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Difference in Leptin Hormone Response to Nutritional Status in Normal Adult Male Albino Rats

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Abstract: The present study investigated the effect of 14 days diet, enriched in butter, vitamin E (vit. E) and green tea, on the major regulators of energy expenditure. Leptin is the product *OB* gene. This 16 KDa protein is produced by mature adipocytes and is secreted in plasma. Its plasma levels are strongly correlated with adipose mass in rodents as well as in humans. Leptin inhibit food intake, reduces body weight and stimulates energy expenditure. In order to evaluate the effect of diet enriched in butter, vit. E and green tea on body weight, adipose tissue weight and organs weight, serum lipids, lipoproteins content and serum leptin levels in male albino rats supplemented for 14 days on the previous diet. This study showed that high fat diet significantly increased body weight and adipose tissue weight, while vit. E and green tea enriched diet significantly lowered body weight and adipose tissue weight, kidney and spleen weights didn't show significant changes in all the experimental groups. While liver weight decreased in diet supplemented with high fat diet. Also, the results showed that high fat diet and vit. E supplemented diet induced significant increase in total cholesterol, LDLc., triglyceride level with significant decrease in HDLc. level as compared to normal control rats. Finally green tea supplemented diet induced significant decrease in total cholesterol, LDLc., triglyceride level with insignificant increase in HDLc. level in control rats. On the other hand, high fat supplemented diet significantly increased serum leptin levels in rats compared to control group, while vit. E and green tea enriched diet significantly lowered serum leptin levels at the end of experimental period. In conclusion, improving the biological activity of leptin by diet modification may exist as a practical strategy for the treatment of obesity and related disorders and a diet rich in green tea to reduce the risk of cardiovascular disease (CVD) obesity and also protect the liver against free radicals.

Key words: Vitamin E, *OB* gene, green tea, adipocytes, leptin, obesity, cardiovascular disease

INTRODUCTION

Obesity is one of the most common chronic diseases that has sever consequence on physical and psychological health and not simply a cosmetic issue (Mantzoros, 1999). The regulation of body weight requires a balance between energy intake and energy expenditure (Mantzoros *et al.*, 1998).

It has been recently recognized that endocrine organ capable of producing biologically active proteins termed adipokines including leptin which may contribute to the development of obesity mediated adverse effects on lipid metabolism (Funahashi *et al.*, 1999).

Leptin is a protein product of obese gene (*OB* gene) and is also referred to the obesity gene product or adipose tissue specific ob gene product (Auwerx and Staels, 1998). Leptin increase energy expenditure and decreases appetite (Jode and Peter, 2001) by decreasing hypothalamic levels of neurotransmitters (Ahima *et al.*, 1996). Leptin may not only reflect peripheral adipose tissue mass but also play a key role in a feedback loop

maintaining energy balance by signaling the state of energy stores to the brain and by influencing the regulation of appetite and energy metabolism (Mantzoros *et al.*, 1998).

Long term high fat diets can induce over consumption of this diet and weight gain, however the mechanism by which this occurs is unknown (Golay and Bobbioni, 1997).

Mice maintained on diet induced obesity have hyperleptinemia (Frederich *et al.*, 1995) and expend less energy, suggesting that dietary fat may cause weight gain by limiting the action of leptin (Deborah *et al.*, 2000).

Also, there are several evidence indicates that glucose is an important regulator of leptin expression and secretion by increasing ob messenger RNA (mRNA) after glucose administration in mice a closely related to plasma glucose concentrations than to plasma insulin concentrations (Wendy *et al.*, 1997).

Vit. E is well documented in the increase of serum triglyceride concentrations, the mechanism of this effect is unclear (David *et al.*, 1991).

It has been suggested that green tea drinking may lower serum cholesterol and LDLc. (Suminori *et al.*, 1996). on the other hand, green tea act as protective agent against cardiovascular disease (CVD) (Imai and Nakachi, 1995). The beneficial effect of drinking green tea is attributed to the content of green tea epicatechins (GTE) (Chan *et al.*, 1999).

Therefore, the aim of this study is to visualize the effect of different nutritional status (high fat, vit. E and green tea enriched diets) on body weight, organs weight, adipose tissue weight, serum lipids and lipoprotein and serum leptin concentrations in normal male rats.

Also, we try to investigate whether these diets can play a role in the protection against obesity and its hazards especially CVD through estimation of body weight changes, serum lipid and lipoprotein and leptin levels.

MATERIALS AND METHODS

The study was conducted in the period between June 2007 to March 2008. Forty adult male albino rats weighting 200 g were used in this study. Animals were divided into 4 equal groups (each 10 rats) according to the type of food.

