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Short-term effect of orlistat on dietary glycotoxins in healthy women and women with polycystic ovary syndrome

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Abstract

Exogenous advanced glycation endproducts (AGEs, known atherogenic molecules) abundant in everyday precooked, rich in fat, overheated meals can possibly contribute to the increased risk for diabetes and cardiovascular disease in women with polycystic ovary syndrome (PCOS). The aim of the present study was to investigate the effect of a lipase inhibitor on absorbed food glycotoxins in healthy women and those with PCOS. A 2-day protocol was followed. In the first day, a meal rich in AGE was provided, which on the second day was followed by two 120-mg capsules of lipase inhibitor, orlistat. Serum AGE levels were evaluated at baseline (0 hours), and at 3 and 5 hours postmeal during the study. Thirty-six women were studied, 15 controls (mean age, 28.80 ± 5.47 years; body mass index, 25.85 ± 6.73 kg/m^2) and 21 with PCOS (mean age, 25.29 ± 5.06 years; body mass index, 30.40 ± 7.51 kg/m^2) (University Hospital, Athens, Greece, institutional practice). Serum AGE levels, on day 1, were significantly increased both in the control group and in the PCOS group as compared with basal values (control group, 14.1%; PCOS group, 6.0%; $P < .001$). The corresponding rise was significantly lower on day 2 when the same meal was combined with orlistat (control group, 4.1%; PCOS group, 2.0%; $P < .01$). A limitation of the study is that it is a nonplacebo, nonrandomized therapeutic trial where each subject is considered as its own control. In conclusion, this study demonstrated the beneficial effect of orlistat on the absorption of food glycotoxins.

1. Introduction

The formation of advanced glycation endproducts (AGEs) has been implicated in the pathogenesis of diabetic complications, aging, and atherosclerosis [1]. Advanced glycation endproducts are highly reactive molecules, formed by nonenzymatic glycation of proteins, lipids, and nucleic acids, which may induce structural and vascular changes [1,2]. The mechanisms by which AGEs lead to these injuries in multicellular structures remain largely unknown; however, the formation of insoluble cross-links, the induction of oxidative stress, and cell activation play a significant role. Interaction of AGE with specific cellular receptors leads to cell activation, and release of cytokines and growth factors, which contribute to abnormal cell/matrix proliferation, such as seen in diabetic renal lesions and atheromas [3-5].

Endogenous and exogenous sources contribute to the serum AGE and tissue levels. There is evidence that AGE content in food is strongly associated with the protein and fat ingredients [6]. Diet has been shown to be a significant source of AGE, and contemporary methods of cooking (precooked fast-food meals heated in high temperatures) dramatically increase AGE concentration.

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With respect to AGE formation in food, questions arise concerning the intake of dietary AGE via the daily food intake and their possible toxic effect on the physiologic systemic functioning either directly or indirectly. The question about the interaction of the exogenous AGE on atherosclerotic process still remains under investigation.

However, recent reports have confirmed oral absorption and tissue incorporation of endproducts originating from mixed foods. Human studies confirmed that about 10% of exogenous AGEs are absorbed and correlate with circulating and tissue AGE levels, exceeding their endogenous production [7]. Data in humans and experimental animals favor that exogenous food-ingested AGE result in elevated serum levels and increased tissue deposition [8,9]. Specifically, ingestion of a meal rich in AGE has led to increased serum levels of AGE in diabetic patients, which were compared with controls, and tissue deposition was enhanced when the AGE meals were rich in fat [7]. Dietary AGE restriction resulted in significant reduction of circulating AGE levels and disease progression in animal models of atherosclerosis [10] and diabetes [11] and in diabetic patients with or without renal impairment [12]. These findings suggest that dietary AGE may constitute a long-term environmental risk factor for tissue injury.

Considering these findings, it could be speculated that high-AGE diets could contribute to the atherosclerotic process in subjects with endogenous elevated AGE, such as diabetic patients or those with PCOS [1,13]. However, studies investigating whether a common fast-food meal alters the serum AGE levels or whether this effect could be reversed partially or totally by an immediate medical intervention have never been performed.

The present study was designed to evaluate the effects of an AGE-rich meal on serum AGE levels and the modulatory effect of orlistat, a lipase inhibitor [14], in a group of healthy women as well as in women with PCOS, a population with elevated endogenous AGE production.

