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Phenolic metabolites in plasma and tissues of rats fed with a grape pomace extract as assessed by liquid chromatography-tandem mass spectrometry

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ABSTRACT

Grape pomace extract (GPE) is a rich and relatively low-cost source of phenolic compounds. However, little is known about the main GPE metabolites in mammals, which could help explain the observed health-promoting effects. This study investigated the presence of parent compounds from flavanol, flavonol and stilbene families and their metabolites in rat plasma and tissues after an acute intake of GPE in doses of 300 and 600 mg kg/body weight. The measurement of free compounds and their metabolites was performed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Results showed the presence of epicatechin, epicatechin methyl-glucuronide, epicatechin methyl-sulphate, catechin, catechin-glucuronide, quercetin methyl-glucuronide, resveratrol-3-glucuronide, resveratrol-4-glucuronide and resveratrol-3-sulphate in plasma, which was dose dependent. The most abundant measured compound in plasma was epicatechin-glucuronide. The presence of glucuronidated and methyl-glucuronidated forms of catechin were observed in the liver at both doses, while epicatechin-glucuronide and methyl-glucuronide were detected only upon intake of 600 mg GPE/kg body weight. At this dose epicatechin-glucuronide and methyl-glucuronide were also detected in muscle, and catechin methyl-glucuronide in adipose tissue. Results show the main GPE metabolites present in rat tissues after oral consumption, contributing to better understand the health benefits of GPE and its potential utilization as a functional ingredient.

1. Introduction

Phenolic compounds are plant secondary metabolites widely distributed in fruits and vegetables with various characterized functions and several proposed beneficial effects [1,2]. These compounds, particularly flavonoids, are currently receiving significant attention because of their health-promoting effects including those against obesity-associated pathologies, such as type 2 diabetes, metabolic syndrome, cardiovascular diseases, and cancer, among others [3–6]. The positive properties demonstrated for functional foods, and the increase of consumers' awareness for healthy foods, highlights the need to find natural alternatives for the food industry [7]. Grape pomace (GP) is obtained from the winemaking process as the residue remaining after fermentation, mainly constituted by skins and seeds of berries. GP is a potentially abundant and relatively low-cost source of a wide range of phenolic compounds including the most abundant monomeric and oligomeric flavanols (catechin, epicatechin, procyanidins), flavonols (quercetin), anthocyanins and stilbenes (resveratrol) with potential biotechnological utilization in food and pharmaceutical industries as natural or functional ingredients [5,8]. In addition, grape pomace extract (GPE) is a concentrate product obtained from GP which is mainly constituted by phenolic compounds present at higher concentrations than those found in the crude by-product.

Phenolic compounds present in foods are highly metabolized before their absorption [9]. After the ingestion of food and beverages rich in phenolic compounds, the mayor absorption occurs in the small intestine. Typically, phenolic compounds except flavanols, are found in glycosylated forms or polymerized and must be hydrolyzed by intestinal enzymes present in the brush border of the small intestine or by the colonic microflora before they could be absorbed. During the course of absorption, polyphenols are conjugated in the small intestine and later in the liver forming sulphate, glucuronidated and/or methylated

Abbreviations: GP, grape pomace; GPE, grape pomace extract; SPE, solid phase extraction; UHPLC-MS/MS, ultra-high performance liquid chromatography-tandem mass spectrometry

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metabolites [10,11]. Throughout digestion, hydrolysis and metabolism change the molecular structure of these compounds, leading to a large number of different molecules [12].

Previous reports showed that GPE can exert beneficial health effects, showing an anti-inflammatory effect in diet-induced obese mice [13], an antioxidant activity in rats [14], counteracting the adiposity and hyperglycemia in Type 2 diabetic mice [15], mitigating hepatic steatosis in db/db mice [16] and lowering plasma triacylglycerides and phospholipids in rats fed a high fat diet [17]. Furthermore, GP or GPE supplementation prevents diet-induced metabolic alterations and adiposity in rats with metabolic syndrome [18–20]. However, little is known about the main GPE-derived phenolic metabolites present in plasma and tissues, their biological targets, their distribution and availability that may account for some of the observed health-promoting effects.

The aim of this study was to assess the availability, distribution and levels of metabolites of the most abundant polyphenol families (flavanols, flavonols and stilbenes) in rat plasma, liver, muscle and visceral adipose tissue after oral intake of two doses of malbec GPE. The measurement of free compounds and their metabolites was performed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS).

