ARAŞTIRMA YAZISI / ORIGINAL ARTICLE

KAFKAS TIP BİLİMLERİ DERGİSİ
Journal of Medical Sciences

Kafkas J Med Sci 2013; 3(1):1–7 • doi: 10.5505/kjms.2013.36855

Effects of Green Tea Extract and Lactobacillus Casei Strain Shirota on Levels of Serum Minerals, Cholesterol, Triglycerides, Glucose and Lactate in Rats Fed on High-carbohydrate and High-lipid Diets

Yeşil Çay Ekstraktı ve Lactobacillus Casei Strain Shirota'nın Yüksek Karbonhidrat ve Lipit İçerikli Yeşil Diyetle Beslenen Ratlarda Serum Mineral, Kolesterol, Trigliserid, Glikoz ve Laktat Seviyeleri Üzerine Etkileri

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ABSTRACT

AIM: *The objective of the present study was to evaluate the effects of green tea extract and Lactobacillus casei strain Shirota on levels of serum minerals (Ca, Cu, Zn, Fe, Mg and Mn), cholesterol, triglyceride, glucose and lactate in rats on high carbohydrate and lipid diets.*

METHODS: *Thirty five healthy Wistar albino rats were used, five in each of seven experimental groups: control (group A), high-carbohydrate diet (group B), high-carbohydrate diet supplemented with probiotic bacteria for 8 weeks - Lactobacillus casei strain Shirota (group C), high-carbohydrate diet supplemented with green tea extract for 8 weeks - in drinking water: 100 mg/kg/day (group D), high-lipid diet for 8 weeks (group E), high-lipid diet supplemented with probiotic bacteria for 8 weeks (group F), high-lipid diet supplemented with green tea extract for 8 weeks- in drinking water: 100 mg/kg/day (group G).*

RESULTS: *A significant increase ($p<0.05$) in serum cholesterol was observed in groups treated with high-lipid, and high-lipid + green tea extracts (E and G) compared with other groups ($P<0.05$). Serum triglyceride levels were significantly lower in the high-carbohydrate + probiotic group (C) than in other groups ($p<0.05$). High-calorie diet, green tea extract and probiotic bacteria had no influence on serum lactate levels in any of the groups. Serum Ca levels decreased significantly only in the high-carbohydrate diet group ($p<0.05$). However, serum Zn and Fe concentrations increased significantly in the high-lipid and high-lipid plus probiotic bacteria groups, respectively ($p<0.05$ and 0.01). Serum Mg levels were significantly lower in all experimental groups compared to the control group ($P<0.01$). L. casei strain Shirota and the green tea extract, significantly lowered serum glucose levels in the high-carbohydrate groups compared to high-lipid groups ($p<0.05$).*

CONCLUSION: *The green tea extract and L. casei strain Shirota decreases serum glucose and triglyceride levels in rats fed on high-calorie diets.*

Key words: *high-calorie diet; green tea; probiotic bacteria; serum mineral levels*

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Geliş Tarihi: 24.12.2012 • Kabul Tarihi: 19.03.2013

ÖZET

AMAÇ: *Bu çalışmanın amacı, yeşil çay ekstraktı ve Lactobacillus casei strain Shirota'nın yüksek karbonhidrat ve lipit içerikli diyetle beslenen ratlarda serum mineralleri (Ca, Cu, Zn, Fe, Mg and Mn), kolesterol, trigliserid, glikoz ve laktat düzeyleri üzerine etkilerinin araştırılmasıdır.*

YÖNTEM: *Otuzbeş sağlıklı Wistar albino rat her grupta 5 hayvan olacak şekilde 7 gruba ayrıldı: Kontrol grubu (A), yüksek-karbonhidratlı diyet grubu (B), yüksek karbonhidratlı diyet ve 8 hafta süreyle probiyotik bakteri ilaveli grup- Lactobacillus casei strain Shirota (C), yüksek karbonhidratlı diyet ve 8 hafta süreyle yeşil çay ekstraktı verilen grup (D), yüksek lipit içerikli diyet grubu (E), yüksek lipit içerikli diyet ve 8 hafta süreyle probiyotik bakteri ilaveli grup- Lactobacillus casei strain Shirota (F) ve yüksek lipit içerikli diyet ve 8 hafta süreyle yeşil çay ekstraktı ilaveli grup (G).*

