Evaluation of the *in vitro* anti-atherogenic activities of goat milk and goat dairy products

Stylianios Poutzalis¹ · Areti Anastasiadou¹ ·
Constantina Nasopoulou² · Kalliopi Megale mou¹ ·
Eleni Sioriki¹ · Ioannis Zabetakis¹

Received: 15 June 2015 / Revised: 12 October 2015 / Accepted: 12 October 2015 /
Published online: 21 October 2015
© INRA and Springer-Verlag France 2015

Abstract Given that goat milk and dairy products should be consumed daily according to suggestions based on Mediterranean diet, the current study evaluates the anti-atherogenic properties of goat milk and goat dairy products (yogurt and white cheese). Total lipids (TLs) of all three samples were extracted by the method of Bligh and Dyer and further separated into total polar lipids (TPLs) and total neutral lipids (TNLs) by counter current distribution. The fatty acid profiles of TPL and TNL of all three samples were determined by gas chromatography analysis. TL and TPL were tested to determine whether they induce platelet aggregation or inhibit platelet aggregation induced by the platelet-activating factor (PAF). The most active lipids were found in goat white cheese (i.e., since they showed lower IC₅₀ values in both TL and TPL samples than corresponding fractions of goat milk and goat yogurt), so the TPL of goat white cheese were further separated by preparative thin-layer chromatography (TLC). The obtained polar lipid fractions after TLC separation were also tested for their biological activity. All the samples' lipids, and especially the polar ones, were found to exhibit strong anti-atherogenic activities. This fact highlights the nutritional value of goat dairy products in terms of cardioprotection, as PAF is a crucial inflammatory mediator that is implicated in the mechanism of atherogenesis.

Keywords Goat · Platelet-activating factor (PAF) · Polar lipids · Atherosclerosis · Platelets

✉ Ioannis Zabetakis
izabet@chem.uoa.gr

¹ Laboratory of Food Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, 15771 Athens, Greece

² Department of Food Science and Nutrition, School of the Environment, University of the Aegean, Myrina, Lemnos 81400, Greece

1 Introduction

The Mediterranean diet has been linked with a protective role against chronic diseases, mainly cardiovascular diseases (CVDs) and cancer (Panagiotakos et al. 2005). Milk and dairy products are important components of the Mediterranean diet (Selvaggi et al. 2014), and it is proposed that they be consumed daily (Kastorini et al. 2010). Additionally, dairy products (i.e., yogurt and cheese) have been shown more beneficially effective against atherogenesis and CVDs than milk (Tsorotioti et al. 2014). This has been attributed to the fact that the microorganisms *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, that are involved in the fermentation of yogurt and cheese, have been found to aid the bioformation of lipids that inhibit PAF-induced platelet activation (Antonopoulou et al. 1996).

Platelet-activating factor (PAF) (1-*O*-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine) is a crucial inflammatory phospholipid mediator (Demopoulos et al. 1979) that is implicated in the mechanism of atherogenesis (Demopoulos et al. 2003). According to this mechanism, PAF is produced during low-density lipoprotein (LDL) oxidation (Liapikos et al. 1994) and causes in situ inflammation. PAF is a compound of atheromatic plaque and is essential for the activation of leukocytes and their binding to endothelial cells (Nasopoulou et al. 2013).

Goats (meat, milk, and dairy products) have lately been at the center of attention due to the increasing interest in the nutritional value of their products (Park and Haenlein 2013). There is also an increasing need to consume goat milk, as a growing part of the population suffer from intolerance and allergy to other types of milk, such as cow milk (Selvaggi et al. 2014).

Goat milk consumption, compared to that of cow milk, decreases plasma cholesterol concentration. Moreover, consumption of this type of milk decreases the plasma concentration of triglycerides and therefore has a positive effect, similar to that of virgin olive oil (López-Aliaga et al. 2005).