2. Material and methods

2.1. Standards and solvents

Standards of catechin (≥99%), epicatechin (≥95%), *trans*-resveratrol (≥99%), quercetin hydrate (95%), procyanidin B1 (≥90%), procyanidin B2 (≥90), the internal standard (IS) catechol and L (+)-ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions (1 mg/ml) of the above compounds were prepared in methanol. Further dilutions were prepared monthly in methanol and stored in dark-glass bottles at −20 °C. Calibration standards were prepared in ultrapure water with 0.1% (v/v) formic acid:acetonitrile 95:5.

HPLC-grade acetonitrile, methanol, acetone, formic acid and glacial acetic acid were purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ, USA). Ethanol was purchased from Merck (Sao Paulo, Brazil). Ortho-phosphoric acid 85% (w/v) was purchased from Sintorgan S.A. (Buenos Aires, Argentina). Ultrapure water was obtained using a Milli-Q system (Millipore, Billerica, MA, USA). OASIS HLB (divinylbenzene-co-*N*-vinylpirrolidone polymer) 60 mg SPE cartridges were purchased from Waters (Milford, MA, USA).

2.2. GP sampling

This study was conducted using GP of *Vitis vinifera* L. cv. Malbec, harvested in 2017. The material was provided by a local winery from the vineyards of the Mendoza's region in Argentina. The winemaking was conducted with daily pumping and contact of the skins and seeds with the juice for 11 days. After this, must was pressed, and the fresh GP was obtained, placed in ice-cooled boxes for transportation to the laboratory, and stored at −20 °C until processing. The recovery of the phenolic compounds from the GP was performed via solid–liquid extraction as previously described [8]. Herein, 80 g of fresh GP was ground with a laboratory mixer, and the powder extracted at a 5:1 solvent-to-sample ratio using ethanol:water, 50:50 v/v) as the solvent. The extraction was carried out for 2 h under continuous stirring at 60 °C. The preparation was filtered through a filter paper and concentrated at low pressure using a rotary evaporator at 40 °C. The concentrated extracts were freeze-dried for 96 h at 0.12 bar and −45 °C in a Free Zone 2.5 equipment (Labconco, Missouri, USA). Freeze dried extracts were placed in sealed tubes and kept in the dark at −20 °C in a dry atmosphere until analysis or preparation of diets.

2.3. Animal studies

All animal studies were conducted in accordance with the Guiding Principles in the Care and Use of Animals of the US National Institute of Health. All procedures were approved by the Institutional Animal Care and Use Committee of the Facultad de Ciencias Médicas, Universidad Nacional de Cuyo (CICUAL, Protocol approval no. 36/2014). Ten-weeks-old male Wistar rats were housed under controlled conditions of temperature (23 ± 1 °C) and light (12 h light/dark cycle), and were fed a standard rat chow (Gepsa-Feeds, Buenos Aires, Argentina) and water ad libitum. Animals were fasted for 16 h with only access to tap water. Rats weighing 320 ± 23 g at dosing were randomly divided into three groups (n = 6 each) receiving: 300 mg GPE/kg of body weight or 600 mg GPE/kg of body weight, or the vehicle (ethanol:water, 50:50, v/v) (control group). The average intake of phenolic compounds in rats that received 300 and 600 mg GPE/kg body weight was 752 and 1503 μg, respectively. Both doses of GPE, dissolved in ethanol:water 50:50 (v/v), and the vehicle were administered by intraesophageal gavage, at a volume of 0.6 ml/rat. Two hours later, animals were anesthetized with ketamine (50 mg/kg body weight) and acepromazine (1 mg/kg body weight), and blood was collected from the abdominal aorta into EDTA-containing tubes. Plasma was obtained after centrifugation at 1000 × g for 15 min at 4 °C. In addition, liver, epididymal adipose tissue and soleus muscle were weighed, flash-frozen in liquid nitrogen and then stored at −80 °C until assayed. Two hours after the acute ingestion of GPE was chosen to sample the plasma and tissues because this is the time that corresponds with previously reported maximum plasma concentration of metabolites [9,21,22].

2.4. Extraction of phenolic compounds and their metabolites from GPE, plasma and tissues

The method used to extract free phenolic compounds and their metabolites from GPE, plasma and tissues was based on Serra et al. [23]. Briefly, 25 mg of GPE were resuspended in 2 ml of ultrapure water with 1% (v/v) of formic acid and extracted by solid-phase extraction (SPE) as described below.

