Effect of Olive Oil Supplementation on PAI-1 Expression in Old Rats

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Abstract: Background: Although Mediterranean diet has grown worldwide due to its link with lower cardiovascular disease rate and greater longevity, the effect of olive oil, which is the principal component of Mediterranean diet, on plasminogen activator inhibitor-1 (PAI-1) concentrations in aged rats is not clear. This study was performed on 28 aged male Wistar albino rats allocated into 2 groups: 1- Olive oil-treated group (14 rats), 2- Control group (14 rats). **Results:** Using Real Time-PCR, the expression of PAI-1 mRNA in the retroperitoneal adipose tissues was decreased significantly in the olive oil treated group versus the control group (32.36%±15.97 versus 100%±6.04 respectively, $p< 0.01$). In parallel, the plasma concentrations of PAI-1 were reduced significantly in the olive oil treated group versus the control group (3.14±1.07 versus 7.16±0.76 respectively, $p<0.01$). Olive oil produced significant decrease in mean serum cholesterol in aged rats (76.32±9.18 mg/dl in the treated group versus 84.03±7.15 mg/dl in the control group, $P<0.05$) and triglycerides (40.73±12.52 mg/dl in the treated group versus 61.17±5.52 mg/dl in the control group, $P<0.01$). No significant difference was seen in mean serum LDL-cholesterol or HDL-cholesterol levels between both groups (31.06±4.88 and 36.5±5.61 mg/dl in the treated group versus 32.24±2.07 and 36.57±5.07 mg/dl in the control group respectively). As regards the BMI, no change was observed after olive oil intake. **Conclusion:** Our results indicate that olive oil intake may reduce the cardiovascular risk in old age via decreasing PAI-1 at level of gene expression.

[Manal L Louka, Haidy Z Habib, Magda H M Youssef, Noha A H Nassef. **Effect of Olive Oil Supplementation on PAI-1 Expression in Old Rats.** *J Am Sci* 2012;8(11):317-321]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 45

Keywords: PAI-1, olive oil, aging, lipid profile.

1. Introduction

Age is a significant risk factor for the development of cardiovascular diseases, such as atherosclerosis ***(Williamson et al., 2012)***. The mechanism of age-related cardiovascular dysfunction remains unclear; yet, an imbalance between procoagulant and profibrinolytic agents appears to play a role. Amongst the procoagulants, plasminogen activator inhibitor-1 (PAI-1) has been linked to coronary heart disease and aging ***(Ruano et al., 2007 and Serrano et al., 2009***).

PAI-1 is expressed in a variety of tissues, but the cellular origin of plasma PAI-1 is likely to be white adipose tissue. Although pharmacological treatments have improved the prognosis of cardiovascular diseases, they remain a leading cause of mortality in aged people. Given the increased life expectancy of the population in developed countries, there is a clear need for alternative treatment strategies ***(Williamson et al., 2012)***.

Mediterranean diet has grown worldwide due to its link with lower cardiovascular disease rate and greater longevity. The actual interest in this dietary model is based in two main premises. First, the high palatability for the consumer, which aids to the adherence to the model on a life-long basis, and second, the mounting evidence on the beneficial properties that its consumption provokes in cardiovascular risk factors, cancer and cognitive age associated decline ***(Lopez-Miranda et al., 2007 and Delgado-Lista et al., 2011).***

Olive oil is the principal component of Mediterranean diet, both by its predominant position as the main energy source, and its presence in almost all cooked and/or seasoned food. Olive oil consumption was found to lower PAI-1 concentrations in hypercholesterolemic subjects ***(Jones et al., 2007)*** and in different experimental situations ***(Lopez-Miranda et al., 2007)***. However, the underlying mechanisms are not clear. Also, the studies that investigated the effect of olive oil consumption on PAI-1 in aged were scarce. Thus, the present work was designed to achieve 2 goals: first to elucidate the effect of olive oil consumption on age-associated increase in plasma PAI-1 levels, second to further investigate the possible underlying mechanisms by observing its effects on gene expression of tPAI-1 in adipose tissue of aged rats.

2. Material and methods

2.1. Animals

This study was carried out on 28 male Wistar albino rats of 24-months age. Rats were purchased from the Research Institute of Ophthalmology (Giza), and were kept in the Physiology Department Animal House under standard conditions of boarding and feeding with free access to water. The animals included in the present study were allocated into 2 groups: 1-Olive oil-treated group (14 rats): Received olive oil which was purchased from the market and administered by gavage in a dose of 1.25 ml/kg for 12 days ***(Stämpfli et al., 2010)***. 2-Control group (14 rats): Received 1.25 ml/kg distilled water by gavage for 12 days. During this period, animals were kept in their cages well ventilated, in 12 hour day/night cycle with no pain. Concept of 3Rs (Replacement, Reduction, Refinement) was considered. Ethical Approval was obtained for the study from Research Ethics Committee, Faculty of Medicine, Ain Shams University.

