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Supplementation with grape pomace in healthy women: Changes in biochemical parameters, gut microbiota and related metabolic biomarkers

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ABSTRACT

This paper reports a comprehensive pilot study of the effects of dietary supplementation with grape pomace (GP) in humans. After an initial washout period, the diet of 10 healthy women was supplemented with a dairy amount of 1.4 g of a red Grape Pomace (GP) extract (Eminol®) for 21 days. Among the different biochemical and immune parameters measured in plasma, a significant decrease ($p < 0.05$) was observed in blood fasting glucose levels after the 21-day supplementation. Overall, the GP supplementation did not lead to significant changes in faecal bacterial populations or, in general, in the content of faecal and urine phenolic metabolites. Nevertheless, significant changes ($p < 0.05$) were observed in the short-chain and medium-chain fatty acid profiles (SCFAs and MCFAs). Due to observed inter-individual differences it was not possible to establish a pattern on the microRNA expression profile associated to GP supplementation, however modulation of the expression of miRNA related to glucose metabolism was perceived after the intervention period.

1. Introduction

Grape pomace (GP) is a winery by-product composed basically of grape seeds, skin and stems. It is rich in dietary fibre and polyphenols including anthocyanins (in red GP), flavan-3-ols, flavonols, phenolic acids and stilbenes (Fontana, Antoniolli, & Bottini, 2013), which provides a wide variety of potential biological activities (Yu & Ahmedna, 2013). In recent years, there has been an increased interest in the use of GP to develop functional ingredients and in other applications for the food industry (Charalampia & Koutelidakis, 2016). But the majority of scientific evidence concerning GP benefits is derived from experiments performed *in vitro* and in animal models, often using a concentration much higher than that contained in biological fluids and in diet, respectively. Moreover, in some cases, GP parent compounds are tested instead of their derived metabolites, which would be the responsible for beneficial effects *in vivo* (D'Archivio, Filesi, Varì, Scazzocchio, & Masella, 2010). Therefore, it is essential to conduct human intervention studies for evaluating the biological properties of GP.

To our knowledge, only two human intervention studies have been published concerning the effects of the inclusion of GP in the human diet. Yubero et al. (2013) evaluated the effects of the supplementation with a GP extract (700 mg/day, 56 days) on cardiovascular risk and oxidative stress indicators in healthy volunteers (n = 60). The results of this study showed that the GP was able to modulate the lipid profile, lowering total blood cholesterol and LDL cholesterol levels (Yubero et al., 2013). Later on, Urquiaga et al. (2015) investigated the effect of the supplementation with a GP flour (20 g/day, 16 weeks) in patients (n = 38) who suffered at least from one component of metabolic syndrome. The results of this study indicated a significant improvement in blood pressure, fasting glucose levels and protein damage after supplementation with GP flour (Urquiaga et al., 2015). On the other hand, some *in vitro* studies have assessed the metabolism and further bioavailability of GP polyphenols. The majority of GP phenolic compounds are present in the form of esters, glycosides or polymers and suffer

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multiple degradation reactions by intestinal enzymes and/or by the colonic microbiota, prior to be absorbed (Cueva et al., 2013). Data currently available on the digestibility and metabolism of GP polyphenols suggest a relatively great bioavailability of their metabolites (Motilva et al., 2016; Sasot et al., 2017).

Another interesting issue about GP functionality is its potential ability to modulate the intestinal microbiota. In this line, the study by Viveros et al. (2011) revealed that broiler chicks fed with GP extracts had higher intestinal populations *Lactobacillus, Clostridium* and *Enterococcus* species than the animals from the untreated control group. Low counts of *Streptococcus* spp. and *Clostridium* cluster XIVa were reported in the faecal microbiota from weaned pigs fed with a GP meal extract (Fiesel, Gessner, Most, & Eder, 2014). Another animal study showed that lambs fed with a diet supplemented with GP presented lower growth of potential pathogenic bacteria, in particular *Enterobacteriaceae* and *E. coli* (Kafantaris et al., 2016). Some of our previous *in vitro* studies indicated changes in some bacterial groups after fermentation of a red GP extract (Eminol®) with faecal microbiota, with increments for *Lactobacillus* and *Bacteroides* groups (Gil-Sánchez et al., 2017; Gil-Sánchez, in press). However, to the best of our knowledge, there are no studies available concerning the impact of GP supplementation on the intestinal microbiota (composition and/or functionality) in humans.

Therefore, the aim of this study was to assess the possible effect of the supplementation with a red GP extract (Eminol®), on different biochemical and molecular biomarkers as well as in the composition and activity of the human gut microbiota. The study was focussed on healthy women to limit inter-individual variability and strengthen further conclusions. A complete biochemical study was performed in blood samples. Populations of the major groups of the intestinal microbiota, as well as short-chain and medium-chain fatty acids (SCFAs and MCFAs) were quantified in faecal samples, and microbial phenolic metabolites were determined in urine and faecal samples. In addition, modulation of 734 miRNAs expression by GP extract intake and validation of five miRNAs related to glucose metabolism was performed in serum.