- Group I:** Male rats maintained on commercial rat chow and served as control
- Group II:** Consisted of rats maintained on commercial rat chow enriched with butter (40 g kg⁻¹ diet) according to Bahceci *et al.* (1999)
- Group III:** Consisted of rats maintained on commercial rat chow enriched with vit. E (200 IU day⁻¹) according to Ozdil *et al.* (2004)
- Group IV:** Consisted of rats maintained on commercial rat chow enriched with green tea powder (40 g kg⁻¹ diet) according to Kazutoshi *et al.* (2000)

Each group was kept for 14 days on the special diet regimen described.

At the end of the experimental period fasting blood samples were obtained from orbital sinus under

light ether anesthesia and allowed to clot at room temperature for 1 h, then centrifuged at 2000 rpm for 15 min. The separated sera were stored at -20°C till time of analysis.

Sera were analyzed for estimation of serum leptin according to Auwerx and Staels (1998), total cholesterol by method of Allain *et al.* (1974), LDLc., HDLc. by method of Levy (1981) and Hino *et al.* (1996) and triglyceride were measured according to the method of McGowan *et al.* (1983).

Also, body weight was measured, kidney, liver, spleen and intraperitoneal adipose tissue were determined.

Statistical analysis of the data was done using Mean±SE and percentage of changes and unpaired students t-test. The criterion for significance was a p-value of less than 0.05. All tested groups were compared to the control one.

RESULTS

High fat diet produced insignificant changes in body weight, spleen weight and kidney weight. On the other hand it induced significant increase in adipose tissue weight with significant decrease in liver weight (Table 1).

Vitamin E supplementation induced insignificant decrease in body weight, adipose tissue weight, spleen weight and kidney weight. While liver weight showed significant decrease.

Green tea induced significant decrease in body weight, liver weight and kidney weight, with insignificant decrease in adipose tissue weight and spleen weight.

Data showed in Table 2 found that, high fat supplemented diet induced significant increase in serum total cholesterol, LDLc. and triglyceride levels with significant decrease in HDLc. level.

Vitamin E supplemented diet induced significant increase in total cholesterol, LDLc. and triglyceride level with significant decrease in HDLc. level.

Finally green tea supplementation induced significant decrease in total cholesterol, LDLc. and triglyceride level with insignificant increase in HDLc.

Table 1: The changes in body weight, adipose tissue and organs weight (g) at the end of experimental period compared to normal control rats

Group	Body weight		Adipose tissue weight		Liver weight		Kidney weight		Spleen weight	
	Mean±SE	p-value and % ch.	Mean±SE	p-value and % ch.	Mean±SE	p-value and % ch.	Mean±SE	p-value and % ch.	Mean±SE	p-value and % ch.
Group I	263.01±0.01	-	3.35±0.24	-	9.88±0.23	-	0.92±0.04	-	0.80±0.03	-
Group II	279.42±20.65	+6.24%	6.35±0.81	+89.55%**	7.80±0.43	-21.50%***	0.85±0.05	-7.61%	0.85±0.81	+6.25%
Group III	257.32±18.00	-2.16%	4.99±0.30	+49.96%	9.05±0.49	-8.40%**	0.93±0.06	+1.09%	0.99±0.30	+23.75%
Group IV	250.78±9.21	-4.65%***	3.82±0.47	+14.03%	6.73±0.29	-31.88%***	0.80±0.03	-13.04%**	0.75±0.47	+6.25%

Values are expressed as Mean±SE. ** Highly significant p<0.01, *** Very highly significant p<0.001

Table 2: The changes in serum lipids and lipoproteins (mg dL⁻¹) at the end of experimental period compared to normal control rats

Group	Total cholesterol		LDL cholesterol		HDL cholesterol		Triglyceride	
	Mean±SE	% of changes compared to control and level	Mean±SE	% of changes compared to control and level	Mean±SE	% of changes compared to control and level	Mean±SE	% of changes compared to control and level
I	88.04±0.67	-	36.33±0.63	-	43.16±0.83	-	51.39±0.96	-
II	129.68±0.86	+47.30***	47.23±0.73	+30.00**	31.78±0.72	-26.37**	65.80±1.53	+28.04***
III	101.45±0.94	+15.23***	57.32±0.49	+57.78**	30.57±0.54	-29.17***	72.90±0.70	+41.86***
IV	65.58±1.08	-25.51***	18.43±0.36	-49.27***	44.19±0.84	+2.39	34.29±0.49	-33.28***

