Chios mastic gum modulates serum biochemical parameters in a human population

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Abstract

Introduction: Current research suggests that *Chios mastic* (*Pistacia lentiscus* var. *chia*) possesses beneficial (antimicrobial, antioxidant, hepatoprotective) properties. This study aims to assess its effects on cardiologic and hepatic biochemical indices of human subjects. *Materials and methods*: Subjects ($n = 133$, aged over 50) were randomly assigned to two groups, the first (high-dose group) ingesting daily 5 g of mastic powder and the second receiving daily a *Chios mastic* solution (low-dose group). Serum biochemical parameters were determined on a monthly basis for an 18-month (high-dose group) and a 12-month (low-dose group) follow-up period. Generalized least squares random-effects linear regression was performed. *Results*: The group ingesting *Chios mastic* powder (high-dose group) exhibited a decrease in serum total cholesterol, LDL, total cholesterol/HDL ratio, lipoprotein (a), apolipoprotein A-1, apolipoprotein B (apoB/apoA-1 ratio did not change), SGOT, SGPT and gamma-GT levels; in the second (low-dose) group, glucose levels decreased in males. *Discussion*: *Chios mastic* powder could have a hepatoprotective/cardioprotective role in vivo in humans.
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Keywords: *Pistacia lentiscus*; *Chios mastic*; Cholesterol; Lipoprotein; Apolipoprotein; Aminotransferase

1. Introduction

Chios mastic gum (CMG) is derived from *Pistacia lentiscus* var. *chia* cv. Anacardiaceae, a plant which is mainly met on the Greek island of Chios.

The beneficial, healing properties of mastic have been known since antiquity. From Dioscurides and Galenus (Kolliaros, 1997) to the 'Jerusalem Balsam' (Moussaieff et al., 2005), *Pistacia lentiscus* has been traditionally considered as a medical agent and is incorporated in Mediterranean cuisine. Interestingly enough, in his essay *De simplicium medicamentorum temperamentis ac facultatibus*, Galenus considers *Chios mastic* as a therapeutic means for hepatic inflammation, and for disorders of the stomach and intestine (Kühn, 1826). Similarly, Dioscorides Pedanius, in his essay *De materia medica*, states that *Chios mastic* positively influences the process of digestion, and additionally possesses cosmetic properties and actions beneficial for the teeth (Wellmann, 1907). Nowadays, and in line with the 2000-year tradition, food products (apart from the well-established chewing gum) and cosmetics based on mastic have been created.

The process of mastic gum production from the plant is simple; longitudinal incisions are made on all over the plant surface, not only on the main trunk, but also on thick branches (Andrikopoulos et al., 2003). Recently, besides the so-called "normal collection", another technique of mastic gum production has evolved, the "liquid collection". In this technique, the tree is not cut and phytohormones are injected to the plant, so that the product is derived in a less viscous form (Andrikopoulos et al., 2003; Assimopoulou and Papageorgiou, 2005).

The biological activity of *Pistacia lentiscus* can be attributed to a variety of compounds. It contains triterpenes of the oleanane, euphane and lupine type (Andrikopoulos et al., 2003; Assimopoulou and Papageorgiou, 2005), alpha-tocopherol (Kivcak and Akay, 2005) and polyphenols (Romani et al., 2002); the latter have been associated with a hypotensive effect of mastic (Sanz et al., 1992).

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Chios mastic possesses anti-bacterial activity (Iauk et al., 1996; Ali-Shtayeh et al., 1998; Magiatis et al., 1999; Koutsoudaki et al., 2005), for which verbenone, alpha-terpineol, and linalool seem to be responsible (Koutsoudaki et al., 2005). *In vivo*, its demonstrated antiplaque action in the oral cavity – also known by the traditional medicine in the Mediterranean region (Saez et al., 2005) since the time of Dioscorides (see above) – has been attributed to its inhibitory action against overall bacterial growth (Takahashi et al., 2003), and especially against *S. mutans* (Aksoy et al., 2006). As far as the effectiveness of *Pistacia lentiscus* against *Helicobacter pylori* and peptical ulcer is concerned, data remain controversial. Clinical studies have initially indicated that *Chios mastic* is effective against gastric and duodenal ulcer (Al-Habbal et al., 1984; Al-Said et al., 1986); then, mastic has been proven bactericidal against *H. pylori in vitro* (Huwez et al., 1998; Marone et al., 2001). However, recent studies show that it is unable to eradicate *H. pylori in vivo* in humans (Bebb et al., 2003; Loughlin et al., 2003).

Pistacia lentiscus has been traditionally regarded also as an anti-cancer agent, especially on tumours of breast, liver, stomach, spleen, and uterus (reported in Assimopoulou and Papageorgiou, 2005). Surprisingly enough, these traditional beliefs are in line with recent studies demonstrating that *Chios mastic* induces apoptosis (Balan et al., 2005) and possesses antiproliferative activity (Balan et al., 2006) in colon cancer cells.