Goat milk is a reliable source of high-quality protein (Raynal-Ljutovac et al. 2008) and has higher protein and fat digestibility than cow milk (Sanz Ceballos et al. 2009) (López-Aliaga et al. 2010). Fat high digestibility is due to smaller fat globule size and higher contents of short- and medium-chain fatty acids. The smaller-sized fat globules result in a better dispersion and a more homogeneous mixture of fat in the milk, and the larger surface/volume ratio of the fat globules enhances further pancreatic lipase activity, making goat milk easier to digest (López-Aliaga et al. 2010).

The prevention of cardiovascular diseases (CVDs) and atherosclerosis, in particular, is a major objective of research in life sciences (Zabetakis 2013). The aim of the current study is, thus, to determine the activities of goat milk and related dairy products against atherogenesis and therefore the onset of CVDs.

2 Materials and methods

2.1 Reagents and instruments

All reagents and solvents were of analytical grade purchased from Merck (Darmstadt, Germany). Fatty acid methyl ester standards bought individually were of GC-quality

and supplied by Sigma-Aldrich (St. Louis, MO, USA), as well as bovine serum albumin (BSA) and PAF.

Chromatographic material used for thin-layer chromatography (TLC) was silica gel G-60 supplied by Merck (Darmstadt, Germany) and polar lipid standards used for TLC was a standard mix of hen egg yolk supplied by Sigma-Aldrich (St. Louis, MO, USA). Platelet aggregation was measured in a Chrono-Log (Havertown, PA, USA) aggregometer (model 400-VS) coupled to a Chrono-Log recorder (Havertown, PA, USA).

2.2 Samples of goat milk and goat dairy products

Three different samples of goat products (milk, yogurt, and white cheese) were purchased from the local market.

2.3 Isolation of lipids

Total lipids (TLs) were extracted from 200 g of each sample (goat milk, goat yogurt, and goat white cheese) according to the Bligh-Dyer method (Bligh and Dyer 1959). One tenth of the TL was weighed and stored in sealed vials under nitrogen atmosphere at -20°C until used, while the rest of it was further separated into total polar lipids (TPL) and total neutral lipids (TNL) by countercurrent distribution chromatography (Galanos and Kapoulas 1962). In brief, TLs were mixed with pre-equilibrated solvents: petroleum ether and 87% aqueous ethanol in ratio 3:1 (v/v). The obtained biphasic solvent system contained TPL distributed in the ethanol phase and TNL distributed in the petroleum ether phase.

The experimental procedure is as follows: petroleum ether and 87% aqueous ethanol were pre-equilibrated in a separatory funnel. The lower phase containing equilibrated 87% ethanol and the upper phase containing equilibrated petroleum ether were collected separately. An amount of dry TL was dissolved in 9 mL pre-equilibrated petroleum ether and then 3 mL pre-equilibrated 87% ethanol were added and stirred. The lower phase, which is the ethanol phase, was collected and transferred to second test tube containing 9 mL pre-equilibrated petroleum ether and was stirred again. Ethanolic phase was then transferred to a third empty test tube. The procedure was repeated eight times in total. Finally, the ethanolic phase (8×3 mL), containing TPL, and the phase of petroleum ether (2×9 mL), containing TNL, were evaporated to dryness, weighed, redissolved in chloroform: methanol in ratio 1:1 (v/v), and stored under nitrogen in sealed vials at -20°C until used—after a short period of time—for further analysis.

TPL of goat white cheese were further separated by preparative TLC. All TLC lipid fractions obtained were stored under nitrogen atmosphere at -20°C for further analysis.