BULGULAR: *Serum kolesterol seviyesinin yüksek lipit ve yüksek lipit + yeşil çay ekstraktı ilaveli gruplarda (grup E ve G) kontrole ve diğer deneme gruplarına göre anlamlı olarak artığı tespit edildi ($p<0.05$). Serum trigliserid seviyesi yüksek karbonhidrat + probiyotik ilaveli grupta (grup C) diğer deneme gruplarından anlamlı olarak düşüktü ($p<0.05$). Yüksek kalorili diyet, yeşil çay ekstraktı ve probiyotik ilavesinin gruplarda serum laktat seviyesi üzerine herhangi bir etkisinin olmadığı görüldü. Serum Ca düzeyinin yanlızca yüksek karbonhidrat içerikli diyet grubunda anlamlı olarak azaldığı tespit edildi ($p<0.05$). Bununla birlikte, serum Zn ve Fe konsantrasyonlarının yüksek lipit ve yüksek lipit + probiyotik ilaveli gruplarda sırasıyla $p<0.05$ ve $P<0.01$ düzeylerinde anlamlı olarak artığı tespit edildi. Serum Mg seviyesinin tüm deneme gruplarında kontrole göre istatistiksel olarak anlamlı derecede azaldığı tespit edildi ($P<0.01$). L. casei strain Shirota ve yeşil çay ekstraktı alınması yüksek karbonhidrat diyetli gruplarda serum glikoz seviyesini yüksek lipid diyetli gruba göre anlamlı olarak azalttığı belirlendi ($p<0.05$).*

SONUÇ: *Yeşil çay ekstraktı ve L. casei strain Shirota kullanımının yüksek enerjili diyetle beslenen ratlarda kan glukoz ve trigliserid seviyelerini düşürücü etkisi vardır.*

Anahtar kelimeler: *yüksek kalorili diyet; yeşil çay; probiyotik bakteri; serum mineral seviyesi*

Introduction

Obesity has been linked to a wide variety of health problems including hypertension, dyslipidemia, cardiovascular diseases, hyperglycemia, diabetes mellitus, inflammation disorders and cancer, based on accumulation of visceral fat[1, 2]. Previous studies have shown that high-fat diets trigger obesity under ad libitum feeding conditions[3]. A high-fat diet increased the sensitivity of insulin to stimulate adipocyte glucose uptake in a mouse strain that was genetically sensitive to developing dietary obesity[4].

Tea *(Camellia sinensis)* is consumed worldwide and it is generally accepted that the tea is the most consumed beverage in the world[5]. Previous studies conducted in humans and animals have reported that the green tea and its components (catechins, flavonols, etc.) have many biological and biochemical advantageous effects of anti-obesity[6], anti-diabetic[7], anti-carcinogenesis[8], hypolipidemic[5], anti-oxidative[9], apoptosis-inducing[10], and anti-angiogenesis[11]. In addition, the consumption of green tea decreases serum levels of triacylglycerols and LDL cholesterol[12], and increases the serum levels of HDL cholesterol[13 ,14].

Probiotic colonies are the natural flora of their host's gastrointestinal track and they play critical roles in promoting health in humans and animals by blocking gastrointestinal pathogens, neutralizing food mutagens produced in colon, enhancing systemic immune response and dissolving intestinal dysfunction[15].

Previous studies showed that oral administration of *Lactobacillus casei* decreased plasma glucose levels in mice [16] and *Bifidobacterium longum* reduced serum cholesterol levels in rats[15]. Similar studies performed on humans showed the similar effect on blood lipids[17].