2.2. Experimental Procedures:

On the day of sacrifice, overnight fasting rats, except for free access to water, were weighed and anaesthetized by intraperitoneal injection of sodium thiopental (EPICO) (40 mg /kg b.wt.). Body length was measured, then a midline abdominal incision was made, the abdominal aorta was exposed and cannulated with a polyethylene catheter and a blood sample was collected in two tubes for plasma and serum. The blood sample was centrifuged at 3000 rpm for 15 minutes, serum and plasma were collected in aliquots and kept frozen at −80°C for determination of serum lipid profile and plasma PAI-1. The retroperitoneal white adipose tissue was removed completely and weighed, immediately frozen in liquid nitrogen, stored at -80°C, and was finally used for PAI-1 mRNA quantification.

BMI was calculated by dividing body weight (grams) by body length squared (square centimeters). Disposal of animal remains was done by incineration.

2.3. Biochemical Analysis:

The levels of serum triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol were analyzed using Biodiagnostic kits according to the methods of **Fassati and Prencipe (1982), Allain *et al.* (1974), Wieland and Seidel (1983) and Lopez-Virella *et al.*(1977)** respectively.

Determination of plasma PAI-1 was done by a commercial ELISA kit according to the manufacturer´s instructions (Rat plasminogen activator inhibitor 1, PAI-1 ELISA Kit, EIAab).

2.4. Real Time Reverse Transcription Polymerase Chain Reaction for the relative quantity of PAI-1

1. Total RNA was extracted using an RNeasy Mini Kit (Qiagen, cat# 74106, Cal. USA) according to the manufacturer.
2. cDNAs were synthesized using RevertAid™ First Strand cDNA Synthesis kit (Fermentas, Life Sciences).
3. cDNA was reversely transcribed from 5 ug of mRNA in transcription buffer, 200U M-MuLV Reverse Transcriptase, 20U RNase inhibitor at 42 ^{o}C for 60 min followed by immediate cooling on ice.
4. Real-time polymerase chain reaction (RT PCR) was performed with 50 ng cDNA per reaction using 25 µL of SYBR Green QPCR Mix (Solis BioDyne) containing 20 uM of specific primers in the Real-Time PCR Detection System (Applied BioSystems 7500).
5. The amplification protocol was as follows: 94°C for 10 min, followed by 40 cycles of 94°C for 30 sec, 1 min at 52°C and 1 min at 72°C, completed by a dissociation curve to identify false-positive amplicons.
6. The SYBER green data were analyzed with a relative quantification to GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) as reference gene.
7. The sets of primers used were as follows: tPAI-1 sense 5'GCCTCCAAAGACCGAAATGTG-3' and antisense 5'GTCGTTGATGATGAATCTGGCTC-3'. GAPDH sense 5’TAGCCCAGGATGCCCTTTAGT-3’ and antisense 5’CCCCCAATGTATCCGTTGTG-3’.
8. The relative expression level of each gene was calculated using the formula $2^{(-\Delta\Delta Ct)}$ according to **VanGuilder, 2008.**

2.5. Statistics

Statistics were done using statistical package for the social sciences (SPSS) program (SPSS Inc., version 19). All data were expressed as mean ± standard deviation (S.D.). The probability value less than 0.05 was taken as the level of statistical significance.

3. Results:

This study included 28 male Wistar albino rats of 24-months age allocated into 2 groups: [Olive oil-treated group (14 rats) and a Control group (14 rats)].

3.1. Influence of olive oil treatment (1.25 ml/kg for 12 days) on serum lipid profile

After the twelve day-treatment period, olive oil produced significant decrease in mean serum cholesterol in aged rats (76.32±9.18 mg/dl in the

treated group versus 84.03±7.15 mg/dl in the control group, $p<0.05$) and triglycerides (40.73±12.52 mg/dl in the treated group versus 61.17±5.52 mg/dl in the control group, $P<0.01$). No significant difference was seen in mean serum LDL-cholesterol levels (31.06±4.88 mg/dl in the treated group versus 32.24± 2.07 mg/dl in the control group). HDL-cholesterol levels also didn't differ between both groups (36.5± 5.61 mg/dl in the treated group versus 36.57±5.07 mg/dl in the control group) table (1).

3.2. Influence of olive oil treatment (1.25 ml/kg for 12 days) on BMI

As regards the BMI, no change was observed after olive oil intake in the treated rats in comparison to the control group, table (1).

Table (1): Effect of olive oil treatment (1.25 ml/kg) on the levels of serum lipid profile and BMI:

parameters	Control group mean ± S.D	Olive oil Group mean ± S.D
BMI (g/cm^2)	0.61± 0.03	0.6±0.03
Cholesterol (mg/dl)	84.03±7.15	76.32± 9.18*
Triglycerides (mg/dl)	61.17±5.52	40.73±12.52**
HDL.Ch. (mg/dl)	36.57±5.07	36.5±5.61
LDL.Ch. (mg/dl)	32.24± 2.07	31.06±4.88

Values are expressed as mean ± S.D. of 14 animals per group.
Values are statistically significant *P <0.05, P **<0.01 when compared with the control group.