2.4 Fractionation of TPL by preparative TLC

The TLC glass plates (20×20 cm) were coated with silica gel G-60 and activated by heating at 120°C for 60 min. The thickness of the TLC plates was 1.0 mm (preparative TLC). Approximately, 50 mg of TPL of the white cheese was applied to the TLC plates. A developing system consisting of chloroform/methanol/water 65:35:6 (v/v/v)

was used. The plates were stained under iodine vapors. Seven bands appeared after the separation of TPL of the white cheese sample. After staining of the TLC plate with iodine vapors, the bands were scraped off, and lipids were extracted from silica gel according to the Bligh-Dyer method (Bligh and Dyer 1959). The chloroform phase was evaporated to dryness under nitrogen, and lipids were weighed, redissolved in 1 mL chloroform/methanol 1:1 (v/v), and stored at -20°C .

2.5 Biological assay on washed rabbit platelets

TL and TPL of all samples, and purified polar lipid fractions of the white cheese, obtained by the above TLC separation, were tested for their biological activity according to the washed rabbit platelet assay (Demopoulos et al. 1979). Briefly, all examined samples and PAF were dissolved in $2.5\text{ mg BSA mL}^{-1}$ saline (0.90% w/v NaCl). Serially increasing amounts of the examined sample were added into the aggregometer cuvette, and their ability to aggregate washed rabbit platelets and/or to inhibit PAF-induced platelet aggregation was determined. Washed rabbit platelet concentration was approximately $500,000\text{ platelets }\mu\text{L}^{-1}$. In order to determine the aggregatory efficiency of either PAF or the examined samples, the maximum reversible PAF-induced aggregation was evaluated, and the 100% aggregation point was determined. The plot of the percentage of the maximum reversible aggregation (ranging from 20 to 80%) versus different concentrations of the aggregatory agent was linear. From this curve, the concentration of the aggregatory agent, which induces 50% of the maximum reversible PAF-induced aggregation, was calculated. This value is defined as the amount of the sample that induces an equivalent to PAF EC_{50} , namely equivalent concentration for 50% aggregation.

In order to determine the inhibitory properties of the samples' lipids, serially increasing amounts of the lipids being examined were added into the aggregometer cuvette, and their ability to inhibit PAF-induced aggregation was determined. The platelet aggregation induced by PAF ($29.59 \times 10^{-11}\text{ M}$, final concentration in the cuvette) was measured as PAF-induced aggregation in washed rabbit platelets before (considered as 0% inhibition) and after the addition of various amounts of the sample being examined. Consequently, the plot of percent inhibition (ranging from 20 to 80%) versus different concentrations of the sample is linear. From this curve, the concentration of the sample, which inhibited 50% the PAF-induced aggregation, was calculated. This value is defined as IC_{50} , namely inhibitory concentration for 50% inhibition.

2.6 Gas chromatographic analysis

Fatty acid methyl esters (FAME) of 35 mg of TPL and 35 mg of TNL of all three samples were prepared using a solution of 0.5 N KOH in CH_3OH (KOH- CH_3OH method, reaction time 5 min) and extracted with *n*-hexane. The fatty acid analysis was carried out using the internal standard method (Nasopoulou et al. 2011). A five-point calibration curve was prepared using five solutions of heptadecanoic (17:0) acid methyl ester and heneicosanoic (21:0) acid methyl ester in ratios of 1000:2000 (v/v), 1000:1000 (v/v), 1000:400 (v/v), 1000:200 (v/v), and 1000:100 (v/v), respectively. Five injections of $1\text{ }\mu\text{L}$ of each solution were analyzed with a

Shimadzu CLASS-VP (GC-17A) (Kyoto, Japan) gas chromatograph equipped with a split/splitless injector and flame ionization detector. The ratio of the mean area of heneicosanoic to that of the internal standard (heptadecanoic) was used as the y-axis variable of the calibration curve, while the concentration (mg.kg^{-1}) of heneicosanoic was used as the x-axis variable of the calibration curve. The equation that described the calibration curve was $y = 0.00012x + 0.0167$ with $r=0.99993$.

