerides and cholesterol levels may be a risk factor for vascular disease (Shahar et al., 2003). Authors reported that feeding polyunsaturated fatty acids and antioxidants results in inhibition of synthesis of triglycerides in liver and consequently its level in plasma (Pereira et al., 1990; Ney et al., 1991)

**HDL-C**

From the result it was noticed that HDL-C in control group was 35.36 mg whereas it was 36.09 mg in olive oil group. Although the HDL-C for sunflower group (31.33 mg) was lesser than control group, it was higher than palm oil (27.88 mg) group. In the vitamin added group there was significant increase in the olive oil group but no significant difference was found between other vitamins added group and control.
This result is in agreement with Kleinveld et al., 1995, who stated that oils rich in oleic acid resulted in increase in HDL-C more compared with oils rich in linoleic acid after adding vitamin E.

**LDL-C and VLDL-C**

Results shows that LDL-C increased in high fat and high cholesterol group compared to control group. For control group the LDL-C was 31.42 mg whereas it was 35.02, 41.84 and 36.94 mg in without vitamin E and C supplemented group and 28.41, 27.34 and 33.20 in vitamin E and C supplemented group in olive oil, sunflower oil and palm oil respectively. Decrease in VLDL-C was observed in vitamin E and C supplemented group and with the consumption of oil rich in PUFA. The vitamin supplemented group resulted in decrease in LDL-C. This result is in agreement with Willingham et al., 1993 who stated that in his study, rabbits that consume high dose of vitamin E resulted in decrease in LDL-C and oxidized cholesterol and also oxidation of triglycerides. Devraj and Jilal, 1996, reported that tocopherols decreases level of LDL-C, guard lipids from oxidation and as a result avert cardiovascular diseases. Studies have shown decrease levels of lipid profile, lipid peroxidation and free radical production by vitamin E and C supplementation (Upritchard et al., 2000). It has been found previously that diets rich in saturated fatty acids increased the LDL-C (van Rooven et al., 2008) and oxidative modification of LDL is an important step in the development of atherosclerosis (Bhadki et al., 2004). The higher the level of LDL cholesterol, the greater is the risk of atherosclerotic heart disease and the higher the level of HDL cholesterol the lower will be the risk of coronary heart disease (Ajayi and Ajayi, 2009; Ratnayake and Gilani, 2004).

**LDL-C/HDL-C and Total Cholesterol/HDL-C**

Studies suggests that changes in the ratios of LDL-C/HDL-C and TC/HDL-C are better predictors of coronary heart diseases risk reduction than changes in their levels (Kinosian et al., 1995; Natarajan et al., 2003, Panagiotakos et al., 2003). Even though in the earlier period an increase in the serum total cholesterol level was associated with higher possibility of atherosclerosis, but recently it has been found that the LDL-C/HDL-C ratio is a stronger index of atherogenicity of the lipoproteins instead of lipid profile of the individual lipoprotein fraction. The higher the ratio of LDL-C/HDL-C; the high will be the chances of atherosclerosis (Wallidus et al., 2001).

From the Fig-3, the LDL-C/HDL-C ratio of high fat and high cholesterol added olive oil and sunflower oil supplemented with vitamin E and C fed rats was lower than the control and this ratio was highest for palm oil group. Similarly no major difference was found in cholesterol/HDL ratio between control and high fat and high cholesterol added olive oil and sunflower oil supplemented with vitamin E and C, although the LDL-C/HDL-C ratio and cholesterol/HDL-C ratio was highest for palm oil either supplemented or not supplemented with vitamin E and C. This might be due to the fact that olive and sunflower oil is enriched with high percentage of unsaturated fatty acids. El-Gegaihi et al., 2004, mentioned that the hypocholesterolemic effect of rocket and borage oil was attributed to the high concentration of unsaturated fatty acids in this oil. It therefore suggests that long term consumption of palm oil as the case is presently could pose a risk in the development of cardiovascular diseases.

**Atherogenic index**

Atherogenic Index (AI) reflects the possibility of atherosclerosis. As AI increases possibility of CVD increases. From the result it was noticed that AI for control group was 0.87. As the percentage of oil increases in the diet in experimental group, increase in the value of AI was noticed but this increase was not statistically significant (p≥0.05). AI of without vitamin added experimental groups were 1.56, 2.09 and 2.72 and for vitamin added experimental groups were 0.91, 1.16 and 2.22 for olive, sunflower and palm oil respectively. Percentage increase in atherogenic index in groups without vitamin E and C supplementation was 79.31, 140.23 and 212.64 and
percentage increase in atherogenic index in group’s supplemented with vitamin E and C was 4.59, 33.33 and 15.17 in olive oil, sunflower oil and palm oil respectively. As for vitamin group, consumption of vitamins in the diet resulted in decrease in AI in high fat and high cholesterol diet. Decrease was significant in olive oil and sunflower group as compared to palm oil group. So from the result it can be concluded that vitamins E and C helps in lowering atherogenic index and high fat may be considered as a risk factor for heart diseases and mainly atherosclerosis. Consumption of high saturated fatty acid may results in high AI, even with low quantity consumption as compared to the diets rich in unsaturated fatty acids. Grundy and Denke, 1990, stated that high fat diet may be considered as risk factor for atherosclerosis.

Conclusion
The result of this study indicates that the addition of vitamin E and C lead to an improvement in the lipid profile and helped in lowering atherogenic index as decrease in the lipid fraction has been associated to the group with vitamin supplementation even when models were fed high fat and high cholesterol diet.

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