An aliquot of 0.75 ml of plasma was diluted 1:1 (v:v) with ultrapure water, then 20 μl phosphoric acid 85% (v/v) and 50 μl catechol (IS) (20 mg/l) were subsequently added prior to the SPE. Freeze-dried liver, muscle and adipose tissue (200 mg) were added with 50 μl of ascorbic acid 1% (w/v), 50 μl catechol (IS) (20 mg/l) and 100 μl phosphoric acid 4% (w/v). Samples were mixed and subsequently extracted with 400 μl of water:methanol: 4% (w/v) phosphoric acid (94:4:1, v:v:v), sonicated during 30 s in a freeze water bath to avoid heating, and centrifuged for 15 min at 15,800 g at 20 °C. This procedure was repeated four times. The supernatants obtained in each extraction were collected, and centrifuged for 3 min, at 13,500 × g at 20 °C. Then, the extracts were treated with SPE to concentrate the compounds of interest before UHPLC-MS/MS analysis. The SPE of GPE, plasma and tissue extracts were performed by using OASIS HLB cartridges (60 mg, Waters, Milford, MA, USA). For this purpose, samples were passed through HLB SPE cartridges previously conditioned with methanol and a 0.2% (v/v) acetic acid solution (5 ml each). The loaded cartridges were washed with 3 ml ultrapure water and 5 ml of 0.2% (v/v) acetic acid solution. The retained analytes were eluted with 2 ml of acetone:Milli-Q water: 0.2% (v/v) acetic acid (70:29.5:0.5, v:v:v) and collected in glass tubes. Afterwards, the extract was evaporated to dryness (SpeedVac concentrator), and the residue re-suspended in 0.2 ml of initial mobile phase and injected into the chromatographic system.

2.5. Phenolic compounds quantification by UHPLC-MS/MS

The analysis of phenolic compounds and their biological metabolites were performed by UHPLC-MS/MS. The UPLC analysis of extracts was performed using a Waters Acquity Ultra-Performance TM liquid

chromatography system (Waters, Milford, MA, USA), equipped with a binary pump system (Waters, Milford, MA, USA). The separations were carried out in reversed-phase Kinetex C18 column (3.0 mm × 100 mm, 2.6 μm) Phenomenex (Torrance, CA, USA). Ultrapure water with 0.1% (v/v) formic acid (A) and acetonitrile (B) were used as mobile phases. Analytes were separated using the following gradient: 0–2.7 min, 5% B; 2.7–11 min, 30% B; 11–14 min, 95% B; 14–15.5 min, 95% B; 15.5–17 min, 5% B: 17–20, 5% B. The mobile phase flow was 0.4 ml/min. The column temperature was 35 °C and the injection volume 5 μl.

The tandem MS analyses were carried out on a TQD mass spectrometer (Waters, Milford, MA, USA) equipped with a Z-spray electrospray interface. For all compounds, the source was operated in a negative mode and the data was acquired in selected reaction monitoring (SRM). The source working conditions were as follows: capillary voltage, 3 kV; source temperature, 150 °C; cone gas flow rate, 80 l/h and desolvation gas flow rate, 800 l/h; desolvation temperature, 400 °C. Ultrapure nitrogen and argon were used as nebulizing and collision gases; respectively. The *m/z*, transitions, cone voltages and collision energies for each analyte were selected based on previous reports with only minor modifications [23–25]. Analytes present in the samples were quantified using an external calibration with pure authentic standards of the analytes. Linear ranges between 0.05 and 25 μg/ml were obtained for all analytes, except for procyanidin B1 and B2 which presented a linear range between 1 and 25 μg/ml. Coefficients of determination (R2) higher than 0.992 were obtained for all the studied phenolic compounds. The absolute recoveries of the overall procedure, considered as an estimation of the accuracy, were 107, 102, 98, 97, 83 and 81% for catechin, epicatechin, procyanidin B1, procyanidin B2, quercetin and *trans*-resveratrol, respectively.