2. Subjects and methods

2.1. Subjects

This was an open-label, single-dose study using orlistat in 36 women divided in 2 groups according to their health status (control and PCOS). All control women were in good health, and none was taking any medications known to affect carbohydrate or sex hormone metabolism for 1 month or more prior the study, including oral contraceptive agents, which were stopped 3 months before the study. The control group consisted of 15 healthy women (mean age, 28.80 ± 5.47 years; body mass index [BMI], 25.85 ± 6.73 kg/m^2) who had regular menstrual cycles every 25 to 30 days and normal plasma androgen levels.

Twenty-one women with the diagnosis of PCOS (mean age, 25.29 ± 5.06 years; BMI, 30.40 ± 7.51 kg/m^2) were studied based on the Rotterdam criteria [15]. Their diagnosis was based on the presence of 2 of the following 3 criteria: (1) oligo- and/or anovulation (menses ≥8 per year); (2) clinical and/or biochemical signs of hyperandrogenism; (3) polycystic ovaries on ultrasonography and exclusion of related disorders (nonclassic congenital adrenal hyperplasia, androgen-secreting neoplasms, thyroid disease, and hyperprolactinemia).

None of the subjects included in this study were current smokers. Regarding their eating habits, women were asked to follow a regular weight-maintaining diet with home-cooked food (avoidance of fast-food meals and snacks), according to dietician instructions for 3 weeks before participation in the experiment.

2.2. Clinical procedures

The study involved a 2-day protocol that was approved by the Research Committee of Aretaieion University Hospital, Athens, Greece (protocol no. 137/22042003), and written informed consent was obtained from each volunteer before enrolment in the study.

On the morning of day 1 (9:00 AM), fasting blood samples were collected from each participant before receiving a high-AGE meal.

The meal consisted of 120 g chicken nuggets overcooked at 240°C for 60 minutes, 70 g potato chips, 25 g meringues, and 250 mL Coca Cola. Each meal is composed of 3534.6 kJ (844.8 kcal), 21.9 g of protein, 38.9 g of fat, 96 g of carbohydrate (10.6%, 42.6%, and 46.7% of total energy content, respectively).

Blood samples were collected before each meal as well as at 3 and 5 hours postmeal. Only water was allowed during the study.

The night of day 1, all participants were asked to have a very light, not fast-food meal. In the morning of the day 2, baseline blood samples were taken. All the participants received the same meal as in day 1 plus 2 capsules of orlistat (2 of 120-mg capsules). Additional blood samples were obtained at 3- and 5-hour postmeal intervals.

Weight, height, and waist and hip circumferences were measured.

Body mass index was calculated by the formula:

$$\text{BMI} = \text{weight (kg)}/\text{height (cm)}^2$$

Waist-to-hip ratio (WHR) was calculated by the formula:

$$\text{WHR} = \text{waist circumference (cm)}/\text{hip circumference (cm)}$$

2.3. Biochemical and hormonal measurements

Serum samples on day 1 were collected from each participant for determination of baseline levels of biochemical and hormonal parameters. Serum levels of total testosterone (nanograms per deciliter, nanomoles per liter), sex hormone–binding globulin (SHBG, nanomoles per liter), serum fasting insulin (micro–international units per liter, picomoles per liter), serum fasting glucose (milligrams

Table 1
Anthropometric characteristics, and hormonal and metabolic profile of patients with PCOS and healthy women

Variable	PCOS group (n = 21)	Control group (n = 15)
Age (y)	25.29 ± 5.06	28.80 ± 5.47
BMI (kg/m^2)	30.40 ± 7.51	25.85 ± 6.73
WHR	0.80 ± 0.07*	0.74 ± 0.07
T (ng/dL, nmol/L)	92.95 ± 41.50*	36.32 ± 14.60
	3.23 ± 1.44	1.26 ± 0.51
SHBG (nmol/L)	37.69 ± 13.07	35.01 ± 9.72
FAI	285.03 ± 189.56*	109.26 ± 49.85
F (mmol/L)	2.23 ± 0.50*	1.72 ± 0.42
FFA (mg/dL, mmol/L)	0.67 ± 0.31	0.52 ± 0.31
TG (mg/dL, mmol/L)	97.14 ± 36.84*	69.33 ± 26.71
	1.09 ± 0.42	0.78 ± 0.30
GLU (mg/dL, mmol/L)	94.62 ± 11.05	90.27 ± 8.22
	5.25 ± 0.61	5.00 ± 0.46
TC (mg/dL, mmol/L)	171.43 ± 37.62	171.93 ± 33.89
	4.44 ± 0.97	4.45 ± 0.88
HDL (mg/dL, mmol/L)	46.27 ± 18.06	41.03 ± 6.26
	1.198 ± 0.47	1.06 ± 0.16
INS (μIU/L, pmol/L)	11.69 ± 3.49*	6.71 ± 4.19
	81.19 ± 24.24	46.60 ± 29.099
UA (mg/dL, μmol/L)	4.35 ± 0.95	3.83 ± 1.09
	258.74 ± 56.51	227.81 ± 64.83
QUICKI	0.33 ± 0.01*	0.37 ± 0.03

