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LEPTIN AND SOME RELATED METABOLITES IN PLASMA OF MALE BROILER CHICKENS TREATED WITH MONOUNSATURATED FATTY ACIDS (PALESTINE OLIVE OIL) AND/OR SATURATED FATS

(With 4 Tables)

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(Received at 29/12/2005)

اللبتين وبعض المواد الأيضية المرتبطة به فى بلازما ذكور الدجاج اللاحم المعامل بالأحماض الدهنية غير المشبعة (زيت الزيتون الفلسطينى) و/أو الدهون المشبعة

بدرية راشد الصويغ

كانت الدراسة الحالية لمعرفة تأثير زيت الزيتون والدهون المشبعة على مستوى اللبتين والبروتينات الدهنية. لذا فقد تم استخدام عدد ٦٠ من ذكور الدجاج ولقد قسمت إلى أربع مجاميع متساوية. المجموعة الأولى عوملت كمجموعة ضابطة. المجموعة الثانية أعطيت زيت الزيتون الفلسطينى المحتوى على أحماض دهنية أحادية غير مشبعة. بينما المجموعة الثالثة أعطيت دهون مشبعة. أما المجموعة الرابعة فقد أعطيت خليط من الدهون المشبعة وأحادية غير المشبعة بنسبة ١:١. ولقد تم أعطاء الحيوانات الدهون عن طريق الفم لمدة عشرة أيام متتالية بعدها تم ذبح ١٠ حيوانات من كل مجموعة مع الابقاء على خمس طيور لكل مجموعة (مجموعة استشفاء) لمدة سبعة أيام. تم جمع عينات الدم لكل مجموعة ، ولقد تبين من النتائج أن المجموعة المعالجة بزيت الزيتون أظهرت انخفاضاً معنوياً لمستوى هرمون اللبتين بينما أرتفع تركيز HDL ارتفاعاً معنوياً مقارنة بالمجموعة المعالجة بالدهون المشبعة ، بينما لم يظهر الكوليسترول وكذلك البروتينات الدهنية الآخرى أى تغيرات معنوية. أما المجموعة المعالجة بالدهون الحيوانية المشبعة فقد أظهرت أرتفاعاً معنوياً فى مستوى اللبتين وكذلك انخفاضاً معنوياً فى مستوى HDL ، بينما لم يظهر الكوليسترول وكذلك البروتينات الدهنية الآخرى أى تغيرات معنوية تذكر. أما خليط الدهون المشبعة وأحادية التشبع فقد أحدثت تعديلاً فى مستوى اللبتين مقارنة بالمجموعة الضابطة والمعالجة بزيت الزيتون ، بينما لم يغير من مستوى الكوليسترول وثلاثى الجليسريدات ، LDL ، VLDL. ولقد خلُصت الدراسة الحالية إلى أن الدهون أحادية غير المشبعة سواء كانت منفردة أو ممزوجة مع الدهون المشبعة أحدثت أرتفاعاً فى مستوى HDL ولم يكن مرتبطاً بالتغيرات فى مستوى هرمون اللبتين بالبلازما.

SUMMARY

The present study was done to investigate the effect of olive oil and saturated fats on plasma leptin and lipoproteins. The total number was sixty male broiler chickens, were used in this study. The birds were divided into four equal groups. The first group served as control, the second group was supplemented with Palestine olive oil "monounsaturated fatty acids, MUFA", while the third one was supplemented with saturated fat. The fourth group was supplied with a mixture of MUFA "olive oil" and saturated animal fat (1:1 ratio). The supplements were given orally in ration, daily for ten successive days, number of each group (10 birds) were then slaughtered and the remained number (5 birds) were allowed to recover " Recovery group" for seven days. Blood samples were collected from all groups. MUFA-treated group (2^{nd} group) showed a significant decrease in plasma leptin levels than control group, while HDL concentrations were increased significantly as compared to saturated fat-treated group. No significant changes were recorded in total cholesterol and other lipoproteins concentration. Saturated animal fat-treated group (3^{rd} group) revealed highly significant increase in plasma leptin level and highly significant decrease in HDL as compared with other treated groups, while there was no significant changes in cholesterol and other lipoproteins. MUFA plus saturated fat-supplemented group (4^{th} group) caused a moderate increase in plasma leptin level as compared to control and MUFA-treated groups. There were no significant alterations in cholesterol, triglycerides, LDL and VLDL concentrations. The present study concluded that MUFA alone or in combination with saturated animal fat resulted in elevation of plasma HDL. This response seemed to be independent to leptin plasma levels in the treated chickens.