Pistacia lentiscus has already been associated with cardiovascular protection and hepatoprotection. It inhibits human LDL oxidation *in vitro* (Andrikopoulos et al., 2003) and, thanks to the triterpenes, it acts on peripheral blood mononuclear cells to elicit an antioxidant/antiatherogenic effect (Dedoussis et al., 2004). Moreover, when an aqueous extract of *Pistacia lentiscus* was administered *per os* to rats intoxicated with carbon tetrachloride (CCl_4), it was proven hepatoprotective, reducing SGOT, SGPT, ALP and bilirubin levels, especially when the extract was not boiled (Janakat and Al-Merie, 2002).

In this study, the biological activity of *Chios mastic* is examined, with the stress put on the liver and the cardiovascular system of human subjects. We have chosen serum parameters, i.e. cholesterol, lipids, apolipoproteins and hepatic function markers, which are easy to measure and unanimously accepted.

2. Materials and methods

2.1. Study design

This study began in January 2003 and included 133 subjects randomly collected from a primary care center in the municipality of Kifissia, Athens, Greece. The sample comprised 93 women and 40 men, all aged over 50. Their medical history was free of cancer, myocardial infarct and hepatic disease. Subjects were randomly assigned to two groups: the high-dose (powder) group (48 patients, follow-up period of 18 months) and the low-dose (solution) group (85 patients, follow-up period of 12 months). A variety of biochemical indices were *a priori* selected to be measured in the patients' serum, monthly in both groups.

During the entire 18-month follow-up period, the high-dose group received daily 5 g of mastic powder, diluted in one glass (250 mL) of water. On a monthly basis, glucose, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, apolipoprotein A-1 (apoA-1), apolipoprotein B (apoB), lipoprotein (a), SGOT, SGPT aminotransferases and gamma-GT levels were measured in the subjects' serum. Furthermore, total cholesterol/HDL and apoB/apoA-1 ratios have been calculated.

During the 12-month period, the low-dose (solution) group ingested per day at most one-seventh of the daily dose taken by the high-dose group. More specifically, the low-dose group followed this procedure: one spoonful (the equivalent amount of the high-dose group) of traditional *Chios mastic* product was left in a glass of water for 24 h at room temperature, so that part of the product spontaneously dissolved in the water; then, the subjects ingested the water, without eating the traditional *Chios mastic* product. The glass (containing the product) was subsequently filled with fresh water; this fresh water would be ingested in turn after 24 h, and so on. The procedure was repeated every day and the remaining product left to dissolve in the glass was finally replaced every 7 days. In this group, glucose, total cholesterol, HDL, LDL, triglycerides, SGOT and SGPT aminotransferases, gamma-GT, alkaline phosphatase, urea, creatinine and uric acid levels were measured in the serum on a monthly basis. Informed consent was obtained from all subjects of the study.

2.2. Biochemical assays

For the measurement of serum urea, uric acid, SGOT, SGPT, gamma-GT, and alkaline phosphatase, *Chema Diagnostica* kits and a spectrophotometer with thermostatic cuvette holder were used. The composition of the urea kit consisted of a Tris buffer 115 mM, pH 7.60, 2-oxoglutarate 7.5 mM, ADP 1.2 mM, urease >8 KU/L, GLDH >800 U/L, NADH 0.25 mM and stabilizers (Burtis and Ashwood, 1998). The kit employed for uric acid contained phosphate buffer pH 7.0 50 mM, TOOS 0.34 mM, 4-aminoantipyrine 0.3 mM, urinase 450 U/L, POD >2500 U/L and surfactant (Fossati et al., 1980; Burtis and Ashwood, 1998). The composition of the SGOT kit was Tris buffer 80 mM pH 7.65, L-aspartate 240 mM, 2-oxoglutarate 12 mM, NADH 0.18 mM, MDH >600 U/L and LDH >900 U/L (German Society for Clinical Chemistry, 1970, 1972; Burtis and Ashwood, 1998; Schumann et al., 2002a,b); respectively, for SGPT, a Tris buffer 100 mM pH 7.15, L-alanine 500 mM, 2-oxoglutarate 15 mM, NADH 0.18 mM, LDH >1700 U/L was used (German Society for Clinical Chemistry, 1970, 1972; Burtis and Ashwood, 1998; Schumann et al., 2002a,b). The γGT kit consisted of a Tris buffer 100 mM pH 8.25, glycylglycine 100 mM, L-glutamyl-3-carboxy-4-nitroanilide 4 mM (Szasz, 1976; Burtis and Ashwood, 1998). The kit of alkaline phosphatase consisted of a DEA buffer pH 9.8 1 M, $MgCl_2$ 0.5 mM, 4-nitrophenylphosphate 10 mM (German Society for Clinical Chemistry, 1970, 1972; Burtis and Ashwood, 1998). For all the above factors, measurement of the optical density