3.3. Effect of olive oil treatment (1.25 ml/kg for 12 days) on the expression of tPAI-1 mRNA in the retroperitoneal adipose tissues

Using Real Time-PCR, the expression of tPAI-1 mRNA in the retroperitoneal adipose tissues was measured. Retroperitoneal adipose tissue mRNA expression levels for tPAI-1 was decreased significantly in the olive oil reated group versus the control rats (32.36% ±15.97 versus 100%±6.04, $P<$ 0.01) table (2).

3.4. Effect of olive oil treatment (1.25 ml/kg for 12 days) on the level of plasma PAI-1 protein

Regarding the plasma concentrations of PAI-1, they were reduced significantly in the olive oil treated rats versus the control rats (3.14±1.07 versus 7.16±0.76, $p<0.01$, table (2).

Table (2): tPAI-1 gene expression (%) in adipose tissues and plasma concentration of PAI-1 in olive oil-treated rats (1.25 ml/kg) and in control group

Parameters	Control group mean ± S.D	Olive oil Group mean ± S.D
Plasma PAI-1 (ng/ml)	7.16±0.76	3.14±1.07**
tPAI-1 gene expression (%) in adipose tissues	100%±6.04	32.36%±15.97**

Data are reported as means ± SD for 14 animals per group.
Values are statistically significant, ** $P<$ 0.01 *vs* control group.

4. Discussion

Olive oil is a functional food that induces favorable changes of lipid profile, improve endothelial function and disclose antithrombotic properties **(Pérez-Martínez *et al.*, 2011).** Traditionally, many beneficial properties associated with this oil have been ascribed to its high oleic acid content which is linked with a reduction in risk of coronary heart disease **(Keys *et al.*, 1986).**

In our work, no change was observed in BMI after olive oil intake in the treated rats compared to the control ones. This was in accordance to some recent human studies (**Pisani *et al.*, 2008 and Benítez-Arciniega *et al.*, 2012**). **De Wit *et al.* (2012)** indicated that olive oil has a less stimulatory effect on weight gain and hepatic lipid accumulation than saturated fat like palm oil. So, we can say that higher olive oil consumption is not predisposing to obesity.

Intake of olive oil (1.25 ml/kg for 12 days) in aged rats included in this study produced significant decrease in mean serum cholesterol (76.32±9.18 mg/dl versus the control group 84.03±.15 mg/dl, $P<$ 0.05) and triglycerides (40.73±12.52 mg/dl versus the control group 61.17±5.52 mg/dl, $P<$ 0.01). LDL-cholesterol levels, however, didn't differ between both groups (31.06±4.88 mg/dl versus the control group 32.24±2.07 mg/dl). This was in agreement with **Alhazza (2007) and Paoli *et al.* (2011**) who reported that the cholesterol and triglyceride levels decreased significantly in rats given olive oil after 4 and 6 weeks. They also, observed no significant difference in LDL concentration in rats given olive oil. **Jones *et***

***al.* (2007)** showed no differences in total cholesterol or LDL-cholesterol concentrations following supplementation of olive oil based diet (4 weeks) while showed significant decrease in triglyceride concentration.

Regarding HDL-cholesterol, we did not find a significant difference between treated and control groups (36.5 ±5.61 mg/dl versus 36.57 ±5.07 mg/dl respectively), yet **Paoli *et al.* (2011)** reported that HDL level was significantly (P<0.05) increased in the rats administrated olive oil after 6 weeks. This controversy may be because of the shorter duration of our study.

No previous work was done to explore the effect of olive oil based diet on the genetic expression level of tPAI-1. We chose adipose tissue to study tPAI-1 gene expression in because it was the site where mice with genetically induced obesity had an increased PAI-1 expression **(Suganami and Ogawa, 2010, Ouchi *et al.*, 2011).** Also, local increase in PAI-1 concentration was being parallel to that in plasma (**Samad and Loskutoff, 1996, Serrano *et al.*, 2009).** We conducted this work in order to prove the association between the tPAI-1 production rate by rat retroperitoneal adipose tissues and plasma PAI-1 levels in the olive oil treated old rats. Using Real Time-PCR, the expression of tPAI-1 mRNA in the rat retroperitoneal adipose tissues was decreased significantly in the olive oil treated group versus the control rats (32.36 % ±15.97 versus 100 % ±6.04, P< 0.01).

Also, the plasma concentrations of PAI-1 were reduced significantly in the olive oil treated group versus the control group (3.14±1.07 ng/ml versus 7.16±0.76 ng/ml, P<0.01). These results were in parallel with the decreased tPAI-1 gene expression level, and in line with **Paoli *et al.* (2011)** who demonstrated low cardiovascular risk with Mediterranean diet rich in olive oil.

Conclusion:

The short (12 days) duration of olive oil administration was an important point of interest in this study because it was enough to bring up the fibrinolytic effect by lowering tPAI-1 gene expression levels and also improved significantly lipid profile by lowering the serum levels of cholesterol and triglycerides. This shows that olive oil based diet has a benefit to be used for its cardioprotective effect.

Competing interests:

The authors have no conflicting interests, including any financial, personal or other relationships with other people or organizations, and are not supported or funded by any company.

Acknowledgment:

We wish to acknowledge both Physiology and Molecular Biochemistry Departments.

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9/12/2012