2.6. Statistical analysis

Variables measured were expressed as mean ± SD. The statistical significance was assessed by one-way ANOVA followed by Bonferroni's multiple comparison post-test. GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Quantification of phenolic compounds in GPE

The concentration of phenolic compounds in GPE was initially determined to characterize the composition of GPE and calculate the rat polyphenol intake at the tested doses. The selection of quantified compounds was based on their abundance and the relevance of their bioactive properties reported in previous works. Table 1 shows the concentrations of phenolic compounds in GPE. With regard to the flavanol family of compounds, the main polyphenol in GPE was catechin at a concentration that doubled that of epicatechin. The dimer procyanidin B1 was also abundant, with a concentration level approximately two-fold higher than that of procyanidin B2. The main flavonol was quercetin, that was found at similarly high concentrations asflavanols. The concentration of the stilbene *trans*-resveratrol was found at lower levels than those of the flavanol and flavonol families.

Table 1
Concentration of phenolic compounds quantified in GPE by UHPLC-MS/MS.

Compound	GPE (μg/g)
Catechin	3138 ± 241
Epicatechin	1474 ± 147
Procyanidin B1	1144 ± 102
Procyanidin B2	645 ± 43
Quercetin	1395 ± 31
***trans*-Resveratrol**	35 ± 1

Values are shown as means ± SD, n = 3 experimental replicates.

3.2. Profile of phenolic compounds and their metabolites in plasma

The concentrations of phenolic compounds and their metabolites were characterized in the plasma of rats receiving two different doses of GPE (300 and 600 mg/kg body weight) (Table 2). After 2 h of acute GPE intake, 10 metabolites derived from catechin, epicatechin, quercetin and *trans*-resveratrol were detected. Only catechin and epicatechin were detected as free phenolic compounds at similar concentrations, showing a different ratio to that observed in GPE. The concentration of most metabolites in plasma were significantly higher at the highest GPE dose consumed.

Regarding the concentration of metabolites, the glucuronidated forms of epicatechin and catechin were the most abundant metabolites in plasma, being approximately 6-fold higher at the highest compared to the lowest GPE dose tested (4363 nM and 4322 nM, respectively). These metabolites were followed in abundance by their methyl-glucuronidated counterparts, while only epicatechin methyl-sulphate was found in plasma. The metabolite quercetin methyl-glucuronide was found in relative low concentrations (244 nM) only at the highest dose of GPE. The conjugated forms of *trans*-resveratrol (glucuronidated and sulphated) were observed at low concentrations in a dose-dependent manner. On the other hand, metabolites of the dimers B1 and B2 were no detected at any concentration tested.

3.3. Profile of phenolic compounds and their metabolites in liver, muscle and epididymal adipose tissue

The phenolic metabolites detected in liver, soleus muscle and epididymal adipose tissue are summarized in Table 3. Five metabolites were detected in the liver, which concentrations were mostly associated with the concentration of their precursors in GPE, including the glucuronidated and methyl-glucuronidated forms of catechin and epicatechin. While the metabolites of catechin were detected at both GPE doses, those of epicatechin were only detected at the highest GPE dose tested.

The glucuronidated and methyl-glucuronidated derivatives of catechin and epicatechin were also detected in the muscle of rats consuming the highest GPE dose. In adipose tissue, detectable levels of catechin methyl-glucuronide were found at the highest GPE dose. Neither quercetin nor *trans*-resveratrol metabolites were detected in muscle or epididymal adipose tissue. Parent compounds were not detected in adipose, liver and muscle tissues at both GPE doses tested.

4. Discussion

GPE constitutes an important source of phenolic compounds with important beneficial effects on health. As far as we know, this is the first study that evaluated the availability and distribution of the most relevant flavanols, quercetin and *trans*-resveratrol from Malbec GPE and their metabolites in rat plasma and tissues. In this regard, there is only one study evaluating parent compounds of flavanols and their biological metabolites in rats after the ingestion of Syrah GPE in a dose of 5 g/kg body weight [26], however, quercetin and *trans*-resveratrol metabolites were not evaluated.

As a result of the extensive metabolism, mainly the conjugated forms (glucuronidated predominant over sulphate and methyl) of the parent compounds catechin, epicatechin, quercetin and *trans*-resveratrol were found in plasma after GPE intake. In humans, metabolites rather than parent compounds appear in plasma after oral consumption of epicatechin [27–29]. These results are in agreement with previous studies where the bioavailability of flavanols and procyanidins were evaluated in rats following the ingestion of a grape seed extract in rats [30,31], or green tea in human, mouse and rats [32,33]. The

Table 2
Plasma concentration of phenolic compounds and their metabolites after an acute intake of GPE.