Key words: *Leptin, Saturated fat, Olive oil, Broiler, Lipogram.*

INTRODUCTION

Leptin hormone produced mainly by adipocytes. It plays an important role as a signal of the body fat content to the brain, where it regulates food intake and energy expenditure (Friedman and Halaas, 1998 and Kratz *et al.,* 2002). Weight loss results in decreased leptin levels, while the weight gain significantly increases circulating leptin concentration. Leptin is not only important in the regulation of food intake and energy balance, but it appears increasingly as a general

metabolic hormone involved in many physiological processes including stimulation of lipolysis in adipocytes and increasing fatty acids synthesis in liver (Cohen *et al.*, 1998).

Korotkova *et al.* (2001) reported that dietary lipid quantity and quality have recently been shown to affect serum leptin levels in adult rats. Although Kratz *et al.* (2002) concluded that both olive oil and sunflower oils did not affect serum leptin concentration in humans, yet a diet with high olive oil reduces total body weight, lean mass, fat mass and increased daily urinary corticosterone (Mai *et al.*, 2003).

However, Sorigure *et al.* (2003) investigated the role of monounsaturated n-9 fatty acids in the lipolytic activity of adipocytes, for this purpose. Male rats were fed variety of dietary fat plamitic acid and olive oil exhibited an increase in tissue monounsaturated fatty acids with an increased lipolytic activity.

Leptin has been detected in several species, like chicken (Taouis *et al.*, 1998 and Ashwell *et al.*, 1999).

The aim of the present study was to investigate the effect of the dietary fatty acids, both saturated and unsaturated "MUFA" on plasma leptin concentration and its relation with lipoproteins in broiler chickens.

MATERIALS and METHODS

Sixty healthy male broiler chickens, weighing 800-1000 g, aged 25 days were housed in laboratory cages in a temperature ranged from 25-30 °C, and controlled light system (12 hr light: 12 hr dark cycle).

Chickens were given a regular special diet for broiler (200-300 g/daily) which consist of protein 17%, fat 2.5%, fiber 2.7% with calcium 1.2%, phosphorus 0.5% (total energy 3000 Kcal/kg b. wt.) with free access to water ad libitum. The chickens were acclimated to their environment for five days before the initiation of each experiment. Chickens were randomly divided into four groups.

Group (1) served as control group, while groups (2), (3) and (4) are experimental groups and treated with different types of semifluid animal fat or oils as shown in Table (1). The olive oil or the semifluid fat was given orally using 1 ml syringe/daily at morning for ten successive days with the basal daily diet.

Ten days later, (10 birds) of both control and treated groups were killed after fasting for eight lasted hour. Remaining chickens of each group were kept as recovery groups (without any treatment) for other one week; then they were killed also.

Table 1:

Groups	Total (n)	Dose ml/kg b. wt (oil or fat)	Control and treated groups	Recovery groups
(1) Control	N=15	----	N=10	N=5
(2) treated with Palestine olive oil (MUFA)*	N=15	0.25	N=10	N=5
(3) treated with saturated animal fat	N=15	0.25	N=10	N=5
(4) treated with Palestine olive oil+ saturated animal fat mixture (1:1)	N=15	0.25+0.25	N=10	N=5
	60		n=40	n=20

MUFA*=monounsaturated fatty acids (olive oil).
N=number of broiler chickens.

Blood samples were collected and plasma was separated immediately using heparinized tubes and then stored at -20 °C until subsequent analysis.

Determination of plasma leptin concentrations were measured using radioimmunoassay kit (multispecies leptin RIA kit, Linco rich in monounsaturated n-9 fatty acids), Research Institute, Charles, MO, USA; after, Kratz *et al.* (2002). Inter and intra assay coefficient of variation were 3% (n=10) and 4% (n=10), respectively.

Plasma cholesterol, HDL, triglycerides concentrations were analyzed by colorimetric methods (Biosystem S. A. Costa Brava, 30 Barcelona (Spain) on a M501 single beam scanning UV/visible spectrophotometer. LDL-cholesterol was calculated as the difference between the cholesterol and that in HDL. VLDL was estimated as one-fifth of the concentration of triglycerides (Friedwald *et al.*, 1972).

Statistical analysis:

All data were subjected to ANOVA to detect inter group differences; comparisons between groups were performed by LSD analysis. All results were expressed as mean±S.E. and the statistical significant difference was considered at $P< 0.05$. Correlation coefficient analysis was adopted among all resultant tested parameters values. It is worth mentioning that all statistical procedures were carried out using SPSS program.

RESULTS

Tables (2 & 3) represent concentrations of leptin and other lipoproteins in treated and recovered broiler chickens groups receiving basal diet in addition to oil or saturated animal fat supplements.