Table 1
Biochemical parameters (mean ± standard deviation) at the beginning of the study

Biochemical parameters	High-dose (powder) group	Low-dose (solution) group
Glucose	108.9 ± 40.5 mg/dL	118 ± 67.2 mg/dL
Total cholesterol	273.3 ± 53 mg/dL	249.7 ± 43.5 mg/dL
Triglycerides	173.1 ± 84.3 mg/dL	139 ± 77.2 mg/dL
HDL	52.2 ± 14.8 mg/dL	59 ± 17.7 mg/dL
LDL	186.5 ± 50.2 mg/dL	160.9 ± 42.7 mg/dL
Apolipoprotein A-1 (apoA-1)	247.9 ± 70 mg/dL	Not measured
Apolipoprotein B (apoB)	160.2 ± 53.7 mg/dL	Not measured
Lipoprotein (a)	51.3 ± 38.5 mg/dL	Not measured
SGPT	23.8 ± 13.8 U/L	23.1 ± 17.3 U/L
SGOT	21.1 ± 7.4 U/L	21 ± 7.4 U/L
Gamma-GT	19.9 ± 10.8 U/L	19.2 ± 9.6 U/L
Urea	Not measured	35.9 ± 10.2 mg/dL
Creatinine	Not measured	0.8 ± 0.2 mg/dL
Uric acid	Not measured	5.1 ± 1.6 mg/dL
Alkaline phosphatase	Not measured	107.3 ± 36 U/L

was subsequently performed according to the manufacturer's instructions.

For the determination of serum glucose and cholesterol, the respective *biosis* kits were used (the one for glucose contained a buffer of pH 7.8, NAD 1.3 mM, Mg^{2+} 4 mM, ATP 1.3 mM, HK >1000 U/L, G6PDH >2000 U/L and conservatives; that for cholesterol contained a buffer of 0.1 M, pH 7.0, CE 0.6 U/mL, CO 1 U/mL, POD 4.0 U/mL, aminophenasone 0.4 mM, phenolic factor 10 mM, cofactors and stabilizers) (Burtis and Ashwood, 1998).

Three *Sentinel Diagnostics* reagents were applied for apoA-1 and apoB measurement. Reagent A contained phosphate buffer 20 mmol/L pH 7.5, poly-ethyleneglycol polymer (PEG) >5%, sodium chloride 150 mmol/L and sodium azide <0.1%. Reagent B contained sodium azide <0.1% and polyclonal antiserum of anti-lipoprotein A-1 and B, respectively. Reagent C consisted of Good's buffer 50 mmol/L pH 7.5, sodium chloride 150 mmol/L and sodium azide <0.1%. After appropriate mixture of the reagents according to the manufacturer's instructions, the absorbance of each sample was read (Kaplan and Pesce, 1996; Burtis and Ashwood, 1998).

Serum lipoprotein (a) [Lp(a)] values were determined with a *DiaSorin* kit, which consisted of an antibody reagent with 2 mL of goat antibody monospecific for lipoprotein (a) and an antibody diluent with 60 mL of a polymeric enhancer solution; both contained <0.1% sodium azide (Burtis and Ashwood, 1998).

Finally, for the determination of serum triglyceride, creatinine, HDL and LDL levels the respective *biosis* kits were employed. In the case of creatinine, equal volumes of NaOH and picric acid with concentrations NaOH 0.4N and picric acid 8.0 mM were mixed; the aforementioned react with creatinine and form a coloured product. For the measurement of triglycerides a buffer of pH 7.5, lipases 300 U/mL, glycerolkinase 0.2 U/mL, glycerol-3-P-oxidase 4 U/mL, ATP 1 mM, aminophenazone 0.4 mM, phenol product 4 mM and cofactors was used. For HDL measurement, additionally polyphosphoremic ions 0.56 mM and Mg^{2+} 25 mM were used. LDL was calculated from the Friedewald formula (LDL = total cholesterol − TG/5 − HDL) (Tremblay et al., 2004).

2.3. Statistical methods

Generalized least squares (GLS) random-effects linear regression was chosen; the aforementioned biochemical parameters were set as dependent variables. Time in months was treated as continuous variable. Univariable regression analysis for the effect of time was firstly done, and subsequently sex and time–sex interaction were checked as covariates for the models. The appropriateness of the random-effects models was checked by Hausman's specification test and Breusch and Pagan Lagrange multiplier test for random effects. Statistical analyses were performed with STATA 7.0 statistical package.