The ratio of the area of the analyte peak to that of the internal standard represented the y value in the above equation, and subsequently, the x value represented the analyte concentration of the fatty acid in the unknown mixture. Separation of fatty acid methyl esters was achieved on an Agilent J&W DB-23 fused silica capillary column ($60 \text{ m} \times 0.251 \text{ mm i.d.}, 0.25 \mu\text{m}$; Agilent, Santa Clara, CA, USA). The oven temperature value sequence was initially 120°C for 5 min, raised to 180°C at $10^\circ\text{C min}^{-1}$, then to 220°C at $20^\circ\text{C min}^{-1}$, and finally isothermal at 220°C for 30 min. The injector and detector temperatures were maintained at 220 and 225°C , respectively. The carrier gas was high purity helium with a linear flow rate of 1 mL.min^{-1} and split ratio of 1:50. Fatty acid methyl esters were identified using Supelco 37-Component FAME Mix in dichloromethane.

2.7 Statistical analysis

All experiment analyses were carried out in triplicate, and all results were expressed as mean value $\pm$ SD. One-way analysis of variance (ANOVA) was used in order to find the statistically significant differences. Differences were considered to be statistically significant when p was lower than 0.05. The data were analyzed using a statistical software package (PASW 18 for Windows, SPSS Inc., Chicago, IL, USA).

3 Results

3.1 TL, TPL, and TNL contents of goat milk, goat yogurt, and goat white cheese samples

The amount of TL, TPL, and TNL of all three samples are shown in Table 1. The yield of the total lipid extraction was found to be 70%. TPL and TNL profiles (both expressed as %TL) of all three samples were found to be similar ($p>0.05$).

Table 1 Content of total lipids (TL), expressed in grams per 100 g sample (mean $\pm$ SD, $n=3$), total polar lipids (TPL), and total neutral lipids (TNL), expressed as percentages of TL in goat milk, goat yogurt, and goat white cheese

Samples	TL (g/100 g sample)	TPL (% TL)	TNL (% TL)
Goat milk	2.18	5.81	87.9
Goat yogurt	2.60	5.78	90.9
Goat white cheese	17.1	6.19	87.8

3.2 Fatty acid profiles of TPL and TNL of goat milk, goat yogurt, and goat white cheese samples

The fatty acid profiles of TPL and TNL of all three samples are presented in Tables 2 and 3, respectively. Significant amounts of saturated fatty acids 6:0, 10:0, 12:0, 14:0, 16:0, and 18:0 have been detected, along with the monounsaturated fatty acid 18:1 *cis* (ω -9) and the polyunsaturated fatty acids 18:2 (ω -6) and 18:3 (ω -3), in both TPL and TNL fractions. The fatty acid profiles of goat milk, goat yogurt, and goat white cheese exhibited statistical significant differences (Tables 2 and 3). Goat white cheese was found to contain statistical significant increased levels of all detected fatty acids in comparison to the ones of goat milk and goat yogurt, which is reasonable due to the higher content of fat per kilogram of goat white cheese than goat milk and yogurt. Fatty acid profile and generally the composition of goat milk and its products are influenced by several factors such as breed, age, genotype, feed, environment, season, and technology (Raynal-Ljutovac et al. 2008).

3.3 Biological activity of TL, TPL of all three samples

The extracted TL and TPL of each sample were tested for their ability to induce washed rabbit platelet aggregation or inhibit PAF-induced platelet aggregation. When they induce platelet aggregation, they act like PAF, but they are thousands of times less active than PAF, thus they are practically acting as PAF inhibitors (Nasopoulou et al. 2013). These PAF-agonists (with PAF-like activity) have been found to have better in vivo anti-atherogenic activity than PAF inhibitors (Tsantila et al. 2007) (Nasopoulou et al. 2010).