Values are expressed as Mean±SE, ** Highly significant p<0.01, *** Very highly significant p<0.001

Table 3: The changes in serum leptin levels (ng mL⁻¹) at the end of experimental period compared to normal control rats

Group	Serum leptin	% of changes in serum leptin level compared to control and level of significant
Group I (Normal control)	18.49±0.35	-
Group II (Normal rats supplemented with high fat diet)	23.96±0.45	+29.58***
Group III (Normal rats supplemented with vit. E)	13.05±0.40	-29.42***
Group IV (Normal rats supplemented with green tea)	12.03±0.32	-34.94***

Values are expressed as Mean±SE, *** Very highly significant p<0.001

Supplementation of rats with high fat diet significantly increased serum leptin level, while vit. E and green tea enriched diet significantly lowered serum leptin levels at the end of the experimental period (Table 3).

DISCUSSION

In this study, feeding rats high fat diet induced significant elevation in the body weight, adipose tissue weight. The weight of kidney and spleen showed insignificant changes, while the liver weight was decreased significantly.

These results are in agreement with Deborah *et al.* (2000) who stated that, the fat rich diet may contribute to early weight gain not only because it provides the substrate for triglyceride accumulation, but also it may increase food intake as a result of reduction in leptin secretion by adipose tissue.

Also, high fat diet lowers satiety, leading to over consumption and weight gain (Margetic *et al.*, 2002). On the other hand, Ahren (1999) was also found that in the mice challenged with a high fat diet, there is increase in the body weight which to a large degree is explained by increased adipose tissue weight.

In addition, Cha Chieh *et al.* (2000) found an increase in body weight gain and abdominal fat weight among rats fed diet enriched with 44% fat.

In the present study, the kidney and spleen weight showed insignificant changes, while the liver weight was significantly decrease. In contrast, Philip (1999) who found that the liver weight of high fat group was significantly higher than that in the control group. These discrepancies may be attributed to the differences in types of fat used, time courses or species and strains.

Feeding of rats on vit. E supplemented diet induced insignificant changes in body, adipose tissue and organ

weights. These results are in agreement with Toshiharu *et al.* (1996) who found that, vit. E contradicted induced elevation of serum cholesterol and this may be due to increased body weight.

This results may be attributed to the conversion of vit. E in the body. Also, vit. E as an antioxidant may reduce the changes of cellular environment and reduce the oxidative stress of tissue without changes in weights. No data are available about the effect of vit. E supplementation on body, organs weight.

Supplementation of diet with green tea powder to rats caused significant reduction in body weight with insignificant change in adipose tissue weight.

This decrease in body weight was supported by Kazutoshi *et al.* (2000) who reported that, green tea powder clearly suppressed the body weight and fatty accumulation in mice when added to the diet at different concentration. These suppressive effects were suggested to be due to the inhibitory effect of green tea on the lipid metabolism and the changes of lipoprotein lipase activity which affects the uptake of lipid into adipose tissue. Also, green tea powder suppressed fatty accumulation and adipose tissue weight.

These suppressive effects were suggested to be due to the anorectic effect of caffeine present in tea (Racotta *et al.*, 1994).

Present study showed insignificant changes in kidney and spleen weight while the liver weight showed significant decrease after green tea supplementation. These data are supported by the study of Kazutoshi *et al.* (2000).

In evaluating the effect of diet on obesity, lipid profile and leptin levels several factors have to be taken into consideration. Among them are animal species, as well as the amount of food supplemented and the duration of treatment.

The result of the present study showed that, high fat supplemented diet and vitamin E supplemented diet, induced significant increase in serum total cholesterol, LDLc. and triglyceride levels, while the HDLc. was significantly decreased.

The cholesterol elevating effect of high fat diet has been shown in several studies using different types of high fat diet, Wanda *et al.* (1997) suggested that 100 mg increase in dietary cholesterol increased plasma cholesterol by 10 mg dL⁻¹ and Yudkin *et al.* (2000) who use butter as a source high fat diet. The level of polyunsaturated fatty acids in the butter diet is probably an important contributing factor to the serum cholesterol response observed.

Moreover, the hypercholesterolemic effect of high fat diet is consistent with Toshiharu *et al.* (1996) who reported that high fat in normal adult male rats induced significant elevation of both serum total cholesterol and triglyceride levels.

Also, the increase in serum concentrations of lipids might, have inhibited glycolyses and reduced insulin action leading to insulin resistance (Seufert, 2004).