3. Results

The initial means of the examined biochemical parameters (before the initiation of *Chios mastic* regimes) are shown in Table 1 for both groups. In the high-dose (powder) group, the univariable analysis revealed a significant decrease in total cholesterol (Fig. 1), LDL, total choles-
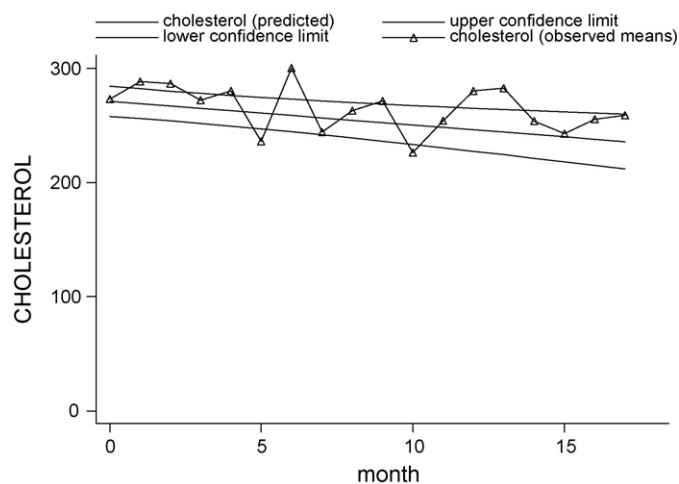

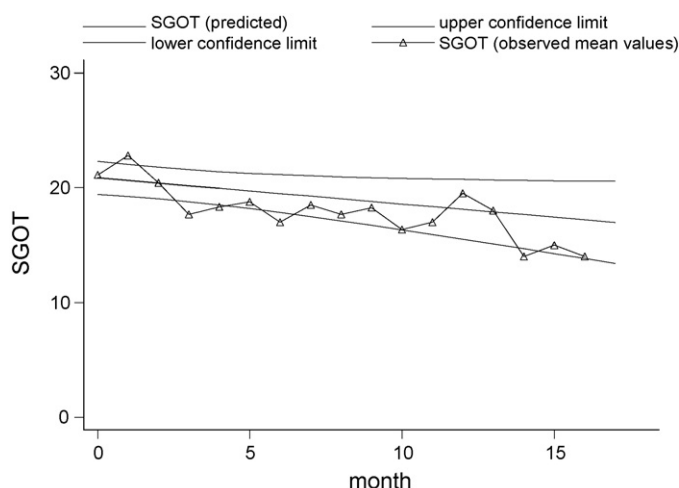Fig. 1. Total cholesterol levels (observed, predicted and 95% prediction bands) in the high-dose group.

Fig. 2. Serum SGOT in the high-dose group (observed, predicted values and 95% prediction bands).
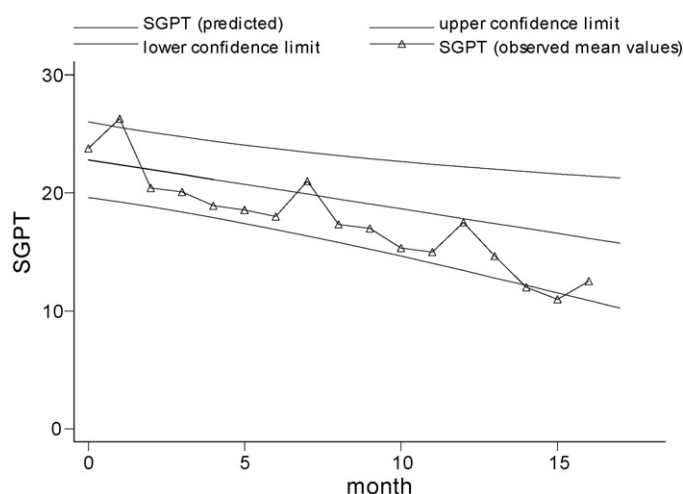

Fig. 3. The pattern of serum SGPT in the high-dose group (observed, predicted values and 95% prediction bands).

terol/HDL ratio, apoA-1, apoB, Lp(a), SGOT (Fig. 2), SGPT (Fig. 3) and gamma-GT levels (Table 2). On the contrary, glucose, HDL and triglyceride levels did not exhibit significant changes during the follow-up period. Since time has been treated as continuous variable (see Section 2.3), the underlying equations for Table 2, i.e. the equations yielding the predicted value for each biochemical parameter in the n-month $(0 < n < 18)$ are (value in the n-month) $=$ (initial value) $- n \times$ (mean change per month).

Subsequently, the inclusion of sex and sex–time interaction in the models allowed the examination of differences in the effect of *Chios mastic* between the two sexes. *Chios mastic* action did not vary with sex for the majority of the factors (LDL, apolipopro-

tein A-1, apolipoprotein B, SGPT, gamma-GT); that means that the pattern of these factors is satisfactorily described by the coefficients presented in Table 2. However, the effect differed along with sex as far as total cholesterol, lipoprotein (a) and SGOT are concerned; the action of *Chios mastic* by sex is shown in Table 3. Thus, the observed beneficial effect on cholesterol and SGOT levels was due to its protective action in male subjects, contrary to what happened for Lp(a) (Table 3).