Although the effects of consumption of green tea and some probiotics on serum mineral levels have been defined, their effects during high caloric diet intake were not thoroughly studied. In this present study we aimed to study the influence of high-fat and high-carbohydrate diets with or without the supplementation of probiotic bacteria and green tea extract on serum Ca, Cu, Zn, Fe, Mg and Mn levels in rats. The second objective of the study was to analyze the effects of consumption of probiotic bacteria and green tea extract on serum cholesterol, triglycerides, glucose and lactate levels during high calorie diet.

Methods

The study was performed in Yüzüncü Yıl University Faculty of Veterinary Medicine. All procedures were conducted according to the Yuzuncu Yil University Ethics Committee Statements on laboratory animals *(Ethical approval decision number 2011-05).*

Animals and diets

Thirty five healthy, male Wistar albino rats, weighting 200–260 g and averaging 8 weeks old were used in this study. Animals were housed three in a polycarbonate cage in temperature controlled rooms (21±2°C) with a 12-hour light/dark cycle, fed with standard rat pellets (Vangölü animal food product co., Van, Turkey), and given water *ad libitum* for an adaptation period of 10 days.

The rats were randomly allocated into one of the seven experimental groups (Table 1): group A (control), group B (high-carbohydrate diet), group C (high-carbohydrate diet supplemented with probiotic bacteria for 8 weeks - *Lactobacillus casei strain Shirota*), group D (high-carbohydrate diet supplemented with green tea extract for 8 weeks - in drinking water: 100 mg/kg/day), group E (high-lipid diet for 8 weeks), group F (high-lipid diet supplemented with probiotic bacteria for 8 weeks), group G (high-lipid diet supplemented with green tea extract for 8 weeks- in drinking water: 100 mg/kg/day). Thus, each group contained five animals.

The control group was fed with the standard pelleted diet (362.0 kcal/100 g) containing a dry weight composition of 18% protein, 76% carbohydrate and 6% lipid. The experimental groups were fed with either a carbohydrate-rich hypercaloric diet or a lipid-rich hypercaloric diet both composed of casein, starch, sucrose, straw, L-methionin, corn oil, animal oil (tallow) and vitamin-mineral mix. The carbohydrate-rich

Table 1. *The study groups and consumed diets.*

Group A	n=5	Control (standard rat pellets feed)
Group B	n=5	High-carbohydrate diet
Group C	n=5	High-carbohydrate diet + Lactobacillus casei strain Shirota
Group D	n=5	High-carbohydrate diet + Green tea extract
Group E	n=5	High-lipid diet
Group F	n=5	High-lipid diet + Lactobacillus casei strain Shirota
Group G	n=5	High-lipid diet + Green tea extract

hypercaloric diet contained a total of 4100 kcal/100 g, with a dry weight composition of 56% carbohydrate. The lipid-rich hypercaloric diet contained a total of 4200 kcal/100 g with a dry weight composition of 50% lipid (Table 2).

At the end of the experiments all animals were sacrificed by decapitation.

Biochemical measurements

Blood samples were obtained at the beginning and the end of the experimental period. The blood samples were carefully collected and stored at -80°C prior to analysis. The levels of Ca, Cu, Zn, Fe, Mg and Mn were measured by using an UV-Spectrophotometer (Shimadzu UV-1201, Japan). The samples were diluted in water before measuring the levels of copper and manganese. All samples were analyzed in triplicate.

Cholesterol (Biolabo, 80106), glucose (Biolabo, 80009) and triglyceride (Ben SRL- TG381, Italy) levels were determined with commercial kits adapted to a Shimadzu UV-1201, UV-Vis Spectrophotometer (Japan). Serum lactate levels were also studied by using commercial kits (Spinreact)[18].

Statistical analysis

The data were expressed as mean ± standard deviation (SD), and analyzed by repeated measures of variance. A Tukey test was used to test the differences among means when an analysis of variance (ANOVA) indicated a statistical significance ($p<0.05$ *or 0.01*).