Regarding LDLc. and triglyceride levels, the results are consistent with Ryu and Cha (2003) who found that, the use of high fat diet in rats induced significant elevation of both LDLc. and triglyceride levels, this indicated by increase in hepatic total lipid in these rats, cholesterol metabolism and receives cholesterol from the diet and lipoproteins (Philip, 1999).

Also, Correia and Haynes (2004) reported that the use of butter diet in human induces significant elevation of serum LDLc. than all other diets.

The increase in serum LDLc. levels may be due to the saturated fat intake in butter that enhances the effect of cholesterol in suppressing receptor mediator hepatic uptake of LDLc. leading to the increase in LDLc. and LDLc. production rate (Singhal *et al.*, 2002).

Also, Toshiharu *et al.* (1996) reported that, high fat diet induced significant increase in serum triglyceride levels.

As regarding HDLc. level, Le Boeuf *et al.* (1990) reported that, use of high fat diet in human and mice induced reduction in the amount of HDLc.

In contrast Randall *et al.* (1993) studied the effect of high fat diet ingestion in human induced nonsignificant increase in HDLc.

There discrepancies in HDLc. response may be attributed to the differences in types of fat, time courses or species.

As well as, the effect of vit. E supplementation on lipid profile, Cuchel *et al.* (1996) reported that the use of tocopherol induces significant increase serum cholesterol,

LDLc. concentration. This effect may be due to the decrease of catabolic rates of cholesterol as a result increased cholesterol levels.

Moreover, Toshiharu *et al.* (1996) who reported that high fat diet rich source of tocopherols in adult male rats induced significant elevation of both serum cholesterol and triglyceride levels and low dose of vit. E for long time have a significant reduction in LDLc. oxidation. This increase most probably attributed to the dose of vit. E used in this study.

In this study, green tea supplementation produced significant reduction in total cholesterol, LDLc. and triglyceride levels, with elevation in HDLc. levels.

These findings are in agreement with Kazutoshi *et al.* (2000) who reported administration of green tea powder to mice induced reduction of both cholesterol and triglyceride levels. In addition, the lowering effect of green tea on cholesterol was supported by the study of Toriunagea *et al.* (2002) who examine the relation between green tea consumption and serum lipids and lipoproteins in humans. They found that consumption of more than 10 cups day⁻¹ was significantly associated with lower levels of cholesterol white its association with serum triglyceride and HDLc. were not statistically significant.

Several mechanisms are reported to explain the hypercholesterolemic activity of green tea powder. One of them is that the active component of green tea is the anti oxidative tea catechins which consists of catechin, epicatechin, epigallocatechin (EGC), epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG), these catechins reduce the solubility of cholesterol in mixed micells and also decrease lymphatic cholesterol adsorption from the intestine in rats (Ikeda *et al.*, 1992). Also epicatechins interfere with the absorption of cholesterol by inhibiting the acyl coA transferase (ACAT) activity, which plays role in the intestinal absorption of cholesterol by esterifying cholesterol before its absorption (Wrenn *et al.*, 1995). On the other hand, Chan *et al.* (1999) reported that dietary GTE had no influence on intestinal ACAT activity, but they increase the fecal output of cholesterol mainly as a result of its binding capacity and acceleration of cholesterol excretion. GTE has been shown to form insoluble coprecipitates with cholesterol and thus decrease cholesterol absorption.

Moreover, green tea catechins exert on inhibitory activity on acetyl coA carboxylase, the first key enzyme of fatty acid biosynthesis (Watanabe *et al.*, 1998). This indicates that lipid synthesis in the liver may be suppressed by the green tea catechins (Kazutoshi *et al.*, 2000).

In addition, green tea extract enhances hepatic metabolism of cholesterol (Suminori *et al.*, 1996). Also, Chan *et al.* (1999) reported that hypocholesterolemic activity of green tea may be due to its inhibition of cholesterol and bile acid absorption.

On the contrary to present results, Brown *et al.* (1993) found that no correlation between tea consumption and plasma cholesterol levels. This contradiction may be related to the difference in the duration of consumption on the dose.

Regarding to the decreased in serum LDLc. levels, the results was supported by the study of Imai and Nakachi (1995) who reported that, there is inverse relation between green tea drinking and LDLc. Levels.

This reduction in serum LDLc. may be due to the hypolipidemic activity of green epicatechins or to potent inhibitory effect of catechins on LDLc. oxidation *in vivo* (Miura *et al.*, 2000).