In the low-dose group, no significant changes were demonstrated, except for a statistically significant decrease in serum glucose levels among male subjects (-3.1 mg/dL per month, $p = 0.003$). Serum glucose in women was not affected.

Table 2
Biochemical parameters influenced by daily consumption of 5 g *Chios mastic* powder

Biochemical parameters	Mean change per month[a]	Time–sex interaction
Total cholesterol	-2.1 mg/dL ($p = 0.002$)	$p = 0.097$
LDL	-2.09 mg/dL ($p = 0.001$)	NS[b]
Total cholesterol/HDL	-0.04 month^{-1} ($p = 0.018$)	$p = 0.047$
Lipoprotein (a)	-1.2 mg/dL ($p = 0.002$)	$p = 0.029$
Apolipoprotein A-1 (apoA-1)	-10.6 mg/dL ($p < 0.001$)	NS
Apolipoprotein B (apoB)	-5.4 mg/dL ($p < 0.001$)	NS
apoB/apoA-1 ratio	NS	
SGOT	-0.2 U/L ($p = 0.039$)	$p = 0.070$
SGPT	-0.4 U/L ($p = 0.005$)	NS
Gamma-GT	-0.3 U/L ($p = 0.014$)	NS

[a] Results from univariable regression analysis.
[b] NS: not statistically significant.

Table 3
Differential effects of *Chios mastic* by sex (in the high-dose group)

Biochemical parameters	Effect in women		Effect in men	
	Initial value	Mean change per month	Initial value	Mean change per month
Total cholesterol (mg/dL)	289.3 ± 52.2	NS (-1.3 mg/dL)	251.5 ± 39.8	Protective (-3.5 mg/dL)
Total cholesterol/HDL	5.37 ± 1.9	NS (-0.019 month^{-1})	6.15 ± 1.67	Protective (-0.096 month^{-1})
Lipoprotein (a) (mg/dL)	57.1 ± 42.4	Protective (-1.9 mg/dL)	42.8 ± 29.8	NS (-0.2 mg/dL)
SGOT (U/L)	19.2 ± 5.0	NS (-0.1 U/L)	24.6 ± 9.9	Protective (-0.5 mg/dL)

4. Discussion

High serum concentrations of total cholesterol and LDL represent well-established risk factors for cardiovascular disease (National Institutes of Health, 2002). Furthermore, the predictive value of total cholesterol/HDL ratio (TC/HDL, reviewed by Criqui and Golomb, 1998) has been demonstrated in men (Kinosian et al., 1995) and women (Hong et al., 1991; Castelli et al., 1992); higher values of TC/HDL are associated with coronary artery disease. To our knowledge, this study is the first one reporting a beneficial action of *Chios mastic* on total cholesterol, LDL and TC/HDL ratio in healthy human subjects. An interesting question that arises is whether *Chios mastic* action depends on the level of blood cholesterol, given the fact that the high-dose group exhibited slightly higher initial levels of total cholesterol (versus the low-dose group, Table 1), despite the random allocation of the subjects to the two groups. However, the concentration of cholesterol *per se* does not seem to predict the extent of *Pistacia lentiscus* action; men within the high-dose group exhibited lower total cholesterol levels, and *Chios mastic* was particularly effective in that subgroup (Table 3).

In agreement with the present study, a variety of recent, *in vitro* data suggest a protective effect of *P. lentiscus* on the cardiovascular system. Andrikopoulos et al. (2003) demonstrated that *Chios mastic* gum effectively protects human LDL from oxidation; another study demonstrated that it might function through restoration of glutathione and downregulation of CD36 receptor on peripheral blood mononuclear cells (Dedoussis et al., 2004). Together with the above results, the present findings support that *P. lentiscus* functions simultaneously in multiple protecting pathways for the cardiovascular system.

Interestingly enough, *Chios mastic* powder led to a reduction of lipoprotein (a) levels in female subjects. Lipoprotein (a) (reviewed by Berglund and Ramakrishnan, 2004), sharing many properties with LDL, is an important independent risk factor for cardiovascular disease (Danesh et al., 2000; Burman et al., 2004; Frohlich et al., 2004). Its predictive value has been proven for both sexes independently of age (Frohlich et al., 2004); nevertheless, a recent study on an elderly population has pointed out that its importance is limited only in the male individuals' subgroup (Ariyo et al., 2003). Given the above controversial studies and the age of our sample, our finding should be interpreted with caution, as far as its clinical significance is concerned.