Data presented in Table (2) revealed that plasma leptin concentration was (2.18 ±0.04 ng/ml) in control chickens of the treated groups that was significantly and drastically dropped into (1.35±0.02 ng/ml) in olive oil "MUFA"-treated group at $P< 0.05$.

However, leptin concentrations showed significant elevated values in chickens-treated with saturated animal fat (3.08±0.01 ng/ml) and those treated with a mixture of "MUFA" plus saturated animal fat (2.44±0.02 ng/ml), respectively, as compared respective control value (2.18±0.04 ng/ml) at $P< 0.05$.

Male broiler chickens that treated with saturated fat and recovered saturated fat-treated groups, showed significant increase in plasma leptin concentrations (3.08±0.01 & 3.03±0.01 ng/ml) as compared to respective control, treated and recovered broiler chickens groups as shown in Tables (2&3).

In male broiler chickens that treated with a mixture of olive oil "MUFA" plus saturated animal fat, the plasma leptin concentration (2.44±0.02 ng/ml) was significantly higher than those of control and olive oil-treated groups (2.18 ±0.04 & 1.35±0.02 ng/ml), respectively at $P< 0.05$ (Tables 2 &3). This significant difference was disappeared in the recovered chickens.

Data presented in Tables (2&3) showed nonsignificant differences in plasma cholesterol concentrations among all groups either in treated or recovered broiler chickens at $P< 0.05$.

Plasma HDL concentrations of olive oil "MUFA"-treated group alone and mixed MUFA plus saturated fat-treated group showed highly significant increase in their values as compared to saturated fat-treated group which were (85.15±8.55, 84.66±11.63 & 38.09±8.74 mg/dl), respectively at $P< 0.05$ (Table 2).

Plasma triglycerides, LDL and VLDL concentrations (Tables 2&3) either in treated or recovered groups did not reveal any significant differences among their values at $P< 0.05$.

Data presented in Table (4) showed either significant positive or negative correlations of the tested parameters of the compiled data of treated and recovered broiler chickens groups.

Table 2:

Groups	Measurements					
	Leptin ng/ml	Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control	$2.18^{a,b,c}$ ± 0.04	113.56 ± 9.02	55.1 ± 6.79	67.56 ± 16.49	46 ± 13.22	11.01 ± 1.35
Treated with olive oil (MUFA)	$1.35^{a,d,e}$ ± 0.02	129.53 ± 11.07	62.79 ± 6.39	85.15^{a} ± 8.55	56.43 ± 12.03	12.55 ± 1.27
Treated with saturated animal fat	$3.08^{b,d,f}$ ± 0.01	120.6 ± 26.16	44.08 ± 3.43	$38.09^{a,b}$ ± 8.74	93.35 ± 27.1	8.81 ± 0.68
Treated with olive oil+ saturated animal fat	$2.44^{c,e,f}$ ± 0.02	131.39 ± 20.11	59.33 ± 11.67	84.66^{b} ± 11.63	60.51 ± 21.59	11.86 ± 2.33

Mean ±SE

Mean values having the same letter (s) in the same column are significantly different from each other at $P< 0.05$

Table 3:

Recovery Groups	Measurements					
	Leptin Ng/ml	Cholesterol mg/dl	Triglycerides mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
R-control	2.14^{a} ± 0.03	133.15 ± 22.59	34.53 ± 8.40	34.28 ± 12.16	97 ± 18.47	6.91 ± 1.68
R- olive oil	2.14^{b} ± 0.06	69.01 ± 8.01	59.68 ± 11.75	41.81 ± 17.35	32.59 ± 6.83	11.93 ± 2.35
R- saturated animal fat	$3.03^{a,b,c}$ ± 0.01	79.64 ± 19.83	34.12 ± 9.00	59.72 ± 14.54	39.37 ± 17.96	6.82 ± 1.80
R- olive oil+ saturated animal fat	2.22^{c} ± 0.07	75.6 ± 24.67	69.59 ± 15.16	49.71 ± 6.55	50.43 ± 17.75	13.91 ± 3.03

Mean ±SE

Mean values having the same letter (s) in the same column are significantly different from each other at $P< 0.05$

Table 4:

		Cholesterol	HDL	LDL	Triglycerides	VLDL	Leptin
Cholesterol	Pearson correlation Sig. (1-tailed) N	1					
HDL	Pearson correlation Sig. (1-tailed) N	–0.102 .175	1				
LDL	Pearson correlation Sig. (1-tailed) N	.884** .000	–.477** .000	1			
Triglycerides	Pearson correlation Sig. (1-tailed) N	0.07 .262	.007 .473	.064 .282	1		
VLDL	Pearson correlation Sig. (1-tailed) N	.069 .266	.004 .486	.064 .281	1** .000	1	
Leptin	Pearson correlation Sig. (1-tailed) N	–.277** .005	–.156 .074	–.172 .059	–.184** .045	–.185* .044	1