The significant decrease in triglyceride levels is consistent with Chan *et al.* (1999) who demonstrated that dietary GTE had triglyceride lowering activity in hamsters. It is related to the inhibitory effect on liver fatty acid synthesis or may be due to the fact that it may enhance the hydrolysis of triglyceride to free fatty acids for oxidation.

Moreover, Kazutoshi *et al.* (2000) reported that the catechins was inhibit the triglyceride accumulation in adipocytes by suppression of lipid synthesis.

As regards HDLc., the insignificant changes in its levels is consistent with Imai and Nakachi (1995) and with Toriunega *et al.* (2002) who found that HDLc. of healthy workers remained unchanged by green tea consumption in one year trial. These results may be explained on the bazas of the different doses and duration used in these trial.

Leptin has been proposed to act as a feed back signal to regulate food intake and energy expenditure. However, the role of leptin in the development of obesity in response to high fat diet remains to be elucidated.

The present study demonstrates that, the administration of high fat diet to normal rats induced significant elevation of serum leptin. This increase is in agreement with several studies which indicated that high fat diet increase the circulating leptin in rats (Stephen *et al.*, 2003) and men (Kratz *et al.*, 2002).

Similarly, in women consuming a diet with 50% energy as fat for 4 weeks, leptin levels were significantly increased (Cnop *et al.*, 2003). Increased body adiposity may account for there elevated leptin levels.

Increased leptin level have been demonstrated in rats fed a diet with 56% energy as fat for 2 days (Linx *et al.*, 1998).

Another mechanism which may contribute to the hyperleptinaemia may be the increase in the circulating insulin by high fat diet (Ahren, 1999). The increased insulin might stimulate leptin expression and secretion (Sainsbury *et al.*, 1996).

In contradiction to our results, Deborah *et al.* (2000) reported that taking high fat diet for 4 weeks is associated with decreased level of leptin due to reduced leptin synthesis. The decreased leptin synthesis may be due to increased lipolysis after high fat diet (Shin and Park, 2007).

The effect of dietary fat on leptin may be dependent on the donation of the dietary treatment: studies have shown that, consumption of high fat diet for 12 days didn't effect circulating leptin concentrations (Chavez *et al.*, 1998), where as consumption of high fat diet by animals for 5 months diet elevate circulating leptin (Stricker *et al.*, 1998), which may have been due to the development of leptin insensitivity. Also feeding rats 60% fat diet induced hyperleptinemia. which began within 24 h and increased progressively after 10 weeks (Lee *et al.*, 2001).

Also, the type of dietary fat consumed may effect the leptin level because different sources of fat can have varying effect on lipolysis and adipose tissue glucose uptake (Fantuzzi and Mazzone, 2007).

Kratz *et al.* (2002) reported that olive oil and the sunflower oil did not effect serum leptin level, while the use of margarine in diet increasing the serum leptin level in rats (Bahceci *et al.*, 1999).

Present results showed that vit. E supplemented diet succeeded in reduction of serum leptin level. This results is in agreement with Ozdil *et al.* (2004) who reported that vit. E as an antioxidant may remove the active free radical and prevent apoptosis (Qin *et al.*, 2001).

As regarding to the effect of green tea powder supplementation on serum leptin level, present results showed that, serum leptin level was decreased. This finding may be attributed to the decrease in leptin production (Considine *et al.*, 1996).

Frederich *et al.* (1995) reported that, in rodents leptin levels reflect the size of adipose stores. This is in agreement with my results, because the decrease in serum leptin level is associated with insignificant changes in adipose tissue weights after the treatment with green tea. Present findings are in agreement with Yung-His *et al.* (2000) who demonstrated that EGCG (which is one of the four major green tea catechin) treatment to rats produce significant decrease in circulating leptin due to diminished fat stores resulting from low food intake or EGCG may interact with leptin receptors and reduced food intake.

In conclusion, from our finding we advice diets of subjects with predisposing causes for atherosclerosis as

those with positive family history, should be supplemented with vit. E with caution to avoid the decrease in HDLc. level which is the predisposing factor for atherosclerosis. Also, green tea is advised to be taken daily (one cup at least) for long period in order to cause reduction of body weight in obese subjects and to treat lipid and lipoprotein disturbances.

At the same time, we advice the normal subjects to use green tea with caution as a prophylactic measurement against obesity and cardiovascular disease to avoid hypoliptinemic effect of green tea supplementation which may be predispose to obesity.

Finally, we needs further investigation to detect other types of diet which may help in the protection and treatment of obesity which still the major problem allover the world and the main cause of death in young and elderly people.

And to study the role of leptin treatment in weight reduction and what are the possible side effects in humans.

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