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Original article

Grape pomace polyphenols improve insulin response to a standard meal in healthy individuals: A pilot study

G. Costabile [a], M. Vitale [a, *], D. Luongo [b], D. Naviglio [c], C. Vetrani [a], P. Ciciola [a], A. Tura [d], F. Castello [e], P. Mena [e], D. Del Rio [e, f, g], B. Capaldo [a], A.A. Rivellese [a], G. Riccardi [a], R. Giacco [h]

[a] Department of Clinical Medicine and Surgery, Federico II University of Naples, Via S. Pansini 5, 80131, Naples, Italy
[b] CNR-Institute of Biostructures and Bioimaging, Via Tommaso De Amicis, 95, 80131, Naples, Italy
[c] Department of Chemical Sciences, Federico II University of Naples, Via Cintia 21, 80126, Naples, Italy
[d] Metabolic Unit, CNR Institute of Neuroscience, Corso Stati Uniti 4, 35127, Padova, Italy
[e] Department of Food & Drugs, University of Parma, Via Volturno 39, 43125 Parma, Italy
[f] Department of Veterinary Science, University of Parma, Via Volturno 39, 43125 Parma, Italy
[g] Microbiome Research Hub, University of Parma, Parco Area delle Scienze 11/A, 43124 Parma, Italy
[h] Institute of Food Science, National Research Council, Via Roma 64, 83100 Avellino, Italy

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SUMMARY

Background & aims: Dietary polyphenols have beneficial effects on glucose/lipid metabolism in subjects at high risk to develop type 2 diabetes; however, the underlying mechanisms are not clear. We aimed to evaluate: 1) the acute effects of the consumption of a drink rich in polyphenols from red grape pomace (RGPD) on glucose/insulin and triglyceride responses to a standard meal in healthy individuals, and, 2) the relationship between plasma levels of phenolic metabolites and metabolic parameters.
Methods: Twelve healthy men, aged 20–40 years participated in a randomized, controlled study according to a cross-over design. After a 3-day low–polyphenol diet, all participants consumed, on two different days and separated by a one week interval, after an overnight fast, a drink rich in polyphenols (1.562 g gallic acid equivalents (GAE)) or a control drink (CD, no polyphenols), followed after 3 h by a standard meal (960 kcal, 18% protein, 30% fat, 52% CHO). Blood samples were taken at fasting, 3 h after the drink, over 5 h after the standard meal and at fasting on the next day to measure plasma concentrations of glucose, insulin, triglyceride and phenolic metabolites.
Results: Glycemic and triglyceride post-meal responses were similar after both the RGPD and the control drink. In contrast, postprandial insulin incremental area ($iAUC_{0-5h}$) was 31% lower ($p < 0.05$), insulin secretion index was 18% lower ($p < 0.016$) and insulin sensitivity (S_I) index was 36% higher ($p = 0.037$) after the RGPD compared to CD. Among phenolic metabolites, gallic acid correlated inversely with the insulin response ($r = -0.604$; $p = 0.032$) and positively with the S_I index ($r = 0.588$, $p = 0.037$).
Conclusions: RGPD consumption acutely reduced postprandial insulin levels and improved insulin sensitivity. This effect could be likely related to the increase in gallic acid levels. This drink, added to usual diet, could contribute to increase the daily intake of polyphenols, with potential health benefits.
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1. Introduction

In recent years, the interest in dietary polyphenols has rapidly increased, due to their potential to improve several biological functions, such as oxidative stress, endothelial function, platelet aggregation, lipid and glucose metabolism [1,2] and, consequently, to prevent chronic diseases such as cardiovascular disease (CVD), type 2 diabetes (T2D) and some types of cancer [3–7]. However,

* Corresponding author. Department of Clinical Medicine and Surgery, Federico II University of Naples, Via S. Pansini 5, 80131, Naples, Italy.
E-mail address: marilena.vitale@unina.it (M. Vitale).