	Control (nM)	GPE 300 mg/kg (nM)	GPE 600 mg/kg (nM)
Catechin	n.d.	196 ± 31	741 ± 314
Catechin-glucuronide	n.d.	672 ± 88	4322 ± 1674
Catechin methyl-glucuronide	n.d.	717 ± 152[a]	4210 ± 521[b]
Catechin methyl-sulphate	n.d.	n.d	n.d
Epicatechin	n.d.	207 ± 34	744 ± 262
Epicatechin-glucuronide	n.d.	723 ± 94	4363 ± 1650
Epicatechin methyl-glucuronide	n.d.	396 ± 106[a]	2631 ± 765[b]
Epicatechin methyl-sulphate	n.d.	130 ± 29	177 ± 65
Dimer B1 y B2	n.d.	n.d	n.d
Quercetin methyl-glucuronide	n.d.	n.d.	244 ± 77
trans-Resveratrol	n.d.	n.d	n.d
Resveratrol-3-glucuronide	n.d.	42 ± 12	74 ± 25
Resveratrol-4-glucuronide	n.d.	55 ± 5	99 ± 22
Resveratrol-3-sulphate	n.d.	33 ± 7	81 ± 26

Plasma concentration (nM) of metabolites after an acute intake of vehicle (Control) or 300 and 600 mg GPE/kg body weight, (n = 6/group). Values are shown as means ± SD. Values having different superscripts are significantly different ($p < 0.05$, one way ANOVA). n.d. = not detected.

glucuronidated forms of epicatechin and catechin were the most abundant metabolites observed in plasma. These results are in agreement with previous studies evaluating extracts rich in procyanidins [9], Syrah GPE [26] or after ingestion of pure catechin and epicatechin [34–36]. Its has been reported that rats show a high ability to conjugate epicatechin with glucuronic acid [36].

Quercetin was found in plasma as a methyl-glucuronide metabolite in rats that received the highest dose of GPE, but lower concentrations compared to those of the flavanols epicatechin and catechin. Some competitive interactions between flavonoids (catechin and quercetin) at the gastrointestinal level was reported by Silberberg et al. [37], showing a reduced intestinal absorption of quercetin when catechin was present. However, important amounts of quercetin methyl-glucuronide were found in liver. Probably, these differences could be due to other metabolites from quercetin not determined in this study or differences in the time of tissue sampling.

It is interesting to mention that sulphate and glucuronidated derivates of *trans*-resveratrol were detected for the first time in rat plasma after GPE administration, no free *trans*-resveratrol was found, suggesting that the absorbed *trans*-resveratrol was extensively converted into those metabolites. These findings are in agreement with previous reports showing the presence of these metabolites in plasma after oral consumption of pure *trans*-resveratrol (5.9 mg/kg body weight) [38]. Our finding is also consistent with earlier studies indicating that glucuronidation and sulphation are the main metabolic pathways of *trans*-resveratrol [39].

After GPE intake, a dose depend increment of plasmatic metabolites was observed. The flavanol metabolites showed an approximately 6-fold plasma increment after consumption of 600 mg GPE/kg body weight compared to the lowest dose. In spite of some reports in human studies suggest a linear and relatively direct relationship between the concentration of epicatechin metabolites excreted in urine and the amount of epicatechin ingested [40,41], this extrapolation could be only possible when pure compounds are ingested. As it is well known, substantial differences in metabolism can be observed when a complex matrix of food or extract is administrated. In the present work, the observed differences between doses for the rest of the metabolites could be related to the complex nature of the GPE administrated to rats.

We also found relatively high concentrations of parent compounds catechin and epicatechin in plasma (13 and 8% in lower and high doses, respectively). Usually parent compounds are not observed in plasma [23] or are present in low concentrations after oral consumption [12,30,42]. These differences could be due to the fact that plasma concentrations reached after polyphenol consumption highly vary according to the dose ingested, the nature of the polyphenol and the food matrix [12]. After 2 h postingestion the concentration of epicatechin present in plasma was similar or even higher than that of catechin, although the amount of catechin compared to epicatechin in the GPE is two times higher. Differences in the bioavailability of these stereoisomers were demonstrated in rats that received a drink containing the same amount (17.2 mmol/l) of catechin and/or epicatechin. This study shows that epicatechin has higher bioavailability than catechin because they might compete for absorption at the gastrointestinal tract of rats [35]. Similar results were reported by Ottaviani et al. [22] in humans consuming equal amounts of pure flavanols (−)-epicatechin, (−)-catechin, (+)-catechin and (+)-epicatechin and showing that plasma

Table 3
Concentration of metabolites in tissues after an acute intake of GPE.