Data are given as means ± SD. T indicates total testosterone; F, fructosamine; FFA, free fatty acid; TG, triglyceride; GLU, serum fasting glucose; TC, total cholesterol; INS, serum fasting insulin; UA, uric acid.
* $P < .05$ vs control.

per deciliter, millimoles per liter), total cholesterol (milligrams per deciliter, millimoles per liter), triglycerides (milligrams per deciliter, millimoles per liter), high-density lipoprotein (HDL) (milligrams per deciliter, millimoles per liter), free fatty acids (FFA, milligrams per deciliter, millimoles per liter), uric acid (UA, milligrams per deciliter, micromoles per liter), and fructosamine (millimoles per liter) were measured. All the parameters, but total testosterone, SHBG, and fructosamine were measured also at 5 hours postmeal, on both days.

2.4. Chemicals and reagents

Bovine serum albumin, D-glucose, alkaline phosphatase–conjugated goat antimouse immunoglobulin G, and antigoat immunoglobulin G–fluorescein isothiocyanate as well as *p*-nitrophenyl phosphate tablets were purchased from Sigma Chemical (St Louis, MO). Superblock blocking buffer was from Pierce (Rockford, IL) and normal goat serum from Gibco BRL (Gaithersburg, MD). Mouse antihuman AGE monoclonal antibody (6D12) was obtained from Research Diagnostics (Flanders, NJ).

2.5. Specificity of anti-AGE antibody (6D12)

6D12 is a mouse antihuman monoclonal antibody with immunospecificity to a common structure among AGE proteins. The epitope of 6D12 is N3-carboxymethyllysine–protein adduct. This antibody also recognizes carboxyethyllysine and does not recognize the early products, Schiff bases, and Amadori products. However, it shows positive reaction to AGE samples obtained from proteins, lysine derivatives, or monoamino-carboxylic acids. Recent immunologic studies using 6D12 demonstrated the presence of AGE-modified proteins in several human tissues, indicating its usefulness in the biochemical quantification of AGE-modified proteins [16].

2.6. Preparation of AGE-modified proteins

Advanced glycation endproduct–modified bovine serum albumin was prepared as previously described by Diamanti-Kandarakis et al [13]. The degree of AGE modification of the protein was further determined by competitive AGE–enzyme-linked immunosorbent assay (ELISA) as described below.

2.7. Competitive AGE-ELISA

The competitive AGE-ELISA procedure was performed as previously described by Diamanti-Kandarakis et al [13].

2.8. Assays

Serum and plasma samples were obtained from the 2 groups and stored at −20°C before analysis. Just before assay by competitive AGE-ELISA, sera were thawed and diluted 1:5 with dilution buffer (phosphate-buffered saline, 0.02% Tween 20, and 1 mmol/L sodium azide).

All measurements were performed using the ChemWell Analyzer (Awareness Technologies, Los Angeles, CA). Plasma glucose was determined by the glucose oxidase colorimetric method (Glucose LR, GOD-PAP, Linear Chemicals, Barcelona, Spain). Total cholesterol was measured by an enzymatic colorimetric method using cholesterol oxidase and peroxidase (Cholesterol LR, CHOD-PAP, Linear Chemicals). High-density lipoprotein cholesterol was assessed enzymatically using a direct method (HDL-Cholesterol, DIRECT, Linear Chemicals). Triglycerides were measured using an enzymatic colorimetric method

Table 2
Serum AGE levels of control and PCOS groups in all time intervals on both days

		Day 1			Day 2		
		Time 0	Time 3	Time 5	Time 0	Time 3	Time 5
Serum AGE levels	C (n = 15)	4.81 ± 0.89	5.44 ± 0.78*	5.49 ± 0.76**	5.83 ± 0.66	5.99 ± 0.70*	6.07 ± 0.88**
	P (n = 21)	9.45 ± 1.23	9.96 ± 1.17*	10.02 ± 1.26**	10.21 ± 1.25	10.36 ± 1.37	10.41 ± 1.43**

