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Postprandial effects of wine consumption on Platelet Activating Factor metabolic enzymes

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ABSTRACT

Platelet Activating factor (PAF) is a potent inflammatory mediator that is involved in the initiation and the prolongation of atherosclerosis. The purpose of the study was to investigate the effect of wine consumption on the activity of PAF metabolic enzymes and on IL-6 levels as a cytokine inflammatory marker. Healthy men participated in 4 daily trials and consumed a standardized meal along with Robola wine (trial R), or Cabernet Sauvignon (trial CS), or ethanol solution (trial E), or water (trial W).

A significant trial effect was found in the activity of lyso-PAF acetyltransferase (Lyso-PAF AT) ($p_{trial} = 0.01$). In specific, R trial decreased enzyme activity compared to E trial ($p = 0.03$) while a trend for differentiation was observed between CS trial and E one ($p = 0.06$) as well as between R trial and W one ($p = 0.07$). Concerning PAF-cholinephosphotransferase (PAF-CPT) activity, a significant trial effect was found ($p_{trial} < 0.00$). Specifically, both R ($p = 0.002$) and CS ($p = 0.001$) trials decreased enzyme activity compared to E trial. Concerning lipoprotein-associated phospholipase A2 (LpPLA2) no time either trial effect was observed. Concerning IL-6 levels a significant time effect was found ($p_{time} < 0.00$) while no trial effect was revealed.

In conclusion, the protective effect of wine consumption could partly be explained through the modulation of PAF metabolism by wine micro-constituents that lead to lower PAF levels.

1. Introduction

Atherosclerosis is considered to be the primary cause of cardiovascular diseases and the major cause of death in the Western World. From the initial stage to the progress of atherosclerosis the underlying dominant mechanism is inflammation [1,2]. The classical risk factors involved are smoking, diabetes mellitus, hyperlipidemia, hypertension and obesity. In fact, a limited set of risk factors seems to explain most part of the cardiovascular risk [3]. In Western societies, a considerable amount of day time is under the postprandial state. Since the report of Zilversmit in 1979 [4], postprandial state and especially hyperglycemia and hyperlipidemia have been associated with the appearance of inflammatory cells in the human organism as well as with endothelium and coagulation system dysfunction that are all included as risk factors for atherosclerosis [5,6]. Postprandial inflammation starts with the increase of remnant lipoproteins and glucose in the circulation. This leads to activation of circulating leukocytes, which interact with the endothelium [6–10]. The increase of circulating cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), following the meal might induce endothelial activation [5]. When stimulated by cytokines and lipid mediators, endothelial cells express adhesion molecules, such as vascular cell adhesion molecule 1 (VCAM-1), which allow activated leukocytes to bind to the vessel wall. Once leukocytes have adhered to the endothelium, they are stimulated by chemoattractant signals, in particular monocyte chemotactic protein-1 (MCP-1), to migrate into the arterial wall [2]. Subendothelial migrated monocytes differentiate into macrophages, which can take up modified lipoproteins, leading to foam cell formation and the development of an atherosclerotic plaque [1].

Platelet Activating Factor (PAF), 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is a potent phospholipid mediator of inflammation [11]. It is involved in the initiation and the prolongation of atherosclerosis [12,13]. PAF induces release of active oxygen species that lead to LDL oxidation which in turn contributes sig-

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nificantly to increased PAF levels [14]. Activated endothelial cells produce PAF and their stimulation with PAF induces changes in the cytoskeletal structure leading to increase vascular permeability [15]. PAF is synthesized by adherent, activated platelets and stimulates a juxtacrine signaling system at the platelet surface which mediates rolling and tight adhesion of neutrophils [16]. The activation of these cells causes their migration in subendothelial space and their aggregation and also the secretion of many lipid mediators, cytokines, enzymes and adhesion molecules, which are all substances that contribute to the atherosclerosis development [12,17].

PAF levels in cells, tissues and blood is regulated *via* its enzymatic biosynthesis and catabolism. PAF can be synthesized by two different enzymatic routes, namely the remodeling and the de novo pathway [18]. The remodeling pathway is believed to produce PAF under inflammatory conditions and involves a structural modification of ether-linked membrane phospholipids. More specifically, the action of cytoplasmic phospholipase A_2 yields lyso- PAF which is then acetylated by acetyl-CoA: lyso-PAF acetyltransferases (Lyso-PAF AT) leading to the formation of PAF [19]. The de novo pathway appears to be responsible for the constitutive production of PAF, maintaining its physiological levels in various tissues and blood. A key step in this route is the conversion of 1-*O*-alkyl-2-acetyl-glycerol to PAF by a specific dithiothreitol l-insensitive CDP-choline: 1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase (PAF-CPT) [20]. As far as PAF catabolism is concerned, the most important enzyme involved is a PAF-specific acetylhydrolase (PAF-AH), which cleaves the short acyl chain at the *sn*-2 position and forms inactive lyso-PAF [21]. The plasma isoform of PAF-AH is known as lipoprotein-associated phospholipase A_2 (LpPLA2), due to its attachment to lipoproteins and mainly LDL-particles, which is believed to be a newly risk factor for cardiovascular diseases [22].