2. Materials and methods

2.1. Grape pomace extract

The GP extract used in this study, trademarked as Eminol® (ABROBIOTEC S.L., Valbuena de Duero, Valladolid, Spain), was obtained according to previous-reported procedures (Gil-Sánchez et al., 2017). Briefly, fresh grape pomace was submitted to a distillation process to remove alcohol and aromatic compounds. Later, the residue was extracted through traditional solid-liquid extraction by diffusion using a hydroalcoholic solution (water:ethanol) as solvent. The resulting product was centrifuged and stabilized in order to delete solid residues. Finally, the final solution was concentrated and dried by a spray-drying process.

The red grapes used in the winemaking process were all of the Tempranillo variety, harvested from vineyards located in the Ribera de Duero Designation of Origin (Spain). The extract contained high amounts of dietary fibre (659.7 mg/g extract) (Table 1), as determined by the methodology reported in Martin-Cabrejas, Waldron, Selvendran, Parker, and Moates (1994). The concentration of total polyphenols was moderate (37.44 mg gallic acid/g extract) (Table 1), as determined by the Folin-Ciocalteu reagent (Singleton and Rossi, (1965). Among phenolic compounds, different phenolic acids, flavonols and flavanols were previously identified in this extract (Gil-Sánchez et al., 2017) (Table 1). In addition, other chemical parameters were previously analysed in the extract such as moisture (4.6%), total protein (8.7%), fat (0.8%) and ash (20.7%) (Gil-Sánchez et al., 2017).

The extract, disposed in capsules containing 700 mg of extract/capsule, was supplied by Grupo Matarromera (Valbuena de Duero, Valladolid, Spain).

Table 1
Content in polyphenols and dietary fibre of Eminol® extract.

	GP Extract
PHENOLIC COMPOSITION (mg/g extract)	
Total polyphenols (mg gallic acid/g)	37.44
Phenolic acids	
Ellagic acid	5.64 ± 0.65
Flavanols	
Catechin	0.28 ± 0.07
Epicatechin	1.03 ± 0.25
Proanthocyanidin	4.62
Flavonols	
Myricetin	0.26 ± 0.01
Quercetin	0.34 ± 0.03
Kaempferol	0.16 ± 0.00
ALCOHOL-INSOLUBLE RESIDUE COMPOSITION (mg/g AIR)	
Total AIR yield	660
Neutral sugars	294.7
Uronic acids	32.3 ± 1.0
Total sugars	328.2
Klason lignin	84.3 ± 1.1

All values are means of three replicates (n = 3).

2.2. Human intervention study design

The intervention study was conducted in accordance with the Helsinki Declaration and was approved by the Ethics Committee from CSIC (Madrid, Spain) (Approval number (23_08_2012). All volunteers gave their written informed consent prior to participate. The study involved 10 healthy women (age ranged 25–65 years, BMI < 25 kg/m^2). The participants were not suffering from diabetes, hypertension, or dyslipidemia, acute or chronic inflammatory disease, infectious disease, cancer, or a previous cardiovascular event at study entry. They had not received any antibiotic therapy, prebiotics, probiotics, synbiotics, or vitamin supplements or any other medical treatment influencing intestinal microbiota during the 6 months before the start of the study or during the study (including the washout period). Volunteers followed an initial washout period of 10 days (baseline) during which they maintained a low-polyphenol diet. After this period, the participants were instructed to take two capsules per day of the GP extract (1400 mg of extract/day) at breakfast over 21 days. Thus, the average daily consumption of fibre and phenolic compounds, in the present study, was 923.58 mg and 54.42 mg respectively. During the intervention period, they also maintained the restrictions of polyphenol rich foods in the diet. Volunteers filled in a questionnaire on their dietary habits to verify the diet.

Blood samples were extracted from volunteers after an overnight fast, by skilled professionals, at three points: (a) after the run-in washout period, (b) after 14 days of consumption of the GP extract and (c) at the end of the GP intervention (21 days). The serum was separated into aliquots and immediately frozen at −80 °C.

At the same time as blood samples were collected, participants provided samples of faeces and 24 h-urine. Faecal samples were immediately frozen and stored at −80 °C awaiting analysis. Urine samples were measured (the total urine volume in 24 h), homogenized, acidified with HCl to achieve a final concentration of 0.2 M, aliquoted, and then aliquots were frozen and stored at −80 °C.

2.3. Serum biochemical and immune parameters measurement

Serum biochemical parameters were measured using an automated biochemical auto-analyser in an accredited external laboratory (Unilabs, Madrid). The tests included the measure of glucose, uric acid, albumin, hepatic enzymes (glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and gamma-glutamyl transferase

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