Table 2 Fatty acid profile of total polar lipids (TPL) of each sample expressed in milligrams per kilogram of sample (mean $\pm$ SD, $n=3$)

Fatty acids	Goat milk	Goat yogurt	Goat white cheese
6:0	8.66 $\pm$ 2.35 ^{a, b}	14.1 $\pm$ 1.02 ^{a, c}	39.3 $\pm$ 2.62 ^{b, c}
8:0	ND	ND	ND
10:0	11.5 $\pm$ 0.63 ^{a, b}	6.52 $\pm$ 0.11 ^{a, c}	108 $\pm$ 0.18 ^{b, c}
12:0	6.16 $\pm$ 0.22 ^{a, b}	7.01 $\pm$ 0.16 ^{a, c}	60.2 $\pm$ 4.61 ^{b, c}
14:0	15.5 $\pm$ 0.05 ^{a, b}	19.7 $\pm$ 0.17 ^{a, c}	134 $\pm$ 2.54 ^{b, c}
15:0	0.18 $\pm$ 0.02 ^{a, b}	0.34 $\pm$ 0.03 ^{a, c}	5.12 $\pm$ 0.43 ^{b, c}
16:0	45.2 $\pm$ 0.45 ^{a, b}	64.4 $\pm$ 0.86 ^{a, c}	373 $\pm$ 0.34 ^{b, c}
16:1 (ω -7)	0.55 $\pm$ 0.09 ^{a, b}	0.39 $\pm$ 0.04 ^{a, c}	ND
18:0	24.3 $\pm$ 0.56 ^{a, b}	41.2 $\pm$ 0.29 ^{a, c}	217 $\pm$ 19.4 ^{b, c}
18:1 <i>cis</i> (ω -9)	58.0 $\pm$ 0.07 ^{a, b}	94.0 $\pm$ 0.47 ^{a, c}	447 $\pm$ 17.1 ^{b, c}
18:2 (ω -6)	10.4 $\pm$ 0.14 ^{a, b}	17.2 $\pm$ 0.25 ^{a, c}	64.1 $\pm$ 0.72 ^{b, c}
18:3 (ω -3)	0.76 $\pm$ 0.03 ^{a, b}	1.44 $\pm$ 0.05 ^{a, c}	15.0 $\pm$ 0.23 ^{b, c}

ND Non-detectable

^a Indicates statistical significance between goat milk and goat yogurt ($p<0.05$), according to ANOVA analysis

^b Indicates statistical significance between goat milk and goat white cheese ($p<0.05$), according to ANOVA analysis

^c Indicates statistical significance between goat yogurt and goat white cheese ($p<0.05$), according to ANOVA analysis

Table 3 Fatty acid profile of total neutral lipids (TNL) of each sample expressed in milligrams per kilogram of sample (mean $\pm$ SD, $n=3$)

Fatty acids	Goat milk	Goat yogurt	Goat white cheese
6:0	83.5 $\pm$ 3.87 ^{a, b}	114 $\pm$ 7.02 ^{a, c}	584 $\pm$ 19.1 ^{b, c}
8:0	ND	ND	ND
10:0	245 $\pm$ 13.8 ^{a, b}	300 $\pm$ 16.2 ^{a, c}	1529 $\pm$ 22.3 ^{b, c}
12:0	137 $\pm$ 6.74 ^{a, b}	165 $\pm$ 7.16 ^{a, c}	808 $\pm$ 10.5 ^{b, c}
14:0	367 $\pm$ 10.7 ^{a, b}	453 $\pm$ 9.63 ^{a, c}	1878 $\pm$ 21.2 ^{b, c}
15:0	11.9 $\pm$ 0.39 ^{a, b}	14.7 $\pm$ 1.08 ^{a, c}	69.2 $\pm$ 1.91 ^{b, c}
16:0	1112 $\pm$ 10.6 ^{a, b}	1373 $\pm$ 21.2 ^{a, c}	5253 $\pm$ 49.8 ^{b, c}
16:1 (ω -7)	3.13 $\pm$ 0.80 ^{a, b}	3.86 $\pm$ 0.99 ^{a, c}	ND
18:0	609 $\pm$ 4.00 ^{a, b}	751 $\pm$ 5.29 ^{a, c}	2885 $\pm$ 38.6 ^{b, c}
18:1 cis (ω -9)	927 $\pm$ 4.76 ^{a, b}	1145 $\pm$ 6.32 ^{a, c}	6507 $\pm$ 51.3 ^{b, c}
18:2 (ω -6)	85.8 $\pm$ 0.57 ^{a, b}	105 $\pm$ 1.55 ^{a, c}	916 $\pm$ 14.1 ^{b, c}
18:3 (ω -3)	13.0 $\pm$ 0.09 ^{a, b}	16.0 $\pm$ 0.13 ^{a, c}	215 $\pm$ 9.50 ^{b, c}