Many epidemiological studies support the notion that alcohol consumption is related with lower risk for cardiovascular diseases [23]. The term "French paradox" was introduced in 1992 by Renaud and De Lorgeril in order to describe the epidemiological observation that the French suffer a relatively low incidence of coronary heart disease, despite having a diet relatively rich in saturated fats [24]. Several theories have been proposed in order to explain this phenomenon and several debates arose [25]. Among them the regular and moderate wine consumption is thought to be predominant. More specific, it is thought that the existence of wine bioactive compounds could have a beneficial effect on the cardiovascular system, preventing or delaying atherosclerosis. In this point of view, our research team supports the idea that the existence of PAF inhibitors in wines could partly explain its protection against cardiovascular diseases [25]. Indeed, biological active compounds with the ability to inhibit PAF induced platelet aggregation were detected in different varieties of wine extracts [26–29]. The most potent wines were a red one with main variety Cabernet Sauvignon and a white one with main variety Robola, demonstrating that the variable of grape is important for the biological activity and not the color. Extracts of these wines also exert potent antioxidant activity and had the ability to inhibit PAF biosynthetic enzymes in U937 monocytes [30–32].

However, no *in vivo* data exist concerning the effect of postprandial state on PAF metabolic enzymes and also in relation to wine consumption. Therefore the aim of the present study was to investigate the acute effect of wine consumption, which contains PAF inhibitors, along with a meal on the activity of PAF biosynthetic enzymes, namely Lyso-PAF AT and PAF-CPT as well as on the catabolic enzyme LpPLA2. Furthermore, the effect of wine consumption on a pro-inflammatory cytokine, namely IL-6 was also evaluated.

2. Materials and methods

2.1. Materials and instruments

All reagents were of analytical grade and were supplied by Sigma (St. Louis, MO, USA). 1-*O*-Hexadecyl-2-*O*-acetyl-*sn*-glycerol was purchased from Biomol International (Plymouth Meeting, PA, USA). 1-*O*-hexadecyl-2-[^{3}H] acetyl-*sn*-glycerol-3-phosphocholine ([^{3}H] PAF) with a specific activity of 10 Ci/mmol was obtained from New England Nuclear (Boston, MA). Centrifugations were performed in a refrigerated superspeed Avanti 30 centrifuge (Beckman). Homogenizations were conducted with the Bandelin Sonoplus sonicator GM 2070 (Germany). Radioactivity was measured with a liquid scintillation counter (1209 Rackbeta, Pharmacia, Wallac, Finland).

2.1.1. Study population

Twelve apparently healthy men were initially recruited to participate in the study. Exclusion criteria were smoking, being on slimming or any other special diet, being an athlete, diagnosis of hypertension, metabolic or endocrine disease, gastrointestinal disorders, or a recent history of medical or surgical events. Ten subjects completed all four trials of the study (one subject dropped out for personal reasons and the other one was excluded for non-adherence). Participating subjects were young, non-obese men (age: 31.3 ± 4.3y, range: 25–39y; Body Mass Index: 24 ± 2 kg/m^2) and none of them was taking drugs or dietary supplements. The study was conducted according to the guidelines laid down in the Declaration of Helsinki. Volunteers gave their informed consent and the University Ethics Committee approved the experimental protocol. The study was undertaken at the Metabolic Unit of the Department of Nutrition and Dietetics, Harokopio University. The ClinicalTrials.gov Identifier for this study is NCT01627912.

2.1.2. Study protocol

Each subject participated in 4 daily trials on separate days, at least 2 weeks apart. The sequence of the trials was randomly selected for each subject. The randomization code was prepared by a staff member who was not involved in running the trial, by using computer-generated random numbers. Three days before the trial, volunteers were given detailed instructions to follow a diet low in vegetables and fruits, generally low in antioxidant and phenolic compounds, to abstain from alcohol, avoid excessive exercise, and sleep at least 7 h/day. In order to ensure that the volunteers complied with the dietary instructions 24 h recalls were performed for 3 days before each daily trial.

After fasting overnight (10–12 h), participants came to the Metabolic Unit around 09:00 am A cannula was inserted into an antecubital vein and a baseline blood sample was obtained 15 min later (−15 min). Participants were asked to consume in 15 min a standardized meal along with (i) white wine (Robola variety – trial R), or (ii) red wine (Cabernet Sauvignon variety – trial CS), or (iii) ethanol solution (12.5% v.v. – trial E) or (iv) water (trial W). All beverages were provided at a standard portion per subject, *i.e.* 4 mL/kg body weight. The standardized meal provided 850 kcal (carbohydrates: 32%, proteins: 13%, lipids: 53% of the total energy intake). Immediately after meal consumption, a second blood sample was obtained (0 min) and subsequently obtained at several time points during the next 6 h. After each blood draw, the cannula was flushed with saline (0.9% NaCl).

2.1.3. Anthropometric measurements

Anthropometry was carried out on each visit. Weight was measured to the nearest 0.1 kg using a digital scale and height to the nearest 0.1 cm using a stadiometer with head in horizontal Frankfurt plane. Both measurements were taken with the subject in light