**Correlation is significant at the 0.01 level (1-tailed)
*Correlation is significant at the 0.05 level (1-tailed)

Table (4) showed:

- Significant positive correlation between cholesterol & LDL.
- Significant negative correlation between cholesterol and leptin.
- Significant negative correlation between LDL & HDL.
- Significant positive one between triglycerides & VLDL.
- Significant negative one between leptin & cholesterol, triglycerides, VLDL

DISCUSSION

The results in Table (2) showed that plasma leptin level of control chickens group was (2.18±0.04 ng/ml). This result was in agreement with (Backus *et al.*, 2000) in domestic cats, and was lower than that obtained by (Kratz *et al.*, 2002) in men (3.22±2.67 ng/ml).

Mean plasma cholesterol level in control male broiler chicken was (113.56±9.02 mg/dl) which in agreement with (Sturkie, 1976 &2000) who found that cholesterol concentration was (116-134 mg/dl) in unsexed White leghorn chicks, aged from 1 to 15 weeks. Circulating lipids in the blood were derived from intestinal absorption, synthesis (mainly in the liver) or mobilization of fat depots. Lipid concentration in birds is influenced by species, age, sex, nutrition; state of health and energy needs including "climate conditions and other factors", Sturkie (2000).

When broiler chickens were given Palestine olive oil "MUFA", the plasma leptin level showed highly significant decrease (1.35±0.02 ng/ml) as compared with control and the other treated groups. This value for plasma leptin level caused by olive oil treatment was associated with highest nonsignificant difference in HDL level

when compared with control value. But plasma leptin concentration of "MUFA"-treated group (1.35±0.02 ng/ml) exhibit higher HDL (85.15±8.55 mg/dl) as compared to HDL of saturated animal fat-treated group (38.09±8.74 mg/dl). In contrast, our results of saturated fat-treated chickens exhibit highest significant increase in plasma leptin value (3.08±0.01 ng/ml) with lowest plasma HDL value (38.09±8.74 mg/dl).

Scarce literatures that study the effect of olive oil "MUFA" on plasma leptin levels in chickens. However, the results of the present investigation have the same lowering action of omega (n-3) polyunsaturated fatty acid "PUFA" on plasma leptin (Ukropec *et al.*, 2003).

In contrast to the results of the present study; (Cha & Jones, 1998) have been observed that rats fed a diet rich in n-6 and n-3 polyunsaturated fatty acids (PUFA) exhibit higher serum leptin levels than those fed a diet rich in both saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Additionally, (Kratz *et al.*, 2002) found that leptin concentrations were not significantly affected by diets rich in olive oil and sunflower oil either in men or in women.

However, the previous researchers (Wang *et al.*, 2002) found that plasma leptin levels were significantly elevated in the saturated fat group compared with low fat control.

In addition, other researchers found that changing the saturated fat group to n-3 PUFA (Pieke *et al.*, 2000 and Wang *et al.*, 2002) and to MUFA for four weeks completely reversed the hyperleptinemia and increased adiposity and neuropeptide changes induced by saturated fat. Their results were in agreement with the results of the present study of broiler chickens that treated with a mixture of olive oil plus saturated fat which exhibit a moderate significant increase of leptin concentration as compared with broilers that treated either with MUFA or with saturated fat alone,

Present results showed the significant effect of "MUFA" olive oil on elevation of plasma HDL and consequently expected reduction in body fat depot.

The present results confirmed the previous results of (Takahashi and Ide, 2000) who used polyunsaturated fatty acids (n-3 PUFA) and concluded that the physiological activities of PUFA prevented body fat accumulation. Because it was found that HDL is a good lipoprotein cholesterol-carrier from blood to liver which metabolite and excrete it, this explains the elevation of blood HDL caused by the direct effect of PUFA (Alwahaibi, 1996).

Results of the present investigation showed no significant effect of the different treatments with "olive oil" and/or with "saturated animal fat" on plasma cholesterol, LDL, and VLDL levels. These results are in agreement with (Sturkie, 2000) who found addition of 1% dietary cholesterol or 10% corn oil to young chickens diet did not alter the plasma concentrations of the aforementioned parameters.

At the end of the recovery period, plasma leptin level in all groups returned to its basal level "control" value with the exception of plasma leptin level of the recovered group that previously treated with saturated animal fat. The latest groups exhibit persistent significant increase in its value than control, MUFA and saturated fat-treated groups.

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