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most of the available studies have focused on single polyphenol classes, mainly flavonoids and their subclasses, and have used pharmacological doses of polyphenols that are difficult to achieve with the habitual diet [8,9]. Moreover, by this approach, the healthful effects of polyphenols in free living conditions cannot be fully appreciated, as they can be better defined when naturally polyphenol-rich food are consumed. Indeed, a clinical trial performed in subjects with metabolic syndrome has shown that a diet rich in polyphenols (about 3 g/day), completely derived from natural foods, exerts beneficial effects on lipid and glucose/insulin metabolism [10,11].

Grapes and red wine are important sources of flavan-3-ols, anthocyanins, and resveratrol, which are the most investigated polyphenols due to their putative cardio-protective and chemopreventive effects [12–14]. The benefits of grapes and wine polyphenols against cardiovascular diseases seem to be mediated by their favorable effects on plasma lipid levels, LDL oxidation, and inhibition of platelet aggregation, inflammation and blood pressure [15]. Limited data is available so far on the effects of grape polyphenols on glucose and insulin metabolism. In acute studies, the supplementation of grape polyphenols was able to reduce plasma glucose concentration in both animals and humans [16,17]. An improvement in glucose metabolism was also observed after a prolonged supplementation of grape polyphenols in people with the metabolic syndrome and type 2 diabetes [18–20]. However, so far, a detailed analysis of the metabolic pathways of the polyphenols involved in the modulation of glucose metabolism and insulin action is not yet available. In fact, as most of the studies investigating the effects of grape polyphenols on clinical outcomes in humans have not measured their metabolites, the relation between the polyphenol metabolic pathways involved in their clinical effects remains unclear.

In our previous publication, we reported that the acute intake of an experimental red grape pomace drink (RGPD) developed by us and prepared in our laboratory was followed by an increase in the plasma levels of several phenolic metabolites, indicating that RGPD polyphenols were absorbed in the intestine and metabolized [21].

In this pilot study, we evaluated the acute effects of the consumption of this RGPD on glucose/insulin and lipid responses to a standard meal in healthy individuals. In addition, we assessed the relationship between circulating phenolic metabolites and the main processes regulating glucose metabolism, i.e., insulin secretion and insulin sensitivity.

2. Material and methods

2.1. Participants

Thirty-one men were recruited through advertisings posted at the Department of Clinical Medicine and Surgery of Federico II University of Naples. Nine candidates were excluded because they did not meet the inclusion criteria, i.e., age 20–40 years, BMI 20–30 kg/m^2, fasting plasma glucose <110 mg/dL, fasting plasma triglycerides ≤200 mg/dL and fasting plasma cholesterol ≤250 mg/dL. Exclusion criteria were cancer and other known chronic illnesses, diabetes, regular intensive physical activity (defined as more than five training units/week), renal failure (serum creatinine>1.7 mg/dL), liver disease, anemia, cardiovascular disease and any other degenerative diseases, use of drugs able to influence glucose and lipid metabolism. Ten of those selected declined to participate; overall, twelve men were studied after providing written informed consent. The main clinical characteristics of the participants are presented in Table 1. The study was approved by the Ethics Committee of the "Federico II" Naples University.

Table 1
Characteristics of participants in the study (n = 12).

Age (years)	26±3[a]
BMI (kg/m^2)	26 ± 2
Systolic Blood Pressure (mmHg)	125 ± 11
Diastolic Blood Pressure (mmHg)	77 ± 6
Fasting plasma glucose (mg/dl)	96 ± 3
Fasting plasma insulin (μU/ml)	12 ± 6
Fasting plasma cholesterol (mg/dl)	152 ± 29
Fasting plasma triglycerides (mg/dl)	86 ± 34
Fasting plasma HDL-cholesterol (mg/dl)	39 ± 5
Homa Index	2.8 ± 1.1

[a] Means ± SD (all such values).