ND Non-detectable

^a Indicates statistical significance between goat milk and goat yogurt ($p<0.05$), according to ANOVA analysis^b Indicates statistical significance between goat milk and goat white cheese ($p<0.05$), according to ANOVA analysis^c Indicates statistical significance between goat yogurt and goat white cheese ($p<0.05$), according to ANOVA analysis

The biological activities of TL and TPL of each sample expressed in micrograms are shown in Fig. 1. For the comparison of IC₅₀ values, we should mention that a lower IC₅₀ value corresponds to a smaller amount of lipids needed for the same biological activity. So, a lower IC₅₀ value is preferable. For the comparison of EC₅₀ values, the opposite applies, i.e., a higher EC₅₀ value is preferable, corresponding to a higher amount of lipids needed for 50% PAF-like aggregation.

According to Fig. 1, TL and TPL exhibited inhibitory activity toward PAF-induced platelet aggregation, while none of the TL or TPL have shown PAF-like aggregatory activity. Additionally, TL of goat white cheese sample had a significantly lower ($p<0.05$) IC₅₀ value compared to TL of the other two samples. Also, TL of goat yogurt sample had significantly lower ($p<0.05$) IC₅₀ value compared to TL of goat milk sample. TPL of goat milk and goat white cheese samples appeared to have similar IC₅₀ values ($p>0.05$), but they were found to be significantly lower ($p<0.05$) than goat yogurt's IC₅₀ value.

3.4 Biological activity of TLC polar lipid fractions from goat white cheese sample

TPL of goat white cheese were further separated with preparative TLC and seven polar lipid fractions were obtained (Fig. 2).

TLC polar lipid fractions of goat white cheese were found to exhibit strong inhibitory activity toward PAF (Fig. 3). TLC polar lipid fractions with the most potent anti-PAF properties were found to be lipid fractions 3 (R_f value similar to

Fig. 1 In vitro biological activity of total lipids (TL) and total polar lipids (TLP) of goat milk, goat yogurt, and goat white cheese samples toward washed rabbit platelet aggregation, expressed in micrograms

that of phosphatidylcholine—PC), 5 (R_f value similar to that of phosphatidylethanolamine—PE), and 6, which had similar IC_{50} values ($p > 0.05$). The IC_{50} values of these three lipid fractions were also significantly lower ($p < 0.05$) when compared to the IC_{50} values of the rest TLC polar lipid fractions. Also, polar lipid fraction 2 was the only fraction that was found to have a PAF-like aggregatory activity (EC_{50} value). This polar lipid fraction had similar R_f value to that of sphingomyelin (SM).

Fig. 2 Preparative TLC plate of goat white cheese sample (stained under iodine vapors). In the photo, the nine bands obtained are shown on the right, whereas on the left, the corresponding elution fronts of standard compounds is given. *L-PC* lyso-phosphatidylcholine, *SM* sphingomyelin, *PC* phosphatidylcholine, *L-PE* lyso-phosphatidylethanolamine, *PE* phosphatidylethanolamine, *CL* cardiolipin

Fig. 3 In vitro biological activity of preparative thin-layer chromatography (TLC) polar lipid fractions of goat white cheese sample toward washed rabbit platelet aggregation, expressed in micrograms

4 Discussion

Our results have demonstrated that TL from goat samples (milk, yogurt, and white cheese) contains PAF inhibitors. TL of goat white cheese and goat yogurt appeared to exhibit stronger anti-atherogenic activities which could be attributed to the fact that microorganisms, such as *S. thermophilus* and *L. bulgaricus*, that are involved in the fermentation of yogurt and cheese, have been found to aid the formation of lipids that inhibit PAF-induced platelet activation (Antonopoulou et al. 1996). According to our results, these PAF inhibitors are mainly present in the TPL fraction.