Tissue	Compound	Control (nM)	GPE 300 mg/kg (nM)	GPE 600 mg/kg (nM)
Liver	Catechin-glucuronide	n.d.	11 ± 1[a]	18 ± 4[b]
	Catechin methyl-glucuronide	n.d.	25 ± 6[a]	125 ± 50[b]
	Epicatechin-glucuronide	n.d.	n.d.	43 ± 7
	Epicatechin methyl-glucuronide	n.d	n.d.	71 ± 15[b]
	Quercetin methyl-glucuronide	81 ± 28	305 ± 57	1300 ± 4
Muscle	Catechin-glucuronide	n.d.	n.d.	32 ± 15
	Catechin methyl-glucuronide	n.d.	n.d.	97 ± 15
	Epicatechin-glucuronide	n.d.	n.d.	52 ± 15
	Epicatechin methyl-glucuronide	n.d.	n.d.	83 ± 4
Adipose tissue	Catechin-glucuronide	n.d.	n.d.	n.d.
	Catechin methyl-glucuronide	n.d.	n.d.	42 ± 2
	Epicatechin-glucuronide	n.d.	n.d.	n.d.
	Epicatechin methyl-glucuronide	n.d.	n.d.	n.d.

Liver, muscle and adipose tissue concentration (nM) of metabolites after an acute intake of vehicle (Control) or 300 and 600 mg GPE/kg body weight, (n = 6/group). Values are shown as means ± SD. Values having different superscripts are significantly different ($p < 0.05$, one way ANOVA). n.d. = not detected.

(−)-epicatechin was higher than those of the others flavanols. On the other hand, dimers B1 and B2 were not detected in plasma or tissues after 2 h of oral ingestion. Accordingly, it has been shown that dimers are poorly absorbed [12,43].

During the course of absorption, polyphenols are conjugated in the small intestine and later in the liver, after this metabolites are distributed in different tissues. In this study, the abundance of phenolic metabolites in tissues was liver > muscle > adipose tissue mainly in the form of glucuronides and methyl-glucuronides. No unconjugated parent compounds were detected in tissues. As expected, the highest concentrations of metabolites were found in the liver. These findings are in agreement with previous reports [23,30,44]. Andres-Lacueva et al. [45] found lower amounts of metabolites from resveratrol in rats muscle in comparison to liver and adipose tissue, after a chronic supplementation with resveratrol (30 and 60 mg/kg body weight). In contrast to our results, they found resveratrol metabolites in muscle, while we found catechin-glucuronide and methyl-glucuronide, and epicatechin-glucuronide and methyl-glucuronide at the highest GPE dose. Borges et al. reported quantifiable amounts of epicatechin in muscle, after an acute intake of the radiolabeled metabolite [2–^{14}C]-epicatechin [46]. On the other hand, data regarding the distribution of polyphenol metabolites in adipose tissue are limited, even in animals. Based on previous studies, some metabolites can reach the subcutaneous and mesenteric adipose tissue after acute or chronic grape seed procyanidin extract ingestion [44,47]. Several studies found in the bibliography have been focused on metabolites in other target tissues such as brain, heart, intestine and kidney, among others [9,24,31].

It is important to note that the nature of the intake and the time of tissue sampling used are relevant, and depend on the kinetics of the accumulation and elimination of phenolic compounds in tissues. Current results indicate that metabolites of the measured polyphenolics, rather than the parent compounds, may be responsible for the reported beneficial actions of GPE. Long-term treatments with GPE are necessary to evaluate the tissue presence of other types of metabolites, e.g. those generated by the microbiota, and will be important to provide new insights related to the kind and concentration of metabolites present in tissues [9,48]. This knowledge will help further understand which metabolites are related to the observed biological effects.

5. Conclusions

The present study is an important step in the understanding of the metabolism of the most relevant GPE phenolic compounds in rats, their absorption, availability and later distribution in tissues. Current results shows that GPE constitutes an interesting matrix or vehicle for delivering polyphenols with potential health benefits.

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