High serum concentrations of apoB, which is mainly associated with the atherogenic lipoproteins (Rader and Hobbs, 2005), have been associated with cardiovascular disease; the observed reduction could thus be beneficial. On the other hand, the observed diminution of apoA-1 levels is not desirable, since the latter is the major apolipoprotein of HDL. Researchers have introduced and proven the predictive value of apoB/apoA-1 ratio (Dunder et al., 2004; Walldius et al., 2004); in our population this ratio did not change significantly, due to the parallel reduction of the numerator and the denominator.

Chios mastic powder also resulted in a decrease in serum SGOT, SGPT and gamma-GT. This finding is in line with its hepatoprotective properties demonstrated in a hepatotoxicity model in rats, to which CCl_4 was administered (Janakat and Al-Merie, 2002). Our results obviously imply hepatic protection in a much wider spectrum (i.e. the mild context of healthy individuals) and thus underline the potential favorable role for the general population. Our finding could be attributed to the antioxidant properties of mastic; *Pistacia lentiscus* was recently found to suppress iron-induced lipid peroxidation in rat liver homogenates, without being toxic (Ljubuncic et al., 2005a).

As expected, the majority of the significant results have been demonstrated in the high-dose group, obviously because of the greater amount of *Chios mastic* ingested; the fact that the action depended on the dose and differed between the two groups further supports that *Chios mastic* is indeed active *in vivo*. The low-dose group represented a different pharmacotechnical form, with considerable variability in the exact daily ingested dose and mimics the widespread low-dose *Chios mastic* consumption in the Greek population; surprisingly enough, a change in glucose in men in the low-dose group was observed. This might indicate that *Chios mastic* might be also effective even at lower concentrations and on other organs, for instance at the level of absorption in the intestine.

It should be stressed that in our sample, no side effects have appeared despite the age of the population. Contrary to a recent report demonstrating a hepatotoxic effect of *Pistacia lentiscus* aqueous extracts in rats (Ljubuncic et al., 2005b), we did not have such evidence in our sample. *Chios mastic* could thus be safe part of a broader health strategy incorporating diet modification and physical exercise.

In the present study, the reported mean changes per month refer to the study period as a whole, with the background assumption of a linear trend in the parameters; they cannot thus be extrapolated to longer time intervals. Larger survival studies with longer follow-up periods would be of particular interest, since they are theoretically able to document a time point, beyond which the daily ingestion does not further improve the biochemical indices (the latter reaching a plateau), and the duration of action after *Chios mastic* cessation (carryover effect). The inclusion of additional age groups and the adoption of experimental designs using standard nutritional protocols seem indispensable in order to confirm the clinical significance of our laboratory findings and the safety of the regime.

Most interestingly, the present cardiovascular and hepatic observations might be related to each other. *Chios mastic* might primarily act on the hepatocyte (as documented by the improvement in aminotransferase levels), and therein could modify the lipoprotein metabolism, either at the level of apolipoprotein biosynthesis or at the level of lipoprotein receptor expression. To answer the question if the present findings are two sides of the same coin, experimental studies on the underlying mechanisms are *sine qua non*. The originality of the present findings could thus be the basis and the stimulus for further, detailed research on *Chios mastic* action.

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References

Aksoy, A., Duran, N., Koksal, F., 2006. In vitro and in vivo antimicrobial effects of mastic chewing gum against *Streptococcus mutans* and mutans streptococci. Archives of Oral Biology 51, 476–481.

Al-Habbal, M.J., Al-Habbal, Z., Huwez, F.U., 1984. A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. Clinical and Experimental Pharmacology and Physiology 11, 541–544.

Ali-Shtayeh, M.S., Yaghmour, R.M., Faidi, Y.R., Salem, K., Al-Nuri, M.A., 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. Journal of Ethnopharmacology 60, 265–271.

Al-Said, M.S., Ageel, A.M., Parmar, N.S., Tariq, M., 1986. Evaluation of mastic, a crude drug obtained from *Pistacia lentiscus* for gastric and duodenal anti-ulcer activity. Journal of Ethnopharmacology 15, 271–278.

Andrikopoulos, N.K., Kaliora, A.C., Assimopoulou, A.N., Papapeorgiou, V.P., 2003. Biological activity of some naturally occurring resins, gums and pigments against in vitro LDL oxidation. Phytotherapy Research 17, 501–507.

Ariyo, A.A., Thach, C., Tracy, R., Cardiovascular Health Study Investigators, 2003. Lp(a) lipoprotein, vascular disease, and mortality in the elderly. The New England Journal of Medicine 349, 2108–2115.

Assimopoulou, A.N., Papageorgiou, V.P., 2005. GC-MS analysis of penta- and tetra-cyclic triterpenes from resins of Pistacia species. Part I. *Pistacia lentiscus* var. *chia*. Biomedical Chromatography 19, 285–311.