C indicates control group; P, PCOS group.
* $P < .03$ vs time 0.
** $P < .001$ vs time 0.

based on hydrolysis of plasma triglyceride to glycerol and free fatty acids by lipoprotein lipase (Triglycerides MR, Linear Chemicals). Serum free fatty acids were estimated using a colorimetric method using ACS-ACOD-MEHA reactions (NEFA C, enzymatic color test, WAKO, Richmond, VA). Plasma uric acid was determined by an enzymatic colorimetric method using uricase and peroxidase (Uric Acid LR, URICASE-PAP, Linear Chemicals).

Quantitative determination of total testosterone was performed with an enzyme immunoassay test kit (Testosterone Enzyme Immunoassay Test Kit, LI7603, Linear Chemicals).

Serum SHBG levels were measured by ELISA (IBL GmbH, Hamburg, Germany). Fructosamine levels were detected by an enzymatic colorimetric method (Fructosamine Reagent Kit, Raichem, San Diego, CA).

2.9. Hyperandrogenism estimation

The free androgen index (FAI, %) was estimated by the formula:

$$\text{FAI} = (\text{total testosterone [ng/dL]}/\text{SHBG [nmol/L]}) \times 100$$

2.10. Insulin resistance estimation

Insulin resistance was estimated by the quantitative insulin sensitivity check index (QUICKI, [17]).

2.11. Statistical analysis

Differences between groups in baseline measurements were estimated using either *t* test or Wilcoxon rank sum test when normality assumption was violated.

Differences before and after the meal for each day as well as their variation from one day to the other (medication effect) were assessed for each group, using paired *t* test or Wilcoxon signed rank test.

A *P* value of less than .05 was taken to indicate statistical significance.

2.12. Funding source

The finding source of the present study was the University of Athens Medical School, which provided the reagents, the equipment, and the appropriate laboratories for the study. No further involvement of the funding source took place.

3. Results

3.1. Demographic profile

There was no statistically significant difference in mean age between the groups (28.80 ± 5.47 years [control group] vs 25.29 ± 5.06 years [PCOS group], $P = .06$) and in BMI (25.85 ± 6.73 kg/m^2 [control group vs 30.40 ± 7.51 kg/m^2 [PCOS group], $P = .07$). Waist-to-hip ratio differed significantly between controls and the PCOS group (control group, 0.74 ± 0.06; PCOS group, 0.84 ± 0.17, $P = .03$) (Table 1).

3.2. Advanced glycation endproduct levels

Serum AGE levels in all time intervals (0, 3, and 5 hours postmeal) of days 1 and 2 are indicated in Table 2. On day 1, a statistically significant difference of serum AGE levels was found between baseline (before meal, 0 hours) and 3 hours postmeal ($P < .03$) as well as between baseline and 5 hours postmeal ($P < .001$) for both groups. On day 2, a statistically significant difference of serum AGE levels was found between baseline and 5 hours postmeal ($P = .001$)

Table 3
Comparison of Δ values of all variables measured between time 5 and time 0 (5-0) on days 1 and 2 in control and PCOS groups

	Day 1	Day 2	*P*
AGE			
C	0.65 ± 0.3	0.24 ± 0.37	<.01
P	0.57 ± 0.28	0.20 ± 0.41	<.01
FFA			
C	0.10 ± 0.29	0.05 ± 0.25	.686
P	0.08 ± 0.47	0.19 ± 0.27	.493
Insulin			
C	0.84 ± 6.41	−1.32 ± 6.36	.213
	5.83 ±44.52	−9.17 ± 44.17	
P	−4.10 ± 8.12	−2.92 ± 9.38	.643
	−28.47 ± 56.39	−20.28 ± 65.14	
Triglycerides			
C	34.73 ± 40.65	44.46 ± 40.35	.176
	0.39 ± 0.46	0.50 ± 0.46	
P	26.57 ± 60.01	33.75 ± 39.86	.615
	0.08 ± 0.68	0.38 ± 0.45	
Glucose			
C	14.07 ± 15.67	12.36 ± 17.36	.924
	0.78 ± 0.87	0.69 ± 0.96	
P	8.81 ± 17.19	23.60 ± 26.71	.010
	0.49 ± 0.954	1.31 ± 1.48	
Total cholesterol			
C	9.07 ± 27.63	37.46 ± 28.22	.064
	0.23 ± 0.72	0.97 ± 0.73	
P	−7.48 ± 55.73	18.53 ± 57.63	.037
	−0.19 ± 1.44	0.48 ± 1.49	
HDL			
C	0.27 ± 13.55	−0.75 ± 12.69	.363
	0.007 ± 0.35	−0.02 ± 0.33	
P	−3.38 ± 19.92	−7.45 ± 16.34	.247
	−0.087 ± 0.52	−0.19 ± 0.42	
QUICKI			
C	−0.02 ± 0.05	0.01 ± 0.04	.043
P	0.04 ± 0.05	0.02 ± 0.04	.080
Uric acid			
C	0.14 ± 1.00	−0.68 ± 0.82	.075
	8.33 ± 59.48	−40.45 ± 48.77	
P	−0.13 ± 1.22	−0.97 ± 1.33	.032
	−7.73 ± 72.56	−57.695 ± 79.11	