Table 2. *The composition of the experimental diets*

	% of total diet	
Ingredients	High-carbohydrate diet	High-lipid diet
Casein	11	11
Starch	15	6
Sucrose	56	50
Straw	2.2	8.2
L-Methionin	0.1	0.1
Corn oil	5.5	10
Animal oil (Tallow)	5.5	10
Vitamin-Mineral Mix	4.7	4.7
Crude protein	10.45	10.48
Metabolic Energy (kcal)	4100/100g	4200/100g

Results

Blood mineral levels

The mineral, cholesterol, triglycerides, glucose and lactate levels of the rats were presented in Tables 3 and 4. Serum Ca levels were significantly decreased after 8 weeks in the carbohydrate-rich diet group (B) ($p<0.05$), however, there were no significant changes within either the control group or the other treatment groups.

Serum concentrations of Cu decreased in groups B and C compared to the other experimental groups (D-G) ($p<0.01$). Serum Zn levels were significantly increased after the lipid-rich diet (E) and the lipid-rich diet plus green tea extract (G) compared with other groups ($p<0.01$). Serum Fe levels were significantly decreased in the carbohydrate-rich diet plus green tea group (D) and lipid-rich plus green tea group (G) ($p<0.01$). In addition, the serum concentration of Mg significantly decreased in the lipid-rich plus green tea extract group (G) compared with other treatment groups (B-F) ($p<0.01$). Serum Mn levels were higher in lipid-rich plus probiotic bacteria group (F), and lipid-rich plus green tea groups (G) compared to other groups ($p<0.01$, Table 3).

Cholesterol and triglyceride levels

Serum cholesterol levels increased in the high-lipid group (E) rats ($p<0.05$), however, there were no changes in other experimental groups. Serum triglyceride levels were significantly increased in the experimental groups compared to control group ($p<0.05$). However, in group D serum triglyceride levels were significantly decreased compared with the other high-calorie and treatment groups ($p<0.01$) (Table 4).

Blood glucose and lactate levels

Serum glucose levels increased in the experimental groups compared to the control group. Serum glucose levels were lower in the carbohydrate plus probiotic (group C), and carbohydrate plus green tea (group D) groups than in the other experimental groups ($p<0.01$). No changes were observed in serum lactate levels in either the high-carbohydrate or the high-lipid diet groups with either the supplementation of green tea or probiotic bacteria in comparison with the control group ($p>0.05$, Table 4).

Table 3. *The effects of high energy diets and the supplementation of the probiotic bacteria and the green tea extracts on some minerals. The data was presented as mean ± standard deviation.*

Group name	Group code	Ca	Cu	Zn	Fe	Mg	Mn
Control	A	90.37±6.0	2.13±0.8[b]	1.59±0.1[e]	3.34±1.0	30.01±8.2[g]	0.09±0.02[i]
Carbohydrate	B	78.52±5.3[a]	1.32±0.01[c]	1.13±0.06	3.46±0.9	20.16±7.4	0.09±0.01[i]
Carbohydrate + Probiotic	C	88.75±10.2	1.42±0.03[c]	1.24±0.02	3.15±1.1	20.28±6.3	0.11±0.01
Carbohydrate + Green tea	D	86.92±12.4	1.65±0.05	1.10±0.04	2.78±0.9[f]	20.02±6.8	0.10±0.02
Lipid	E	89.02±9.8	1.74±0.02	2.09±0.7[d]	3.49±1.0	20.21±5.4	0.11±0.03
Lipid+Probitic	F	93.92±11.4	1.71±0.1	1.85±0.03	4.05±0.9*	21.12±6.8	0.18±0.04[h]
Lipid+green tea	G	88.71±6.3	1.55±0.01	2.41±0.9[d]	2.26±0.8[f]	10.71±3.1[¶]	0.18±0.01[h]

[a]: $P<0.05$ compared to control and other experimental groups; [b]: $P<0.01$ compared to experimental groups; [c]: $P<0.05$ compared to other groups; [d]: $P<0.01$ compared to other groups; [e]: $P<0.01$ compared to B-D groups. [f]: $P<0.01$ compared to control and other experimental groups; [g]: $P< 0.01$ compared to experimental groups; [h],*,[¶]: $P< 0.01$ compared to control and other experimental groups; [i]: $P<0.05$ compared to groups C-G.