Balan, K.V., Demetzos, C., Prince, J., Dimas, K., Cladaras, M., Han, Z., Wyche, J.H., Pantazis, P., 2005. Induction of apoptosis in human colon cancer HCT116 cells treated with an extract of the plant product, Chios mastic gum. In Vivo 19, 93–102.

Balan, K.V., Prince, J., Han, Z., Dimas, K., Cladaras, M., Wyche, J.H., Sitaras, N.M., Pantazis, P., 2006. Antiproliferative activity and induction of apoptosis in human colon cancer cells treated in vitro with constituents of a product derived from *Pistacia lentiscus* L. var. *chia*. Phytomedicine [Epub ahead of print].

Bebb, J.R., Bailey-Flitter, N., Ala'Aldeen, D., Atherton, J.C., 2003. Mastic gum has no effect on *Helicobacter pylori* load in vivo. Journal of Antimicrobial Chemotherapy 52, 522–523.

Berglund, L., Ramakrishnan, R., 2004. Lipoprotein(a): an elusive cardiovascular risk factor. Arteriosclerosis, Thrombosis, and Vascular Biology 24, 2219–2226.

Burman, A., Jain, K., Gulati, R., Chopra, V., Agarwal, D.P., Vasisht, S., 2004. Lipoprotein(a) as a marker of coronary artery disease and its association with dietary fat. Journal of the Association of Physicians of India 52, 99–102.

Burtis, C.A., Ashwood, E.R. (Eds.), 1998. Tietz Textbook of Clinical Chemistry, 3rd ed. WB Saunders, Philadelphia, PA.

Castelli, W.P., Anderson, K., Wilson, P.W., Levy, D., 1992. Lipids and risk of coronary heart disease. The Framingham Study. Annals of Epidemiology 2, 23–28.

Criqui, M.H., Golomb, B.A., 1998. Epidemiologic aspects of lipid abnormalities. The American Journal of Medicine 105, 48S–57S.

Danesh, J., Collins, R., Peto, R., 2000. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 102, 1082–1085.

Dedoussis, G.V., Kaliora, A.C., Psarras, S., Chiou, A., Mylona, A., Papadopoulos, N.G., Andrikopoulos, N.K., 2004. Antiatherogenic effect of *Pistacia lentiscus* via GSH restoration and downregulation of CD36 mRNA expression. Atherosclerosis 174, 293–303.

Dunder, K., Lind, L., Zethelius, B., Berglund, L., Lithell, H., 2004. Evaluation of a scoring scheme, including proinsulin and the apolipoprotein B/apolipoprotein A1 ratio, for the risk of acute coronary events in middle-aged men: Uppsala Longitudinal Study of Adult Men (ULSAM). American Heart Journal 148, 596–601.

Fossati, P., Prencipe, L., Berti, G., 1980. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clinical Chemistry 26, 227–231.

Frohlich, J., Dobiasova, M., Adler, L., Francis, M., 2004. Gender differences in plasma levels of lipoprotein (a) in patients with angiographically proven coronary artery disease. Physiological Research 53, 481–486.

German Society for Clinical Chemistry, 1970. Recommendations of the German Society for Clinical Chemistry. Standardization of methods for the determination of enzyme activities in biological fluids. Zeitschrift fuer Klinische Chemie und Klinische Biochemie 8, 658–660.

German Society for Clinical Chemistry, 1972. Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. Zeitschrift fuer Klinische Chemie und Klinische Biochemie 10, 281–291.

Hong, M.K., Romm, P.A., Reagan, K., Green, C.E., Rackley, C.E., 1991. Usefulness of the total cholesterol to high-density lipoprotein cholesterol ratio in predicting angiographic coronary artery disease in women. The American Journal of Cardiology 68, 1646–1650.

Huwez, F.U., Thirlwell, D., Cockayne, A., Ala'Aldeen, D.A., 1998. Mastic gum kills *Helicobacter pylori*. The New England Journal of Medicine 339, 1946.

Iauk, L., Ragusa, S., Rapisarda, A., Franco, S., Nicolosi, V.M., 1996. In vitro antimicrobial activity of *Pistacia lentiscus* L. extracts: preliminary report. Journal of Chemotherapy 8, 207–209.

Janakat, S., Al-Merie, H., 2002. Evaluation of hepatoprotective effect of *Pistacia lentiscus*, *Phillyrea latifolia* and *Nicotiana glauca*. Journal of Ethnopharmacology 83, 135–138.

Kaplan, L.A., Pesce, A.J. (Eds.), 1996. Clinical Chemistry: Theory, Analysis, Correlation, 3rd ed. Mosby, St. Louis, MO.

Kinosian, B., Glick, H., Preiss, L., Puder, K.L., 1995. Cholesterol and coronary heart disease: predicting risks in men by changes in levels and ratios. Journal of Investigative Medicine 43, 443–450.