Positive values indicate a rise and the negative values a drop.

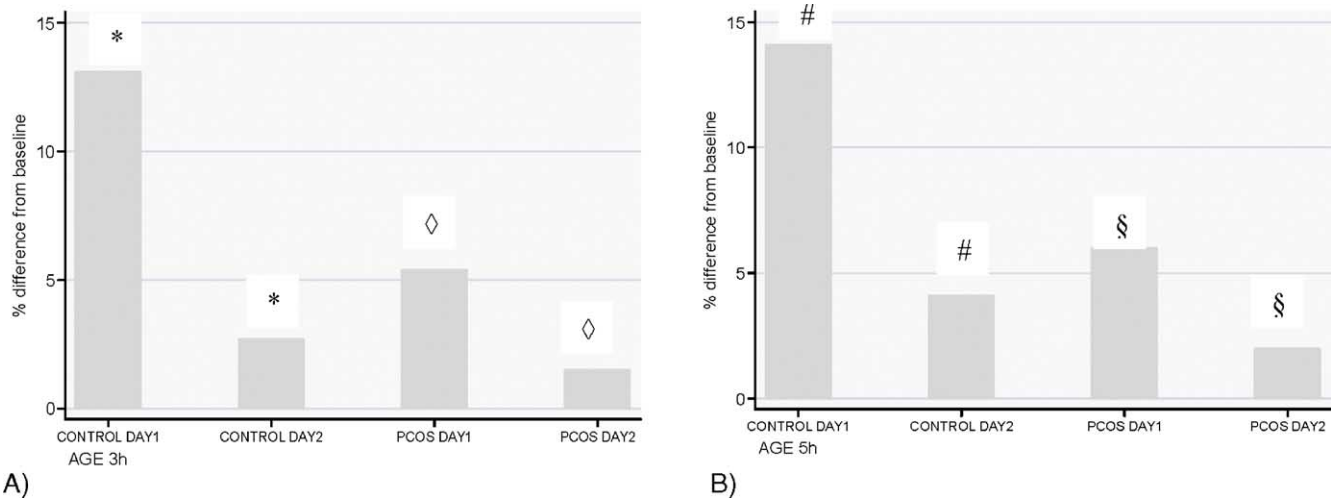

Fig. 1. The percentage of changes in AGE levels at 3 hours (A) and at 5 hours (B) postmeal for the 2 groups (control, PCOS) on day 1 (high-AGE meal) and day 2 (high-AGE meal + orlistat). *C, day 2 − day 1 (0-3 hours) = −73.3%; $^{\diamond}$P, day 2 − day 1 (0-3 hours) = −70.6%; $^{\#}$C, day 2 − day 1 (0-5 hours) = −63.1%; §P, day 2 − day 1 (0-5 hours) = −64.9%.

and at baseline and 3 hours postmeal (P = .03) for the control group, but not for women with PCOS (P = .10).

Regarding the percentage of rise in AGE levels over baseline at 3 and 5 hours postmeal for the 2 groups on both days, some observations were made.

On day 1, in the controls, a 13.1% rise in AGE levels was shown at 3 hours and 14.1% at 5 hours postmeal, whereas a 5.4% and 6.0% rise, respectively, was observed in the women with PCOS.

On day 2, a 2.7% rise in AGE levels was shown 3 hours postmeal and 4.1% 5 hours postmeal in the controls and 1.5% and 2.0%, respectively, in the women with PCOS.