Further fractionation of TPL from goat white cheese sample by preparative TLC and biological assay of the seven polar lipid fractions obtained showed that polar lipid fractions with R_f values similar to those of SM, PC, and PE exhibited strong biological activity. Those phospho- and sphingolipids are present in various dairy products (Rombaut et al. 2007).

The presence of PAF inhibitors in various foods is very important in terms of their capacity to prevent the onset of CVDs and thus this parameter affects their overall nutritional value (Nomikos et al. 2007). Lipid microconstituents of specific foods animal-origin, i.e., cheese (Tsorotioti et al. 2014) and eggs (Nasopoulou et al. 2013), that constitute important ingredients of the Mediterranean diet, have been found to exert in vitro important biological activities by inhibiting PAF actions. These lipid microconstituents could inhibit the onset of the atherosclerosis and the development of CVDs, as several in vivo studies have shown (Karantonis et al. 2006; Tsantila et al. 2007; Nasopoulou et al. 2010; Tsantila et al. 2010).

5 Conclusion

In conclusion, our results underline the nutritional value of goat milk, yogurt, and white cheese and demonstrate that the TL and the TPL of all three products appeared to have inhibitory activities against PAF-induced platelet activation. The novelty of this work is on the comparison of these anti-atherogenic activities; the three products in terms of increased cardioprotection could be classified as milk < yogurt < white cheese. It could be thus interpreted that the regular consumption of these dairy foods offers anti-

atherogenic protection and therefore protection against the development of CVDs. Future dietary guidelines should take into account data like the ones presented here when redefining the suggested consumption (frequency and servings per day) of dairy products, especially the ones originated from goat milk.

Compliance with ethical standards

Conflict of interest Stylianos Poutzalis, Areti Anastassiadou, Constantina Nasopoulou, Kalliopi Megalemou, Eleni Sioriki, and Ioannis Zabetakis declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain studies with human or animal subjects performed by any of the authors.