Kivcak, B., Akay, S., 2005. Quantitative determination of alpha-tocopherol in *Pistacia lentiscus*, *Pistacia lentiscus* var. *chia*, and *Pistacia terebinthus* by TLC-densitometry and colorimetry. Fitoterapia 76, 62–66.

Kolliaros, G., 1997. *Chios mastic* from antiquity to today. In: *Chios mastic*. Tradition and Current Practice (Acta of the International Symposium held in Chios, 3–5 October 1997), Athens, pp. 242–243 [reference in Greek].

Koutsoudaki, C., Krsek, M., Rodger, A., 2005. Chemical composition and antibacterial activity of the essential oil and the gum of *Pistacia lentiscus* Var. *chia*. Journal of Agricultural and Food Chemistry 53, 7681–7685.

Kühn, C.G. (Ed.), 1826. Claudii Galeni opera omnia, vol. 12. Leipzig, Knobloch.

Ljubuncic, P., Azaizeh, H., Portnaya, I., Cogan, U., Said, O., Saleh, K.A., Bomzon, A., 2005a. Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel. Journal of Ethnopharmacology 99, 43–47.

Ljubuncic, P., Song, H., Cogan, U., Azaizeh, H., Bomzon, A., 2005b. The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental liver disease. Journal of Ethnopharmacology 100, 198–204.

Loughlin, M.F., Ala'Aldeen, D.A., Jenks, P.J., 2003. Monotherapy with mastic does not eradicate *Helicobacter pylori* infection from mice. Journal of Antimicrobial Chemotherapy 51, 367–371.

Magiatis, P., Melliou, E., Skaltsounis, A.L., Chinou, I.B., Mitaku, S., 1999. Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. *chia*. Planta Medica 65, 749–752.

Marone, P., Bono, L., Leone, E., Bona, S., Carretto, E., Perversi, L., 2001. Bactericidal activity of *Pistacia lentiscus* mastic gum against *Helicobacter pylori*. Journal of Chemotherapy 13, 611–614.

Moussaieff, A., Fride, E., Amar, Z., Lev, E., Steinberg, D., Gallily, R., Mechoulam, R., 2005. The Jerusalem Balsam: from the Franciscan Monastery in the old city of Jerusalem to Martindale 33. Journal of Ethnopharmacology 101, 16–26.

National Institutes of Health, 2002. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).

Rader, D.J., Hobbs, H.H., 2005. Disorders of lipoprotein metabolism. In: Kasper, D.L., Braunwald, E., Fauci, A.S., Hauser, S.L., Longo, D.L., Jameson, J.L. (Eds.), Harrison's Principles of Internal Medicine, 16th ed. McGraw-Hill Companies, Inc., New York, pp. 2286–2298.

Romani, A., Pinelli, P., Galardi, C., Mulinacci, N., Tattini, M., 2002. Identification and quantification of galloyl derivatives, flavonoid glycosides and

anthocyanins in leaves of *Pistacia lentiscus* L. Phytochemical Analysis 13, 79–86.

Saez, J.M., Lopez, J., Romero, M., 2005. The historical roots of popular practices in oral health: *Pistacia lentiscus* in Cartagena, Murcia (Spain). Journal of the History of Dentistry 53, 109–112.

Sanz, M.J., Terencio, M.C., Paya, M., 1992. Isolation and hypotensive activity of a polymeric procyanidin fraction from *Pistacia lentiscus* L. Pharmazie 47, 466–477.

Schumann, G., Bonora, R., Ceriotti, F., et al., 2002a. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. Clinical Chemistry and Laboratory Medicine 40, 718–724.

Schumann, G., Bonora, R., Ceriotti, F., et al., 2002b. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 5. Reference procedure for the measurement of catalytic concentration of aspartate aminotransferase. Clinical Chemistry and Laboratory Medicine 40, 725–733.

Szasz, G., 1976. Reaction-rate method for gamma-glutamyltransferase activity in serum. Clinical Chemistry 22, 2051–2055.

Takahashi, K., Fukazawa, M., Motohira, H., Ochiai, K., Nishikawa, H., Miyata, T., 2003. A pilot study on antiplaque effects of mastic chewing gum in the oral cavity. Journal of Periodontology 74, 501–505.

Tremblay, A.J., Morrissette, H., Gagne, J.M., Bergeron, J., Gagne, C., Couture, P., 2004. Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population. Clinical Biochemistry 37, 785–790.

Walldius, G., Jungner, I., Aastveit, A.H., Holme, I., Furberg, C.D., Sniderman, A.D., 2004. The apoB/apoA–I ratio is better than the cholesterol ratios to estimate the balance between plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk. Clinical Chemistry and Laboratory Medicine 42, 1355–1363.

Wellmann, M. (Ed.), 1907. Pedanii Dioscuridis Anazarbei de materia medica libri quinque, vol. 1. Weidmann, Berlin.