The Δ values between 5 and 0 hours on day 1 compared with the same Δ values on day 2 showed a statistically significant difference for controls and the PCOS group (Table 3).

Fig. 1 summarizes the percentage of changes in AGE levels observed in the 2 groups on both days. For the control group, the postprandial rise in AGE levels was 73.3% lower 3 hours after the orlistat-supplemented meal than after the control meal. Similarly, in women with PCOS, the postprandial rise was 70.6% lower at 3 hours after orlistat meal than after the control meal (Fig. 1A). Accordingly, the control group presented a 63.1% lower postprandial rise in AGE levels at 5 hours after orlistat meal than after the control meal, whereas for the PCOS group, the postprandial rise in AGE levels was 64.9% lower at 5 hours after orlistat-supplemented meal than after the control meal (Fig. 1B).

3.3. Metabolic profile

The 2 groups differed in baseline values of insulin, triglycerides, fructosamine, and QUICKI index (Table 1).

The Δ values at 5 hours between day 1 and day 2 are indicated in Table 3. A statistically significant drop of uric acid levels (P = .03) and a rise of total cholesterol (P = .03) and glucose levels (P = .01) in PCOS group was observed. Furthermore, a statistically significant rise of QUICKI was observed in the control group (P = .04) (Table 3).

3.4. Hormonal profile

The PCOS group differed in total testosterone levels compared with controls (36.32 ± 14.60 ng/dL [control group] vs 92.95 ± 41.50 ng/dL [PCOS group], P < .001). The PCOS group did not differ in SHBG compared with controls (35.01 ± 9.72 nmol/L [control group] vs 37.69 ± 13.07 nmol/L [PCOS group], P = .50). Free androgen index differed with statistically significant difference between groups (109.25% ± 49.84% [control group] vs 285.03% ± 189.55% [PCOS group], P = .50) (Table 1).

4. Discussion

In the present study, it is demonstrated that orlistat administration resulted in a decrease of AGE absorption after ingestion of an AGE-rich meal in control as well as in women with PCOS.

The interventions in reducing exogenous AGE intake that have been studied so far are low-AGE diet [12] and administration of aminoguanidine [18]. The finding that orlistat has a significant inhibitory effect on AGE absorption is another alternative method and could be used as a tool in preventing the overabsorption of glycotoxins from AGE-rich foods. This may be of particular importance in populations with endogenous load of AGE, such as diabetic patients, obese individuals, patients with renal failure in dialysis, and young women with PCOS [1,12,13,19].

It has been shown recently that women with PCOS have higher serum levels of AGE, compared with controls, which were positively correlated with insulin resistance indices and hyperandrogenemia [13].

This syndrome affects 6% of women of reproductive age, and it is also known to be associated with increased

atherogenic risk factors and type 2 diabetes mellitus [20,21], where endogenous AGEs have been shown to play a significant role [22-24]. Considering that 10% of AGEs in food are absorbed from exogenous sources [7], reducing their absorption by orlistat could minimize the presence of a potentially aggravating environmental/ nutritional factor.

Particularly noteworthy in this study is that a lipase inhibitor reduces the proportion of absorbed AGE, in normal as well as in women with PCOS. The use of this medication is justified and might be proven clinically useful because the fat content plays a major role in AGE generation during cooking [6]. Fats more than proteins and proteins more than carbohydrates influence, by their relative content and type, the absorption of food glycotoxins [6]. Among several selected foods, lipid-rich poultry skin contained far greater amounts of AGE than foods that were high in proteins [7]. A recent very interesting study looking into AGE content of commonly consumed foods revealed that the highest mean values of food AGE are found in the fat-containing group of foods, intermediate values were found in meat and meat-substitute groups of food, and the lowest in the carbohydrate-containing group of foods [6,7]. This may be attributed to the radicals generated most efficiently from lipids during the heating of cutaneous fat, catalyzing the glucose-induced glucoxidative modification of free amine-containing lipids [25]. Considering the prominence of foods highly concentrated in fat in the westernized diet, and the effect of fat on glycotoxin absorption, the findings of the current study may be used as a tool in lifestyle modification.

The pathogenetic role of dietary glycol-lipotoxins is not clear. However, AGEs have been implicated in insulin resistance mechanisms including induction of cytokines, such as tumor necrosis factor α [26], activation of human endothelial cells in vivo, marked increase of mitogen-activated protein kinase (MAPK) phosphorylation, nuclear factor κB activity, and vascular cell adhesion molecule-1 (VCAM-1) secretion [27,28]. This may prove to be particularly ominous in PCOS where AGE may have a direct or indirect effect on pathogenetic mechanisms like insulin resistance because it has been shown that both cytokines and inflammatory factors are elevated [29-32].