Table 4. *The effects of high energy diets and the supplementation of the probiotic bacteria and the green tea extracts on serum glucose and lipid levels. The data was presented as mean± standard deviation.*

Group name	Group code	Cholesterol	Triglycerides	Glucose	Lactate
Control	A	108.7±8	40.1±12[c]	88.5±10[d]	10.13±3
Carbohydrate	B	114.6±15	129.6±23	166.5±33	11.03±2
Carbohydrate + Probiotic	C	111.2±20	127.2±16	114.8±11.5[e]	14.56±5
Carbohydrate + green tea	D	118.3±14	51.2±9[b]	111.6±8[e]	12.55±2
Lipid	E	137.7±10[a]	146.1±19	155.9±20	14.08±4
Lipid + probitic	F	123.4±13	130.7±21	133.6±25	11.57±3
Lipid + Green tea	G	128.1±9[a]	135.8±24	135.6±27	8.89±2

[a]: $P<0.05$ compared to group F; [b]: $P<0.05$ compared to experimental groups; [c,d]: $P<0.01$ compared to experimental groups; [e]: $P<0.05$ compared to other experimental groups.

Discussion

Obesity is an important health problem, and its prevalence is increasing worldwide. Obese individuals have been shown to have a higher incidence of infections and infection related mortality, and several types of cancer occur more frequently in obese populations[2]. In a previous study it was suggested that high fat diet-induced overweight rats present an altered immune response[19]. Milagro et al. showed that a high fat diet might develop obesity, hyperglycemia, and fatty liver and oxidative stress[20].

In our study, we observed that a high-calorie diet had also an impact on serum mineral levels. Group B (high-carbohydrate diet) displayed decreased serum Ca levels compared to the control and other high-calorie diet groups ($p<0.05$). However, supplementation of probiotic bacteria significantly increased serum Fe levels in the high-lipid diet group (F) ($p<0.01$). In addition, supplementation of green tea extract significantly decreased serum Fe levels in the high-carbohydrate group (D) and high-lipid diet group (G) ($p<0.01$).

High-lipid diet groups supplemented with green tea in their drinking water had an approximately 1.5-fold decreased levels of serum Mg after 8 weeks compared with rats fed the basal diet. Our data also showed that feeding the rats with a high-carbohydrate diet decreased serum Ca, Cu, Zn and Mg levels.

In our study the serum Cu levels were significantly lower in all of the high-calorie and supplementation diet groups compared with the control group. However, serum Zn levels decreased in carbohydrate-rich diet groups with or without probiotic bacteria and green tea supplementation. The oxidant effect of a high-calorie diet has been associated with the impairment of obesity related issues such as suppressor effect on immunity. Lamas et al. suggested that weight loss after caloric restriction may restore immune function in rats[2].

Copper and manganese are required in small amounts as components of antioxidant enzymes. The primary role for copper is catalytic, as it is found in many copper metalloenzymes, e.g., ferroxidase, CuZn–superoxide dismutase (CuZn–SOD), cytochrome C oxidase, dopamine hydroxylase, and diamine oxidase[21]. Ferroxidases are copper-containing enzymes found in plasma that oxidize ferrous iron, i.e., $Fe^{2+} \rightarrow Fe^{3+}$. Therefore, determining their plasma level may contribute to better assessing the health and nutritional status of certain populations[22]. Under conditions of oxidative stress, plasma copper increases predominantly in the form of ferroxidase I and assists with the removal of any unbound iron in the plasma[21,23].