2.2. Study design

Participants were asked to consume a low-polyphenol diet during the experimental period (at least 3 days before and 2 days after the 2 test days) as reported in our previous publication [21]. To facilitate adherence, the volunteers were given a list of permitted and forbidden foods. To assess polyphenols intake, participants filled in a 3-day food record before each test day. Moreover, they were instructed to abstain from performing vigorous physical activities 24 h before each test day. The study was conducted with a randomized, controlled, cross-over design (Fig. 1). On the two experimental days, at one-week interval, participants were admitted to the research center after a 12 h overnight fast, for baseline blood drawing; thereafter, they consumed in random order 250 mL of a red grape pomace drink [RGPD; 24 g of soluble carbohydrates, 92 kcal and 1562 g of total polyphenols, as gallic acid equivalents (GAE)] or 250 mL of a control drink without polyphenols (CD; 24 g of soluble carbohydrates, 92 kcal). Randomization was made by a web-based program. Three hours after RGPD or CD consumption, participants consumed a standard low-polyphenol meal, consisting of white bread (150 g), fatless ham (70 g), spreadable cheese (80 g) and plumcake (33 g) (903 kcal, 18% protein, 30% fat, 52% carbohydrates). At the end of the test day, participants received another standard meal (pasta omelet) (905 kcal, 14% protein, 46% fat, 40% carbohydrates) to be consumed at 9.00 p.m. for dinner.

2.3. Polyphenol composition of red grape pomace drink

A total of 25 phenolic compounds were identified and quantified in the RGPD. The retention times and mass spectra of the compounds detected are reported in our previous publication (21). The 250 mL of the RGPD used in this study contained 3.7 mmol of total polyphenols. Anthocyanins were the most abundant class of phenolic compounds (70%), followed by flavan-3-ol monomers (23%) and procyanidins (4%). Small amounts of flavonols, galloyl glucose, and gallic acid were also present in the drink.

2.4. Blood sampling and analytical methods

Blood samples were collected from an antecubital arm vein at fasting before RGPD/CD consumption. After 3 h, a standard meal was administered and blood samples were collected immediately before and at 30 min intervals for 5 h. In addition, the day after the test, a fasting sample was also collected in the morning, 24 h after the test drink.

Blood drawn in EDTA tubes was centrifuged and plasma was stored at −80 °C until the analyses. Plasma glucose and triglyceride concentrations were assayed by enzymatic colorimetric methods (ABX Diagnostics, Montpellier, France) on an ABX Pentra 400 Autoanalyzer (ABX Diagnostics, Montpellier, France). Plasma insulin concentration was measured by sandwich enzyme-linked