The other metabolic parameters studied did not show any significant beneficial change after immediate orlistat administration, apart from plasma uric acid levels. The decrease of uric acid by orlistat has also been reported in long-term studies [33,34]. Considering the association of hyperuricemia with insulin resistance and other atherogenic factors, this reduction could be of clinical importance too [35,36].

However, no difference was found in a 6-month study with orlistat in a Greek diabetic population regarding insulin, HDL, triglyceride, and uric acid plasma levels, apart from a reduction in glucose and total cholesterol levels [34].

Among the limitations of our study, it should be considered that this is a nonplacebo, nonrandomized therapeutic trial. However, the fact that each subject was considered as its own control may decrease the bias of the above limitation. The presence of other glycol- or lipotoxins can be underestimated in our study because we measured only a limited number of AGE structures. Another limitation is that orlistat was given as a single dose, and therefore, long-term experiments are needed. Finally, we failed to demonstrate an immediate effect of orlistat on free fatty acid and plasma triglyceride levels. However, concentrations of free fatty acids in the blood are highly variable over the course of the day, precluding their practical use in clinical medicine [36], and triglycerides did not change even in long-term studies [34].

In this study, we present data suggesting that orlistat may reduce the absorption of food glycotoxins found in an everyday meal and that it could favorably contribute to the lifestyle modification. This observation has a potential of clinical importance especially for subjects prone to accelerated atherogenic process covering a broad spectrum from metabolic syndrome to diabetes.

References

[1] Vlassara H, Palace MR. Diabetes and advanced glycation end-products. J Intern Med 2002;251:87-101.

[2] Baynes JW. The Maillard hypothesis on aging: time to focus on DNA. Ann N Y Acad Sci 2002;959:360-7.

[3] Vlassara H, Bucala R, Striker L. Pathogenic effects of advanced glycosylation: biochemical, biologic, and clinical implications for diabetes and aging. Lab Invest 1994;70:138-51.

[4] Makino H, Shikata K, Hironaka K, et al. Ultrastructure of non-enzymatically glycated mesangial matrix in diabetic nephropathy. Kidney Int 1995;48:517-26.

[5] McCance DR, Dyer DG, Dunn JA, et al. Maillard reaction products and their relation to complications in insulin-dependent diabetes mellitus. J Clin Invest 1993;91:2470-8.

[6] Goldberg T, Cai W, Peppa M, et al. Advanced glycoxidation end products in commonly consumed foods. J Am Diet Assoc 2004;104:1287-91.

[7] Koschinsky T, He C-J, Mitsuhashi T, et al. Orally absorbed reactive glycation products (glycotoxins): an environmental risk factor in diabetic nephropathy. Proc Natl Acad Sci U S A 1997;94:6474-9.

[8] Lin RY, Dore AT, Ghodsi N, Fallon JT, Fisher EA, Vlassara H. Lowering of dietary advanced glycation endproducts (AGE) reduces neointimal formation after arterial injury in genetically hypercholesterolemic mice. Atherosclerosis 2002;163:303-11.

[9] Zheng F, He C, Cai W, Hattori M, Steffes M, Vlassara H. Prevention of diabetic nephropathy in mice by a diet low in glycoxidation products. Diabetes Metab Res Rev 2002;18:224-37.

[10] Vlassara H, Cai W, Crandall J, et al. Inflammatory markers are induced by dietary glycotoxins: a pathway for accelerated atherosclerosis in diabetes. Proc Natl Acad Sci U S A 2002;99:15596-601.

[11] Hofmann SM, Dong HJ, Li Z, et al. Improved insulin sensitivity is associated with restricted intake of dietary glycoxidation products in the *db*/*db* mouse. Diabetes 2002;51:2082-9.

[12] Uribarri J, Peppa M, Cai W, et al. Restriction of dietary glycotoxins reduces excessive advanced glycation end products in renal failure patients. J Am Soc Nephrol 2003;14:728-31.

[13] Diamanti-Kandarakis E, Piperi C, Kalofoutis A, Creatsas G. Increased levels of serum advanced glycation end-products in women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 2005;62:37-43.