Previous studies have suggested that, under acute hyperglycemic state, plasma zinc status alters the interrelationship between plasma glucose and zinc concentrations during oral glucose tolerance test (OGTT) in obese individuals[24]. However, the same study showed that plasma zinc levels were not changed during OGTT in obese individuals[24]. Sambol et al. showed that Fe plasma levels were lower in non-vegetarians than in vegans and lacto-ovovegetarians[25]. In the present study, serum iron levels were statistically decreased in carbohydrate rich diet plus green tea, and lipid rich diet plus green tea compared to carbohydrate and lipid rich diet groups (Table 3). In addition the lipid-induced diet caused a marked increase in serum Zn levels that was speculated to be responsible for inflammatory changes.

Serum zinc is the most commonly used indicator and it rapidly decreases in the presence of inflammation. Serum insulin-like growth factor (IGF) concentrations are low in zinc-deficient rats[26]. Similarly, zinc supplementation can raise IGF concentrations in children considered undernourished or moderately zinc deficient[27].

The main indices of iron status are blood hemoglobin and concentrations of serum ferritin and transferrin receptor. Total serum iron is also used as a measure of iron status, however as in the case of zinc, serum iron is strongly depressed by inflammation[21,28]. The antihypertensive effects of magnesium supplements in hypertensive individuals are controversial[29].

Karmakar et al. suggested that the high-fat diet induced increases in serum glucose, cholesterol, triglyceride, LDL and VLDL levels, and a decrease in serum HDL levels[5]. However, all these increases were found to be reversed significantly with black tea extract supplementation. The previous studies reported that the hypoglycemic effect of tea was attributed to the presence of polyphenols, catechins and a water-soluble polysaccharide fraction[6, 30, 31]. Similar studies reported that black and green tea extract have been found to decrease plasma triglycerol, cholesterol and LDL-cholesterol in rats, and green tea epigallocatechin gallate inhibited obesity[32].

Table 4 summarizes the concentrations of cholesterol, triglycerides, glucose and lactate in all study groups. In our study, the levels of glucose, triglycerides, lactate and cholesterol were measured after 8 weeks. Blood glucose levels of the carbohydrate plus probiotic (C) and carbohydrate plus green tea diet groups (D) were lower than of the other experimental groups. These results suggest that the dietary supplementation of *L. casei strain Shirota* and green tea extract may prevent or delay the development of hyperglycemia in obese rats induced by a high-carbohydrate diet. The serum glucose values were significantly higher in the B, E, F and G groups ($p<0.05$) in comparison with these two groups.

The serum cholesterol levels were higher in the high-lipid diet group (E) compared with the other experimental groups ($p<0.01$). The serum triglyceride levels of group D were lower than the serum triglyceride levels of all other experimental groups ($p<0.05$).

Diets containing high amounts of carbohydrate, such as fructose, have been shown to cause weight gain and increase plasma triglyceride concentrations[33, 34]. In a previous study it was shown that the rats fed on high-carbohydrate diets containing either a fructose/glucose mixture or honey had a hypertriglyceridemia reaction and an increased lipid peroxidation[35].

Previous studies in humans and animals have shown that probiotics or the products of its fermentation may have beneficial hypocholesterolemic and hypoglycemic effects on lipid metabolism of their hosts'[36,37]. Rodas et al. reported that L. acidophilus ATCC 43121 supplementation was beneficial in lowering serum cholesterol and LDL cholesterol concentrations in pigs. Our study indicated that the rats fed on a high-carbohydrate diet and supplemented with *L. casei strain Shirota* had a decrease in their serum glucose levels. However, the rats fed on a high-lipid diet did not show changes in serum cholesterol or triglyceride levels[38]. Wang et al. suggested that L. plantarum MA2 decreased serum levels of total cholesterol, LDL cholesterol and triglyceride levels in rats fed on high-cholesterol diets[39].

In conclusion, according to our experimental study a high-carbohydrate diet significantly decreased the serum Ca levels in rats. A high lipid and a high lipid with probiotics diet increased the Zn and Fe concentrations. The consumption of the green tea extract and L. *casei strain Shirota* lower the serum glucose and triglyceride levels in rats on high-calorie diets.

Acknowledgement

The study was supported by a grant from the Head of the Scientific Research Projects, University of Yuzuncu Yil, with a Grant Number VF-2009-B020.

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