References

- Antonopoulou S, Semidalas CE, Koussisis S, Demopoulos CA (1996) Platelet-activating factor (PAF) antagonists in foods: a study of lipids with PAF or anti-PAF-like activity in cow's milk and yogurt. *J Agric Food Chem* 44:3047–3051
- Bligh E, Dyer W (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 37: 911–917
- Demopoulos CA, Pinckard RN, Hanahan DJ (1979) Platelet-activating factor. Evidence for 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). *J Biol Chem* 254:9355–9358
- Demopoulos CA, Karantonis H, Antonopoulou S (2003) Platelet activating factor—a molecular link between atherosclerosis theories. *Eur J Lipid Sci Tech* 105:705–771
- Galanos DS, Kapoulas VM (1962) Isolation of polar lipids from triglyceride mixtures. *J Lipid Res* 3:134–137
- Karantonis HC, Antonopoulou S, Perrea DN, Sokolis DP, Theocharis SE, Kavantzaz N, Iliopoulos DG, Demopoulos CA (2006) In vivo antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. *Nutr Metab Cardiovasc* 16:174–185
- Kastorini CM, Milionis HJ, Goudevenos JA, Panagiotakos DB (2010) Mediterranean diet and coronary heart disease: is obesity a link?—a systematic review. *Nutr Metab Cardiovasc Dis* 20:536–551
- Liapikos TA, Antonopoulou S, Karabina SAP, Tsoukatos DC, Demopoulos CA, Tselepis AD (1994) Platelet-activating factor formation during oxidative modification of low-density lipoprotein when PAF-acetylhydrolase has been inactivated. *Biochim Biophys Acta* 1212:353–360
- López-Aliaga I, Alférez MJM, Nestares T, Ros PB, Barrionuevo M, Campos MS (2005) Goat milk feeding causes an increase in biliary secretion of cholesterol and a decrease in plasma cholesterol levels in rats. *J Dairy Sci* 88:1024–1030
- López-Aliaga I, Diaz-Castro J, Alférez MJM, Barrionuevo M, Campos MS (2010) A review of the nutritional and health aspects of goat milk in cases of intestinal resection. *Dairy Sci Tech* 90:611–622
- Nasopoulou C, Karantonis HC, Perrea DN, Stamatios TE, Iliopoulos DG, Demopoulos CA, Zabetakis I (2010) In vivo anti-atherogenic properties of cultured gilthead sea bream (*Sparus aurata*) polar lipid extracts in hypercholesterolaemic rabbits. *Food Chem* 120:831–836
- Nasopoulou C, Stamatakis G, Demopoulos CA, Zabetakis I (2011) Effects of olive pomace and olive pomace oil on growth performance, fatty acid composition and cardio protective properties of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). *Food Chem* 129:1108–1113
- Nasopoulou C, Gogaki V, Panagopoulou E, Demopoulos CA, Zabetakis I (2013) Hen egg yolk lipid fractions with antiatherogenic properties. *Anim Sci J* 84:264–271
- Nomikos T, Fragopoulou E, Antonopoulou S (2007) Food ingredients and lipid mediators. *Curr Nutr Food Sci* 3:255–276
- Panagiotakos DB, Pitsavos C, Matalas AL, Chrysohoo C, Stefanadis C (2005) Geographical influences on the association between adherence to the Mediterranean diet and the prevalence of acute coronary syndromes, in Greece: the CARDIO2000 study. *Int J Cardiol* 100:135–142

- Park YW, Haenlein GFW (2013) Milk and dairy products in human nutrition: production, composition and health. Wiley, London
- Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliard Y (2008) Composition of goat and sheep milk products: an update. *Small Rumin Res* 79:57–72
- Rombaut R, Dewettinck K, Van Camp J (2007) Phospho- and sphingolipid content of selected dairy products as determined by HPLC coupled to an evaporative light scattering detector (HPLC–ELSD). *J Food Compos Anal* 20:308–312
- Sanz Ceballos L, Ramos Morales E, De La Torre Adarve G, Díaz-Castro J, Pérez-Martínez L, Sanz Sampelayo MR (2009) Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. *J Food Compos Anal* 22:322–329
- Selvaggi M, Laudadio V, Dario C, Tufarelli V (2014) Major proteins in goat milk: an updated overview on genetic variability. *Mol Biol Rep* 41:1035–1048
- Tsantila N, Karantonis HC, Perrea DN, Theocharis SE, Iliopoulos DG, Antonopoulou S, Demopoulos CA (2007) Antithrombotic and antiatherosclerotic properties of olive oil and olive pomace polar extracts in rabbits. *Mediators Inflamm*. doi:[10.1155/2007/36204](https://doi.org/10.1155/2007/36204)
- Tsantila N, Karantonis HC, Perrea DN, Theocharis SE, Iliopoulos DG, Iatrou C, Antonopoulou S, Demopoulos CA (2010) Atherosclerosis regression in rabbits upon olive pomace polar lipid extract administration. *Nutr Metab Cardiovasc* 2010:740–747
- Tsorotioti SE, Nasopoulou C, Detopoulou M, Sioriki E, Demopoulos CA, Zabetakis I (2014) In vitro anti-atherogenic properties of traditional Greek cheese lipid fractions. *Dairy Sci Tech* 94:269–281
- Zabetakis I (2013) Food security and cardioprotection: the polar lipid link. *J Food Sci* 78:1101–1104