[14] Hauptman JB, Jeunet FS, Hartmann D. Initial studies in humans with the novel gastrointestinal lipase inhibitor Ro 18-0647 (tetrahydrolipstatin). Am J Clin Nutr 1992;55:309S-13S.

[15] Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group Revised 2003 consensus on diagnostic criteria and long-term

health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81:19-25.
[16] Sakata N, Imanaga Y, Meng J, et al. Immunohistochemical localization of different epitopes of advanced glycation end products in human atherosclerotic lesions. Atherosclerosis 1998;141:61-75.
[17] Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.
[18] He C, Sabol J, Mitsuhashi T, Vlassara H. Dietary glycotoxins: inhibition of reactive products by aminoguanidine facilitates renal clearance and reduces tissue sequestration. Diabetes 1999;48:1308-15.
[19] Carmina E, Legro RS, Stamets K, Lowell J, Lobo RA. Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet. Hum Reprod 2003;18:2289-93.
[20] Miranda PJ, DeFronzo RA, Califf RM, Juyton G. Metabolic syndrome: definition, pathophysiology, and mechanisms. Am Heart J 2005;149:33-45.
[21] Diamanti-Kandarakis E, Kouli CR, Bergiele AT, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab 1999;84:4006-11.
[22] Xu X, Li Z, Luo D, et al. Exogenous advanced glycosylation end products induce diabetes-like vascular dysfunction in normal rats: a factor in diabetic retinopathy. Graefe Arch Clin Exp Ophthalmol 2003;241:56-62.
[23] Tan KC, Chow WS, Ai VH, Metz C, Bucala R, Lam KS. Advanced glycation end products and endothelial dysfunction in type 2 diabetes. Diabetes Care 2002;25:1055-9.
[24] Nakamura Y, Horii Y, Nishino T, et al. Immunohistochemical localization of advanced glycosylation end products in coronary atheroma and cardiac tissue in diabetes mellitus. Am J Pathol 1993; 143:1649-56.
[25] Uribarri J, Peppa M, Cai W, et al. Dietary glycotoxins correlate with circulating advanced glycation end product levels in renal failure patients. Am J Kidney Dis 2003;42:532-8.
[26] Yui S, Sasaki T, Araki N, Horiuchi S, Yamazaki M. Induction of macrophage growth by advanced glycation end products of the Maillard reaction. J Immunol 1994;152:1943-9.
[27] Cai W, He JC, Zhu L, et al. High levels of dietary advanced glycation end products transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients. Circulation 2004;110:285-91.
[28] Vlassara H, Fuh H, Makita Z, Krungkrai S, Cerami A, Bucala R. Exogenous advanced glycosylation end products induce complex vascular dysfunction in normal animals: a model for diabetic and aging complications. Proc Natl Acad Sci U S A 1992;89:12043-7.
[29] Paradisi G, Steinberg HO, Hempfling A, et al. Polycystic ovary syndrome is associated with endothelial dysfunction. Circulation 2001; 103:1410-5.
[30] Diamanti-Kandarakis E, Palioniko G, Alexandraki K, Bergiele A, Koutsouba T, Bartzis M. The prevalence of 4G5G polymorphism of plasminogen activator inhibitor (PAI-1) gene in polycystic ovarian syndrome and its association with plasma PAI-1 levels. Eur J Endocrinol 2004;150:795-800.
[31] Diamanti-Kandarakis E, Paterakis T, Alexandraki K, et al. Adhesion molecules in PCOS. Indices of low grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. Hum Reprod [submitted for publication].
[32] Diamanti-Kandarakis E, Spina G, Kouli C, Migdalis I. Increased endothelin-1 levels in women with polycystic ovary syndrome and the beneficial effect of metformin therapy. J Clin Endocrinol Metab 2001; 86:4666-73.
[33] Gokcel A, Gumurdulu Y, Karakose H, et al. Evaluation of the safety and efficacy of sibutramine, orlistat and metformin in the treatment of obesity. Diabetes Obes Metab 2002;4:49-55.
[34] Didangelos TP, Thanopoulou AK, Bousboulas SH, et al. The ORLIstat and CArdiovascular risk profile in patients with metabolic syndrome and type 2 DIAbetes (ORLICARDIA) Study. Curr Med Res Opin 2004;20:1393-401.
[35] Vuorinen-Markkola H, Yki-Jarvinen H. Hyperuricemia and insulin resistance. J Clin Endocrinol Metab 1994;78:25-9.
[36] Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971-1992. National Health and Nutrition Examination Survey. JAMA 2000;283: 2404-10.