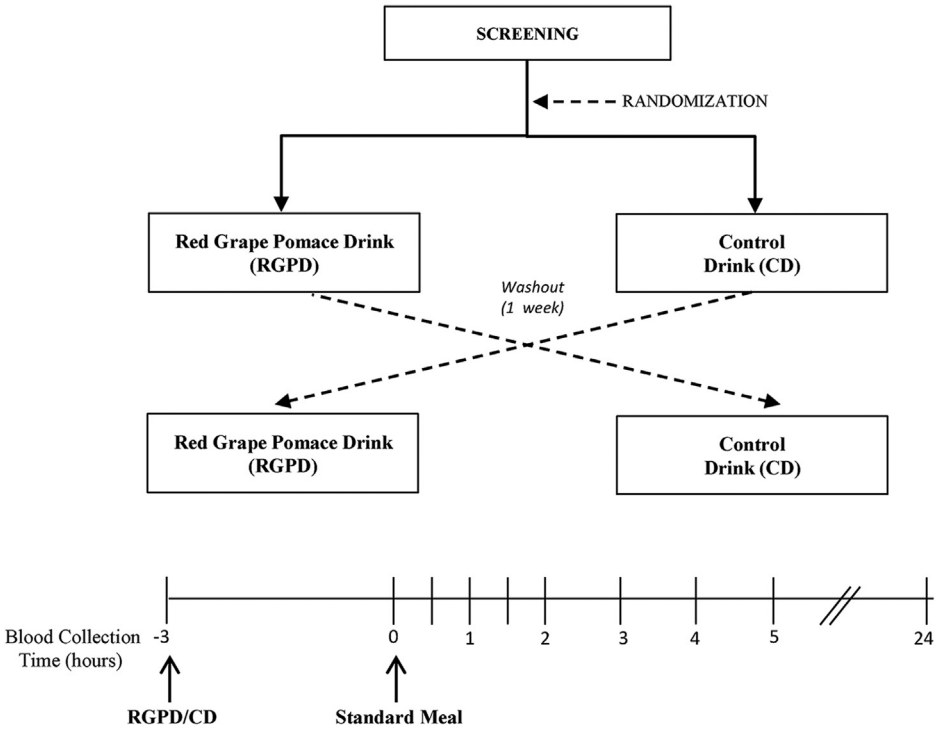

Fig. 1. Study design.

immunosorbent assay methods (ELISA; DIAsource ImmunoAssays S.A., Nivelles, Belgium; Merck-Millipore, Darmstadt, Germany) on Triturus Analyzer (Diagnostics Grifols, S.A., Barcelona, Spain). Plasma phenolic metabolites analysis was performed only in ten participants by UHPLC-ESI-MS/MS techniques, as reported previously [21].

2.5. Data analysis and statistics

The primary outcome of the present study was the differences in insulin sensitivity after RGPD and CD consumption. Due to the preliminary nature of the study, it was not possible to calculate the sample size needed to obtain a power greater than 80% with an α error less than 5%.

All data are expressed as mean ± SEM unless otherwise stated.

The post-meal response of glucose, insulin, triglycerides, polyphenols and their metabolites was expressed as incremental area under the curve above the fasting value (iAUC). Fasting insulin resistance was assessed by the Homeostatic Model Assessment (HOMA) method calculated as: [fasting glucose (mg/dl) x fasting insulin (μU/ml)/405]. The insulin sensitivity index (S_I) was calculated from the meal glucose tolerance test (MGTT) over 2 h according to Caumo et al. [22].

The insulin secretion index was calculated as ratio between insulin and glucose total areas under the curve (insulin tAUC $_{0-5\ h}$)/(glucose tAUC $_{0-5\ h\ min}$).

A repeated measures ANOVA was performed to examine the effects of RGPD/CD on postprandial glucose, insulin and triglycerides responses. In this analysis, post-meal values measured after the standard meal were included as levels of the within-subject "time" factors, and RGPD and CD were included as levels of the within-subject "drink" factors. Differences between time-points of post-meal glucose, insulin and triglycerides responses and the corresponding iAUC, S_I index, and Insulin Secretion were tested by paired sample t-test.

The associations between plasma phenolic metabolites and the main clinical outcomes in the participants consuming RGPD were explored by bivariate associations using Pearson's correlation. A stepwise linear regression analysis, using the metabolic parameters as dependent variables and plasma phenolic metabolites as independent variables, was also performed to assess the plasma phenolic metabolites that best predicted the outcomes. The regression model was also used to test for multicollinearity through tolerance and variance inflation factor (VIF). If the value of tolerance was more than 0.2 and, simultaneously, the value of VIF less than 10, then the multicollinearity was considered not problematic.

For all analyses, the level of statistical significance was set at $p = 0.05$ (two tails). Statistical analysis was performed using the SPSS software 20.0 (SPSS/PC; IBM Armonk, NY, USA).

3. Results

3.1. Post-meal metabolic response

Glucose, insulin and triglyceride levels at fasting and in response to the standard meal and the corresponding iAUC are reported in Fig. 2. On the two experimental days, there were no differences in fasting values; after RGPD or CD consumption, the post-meal glucose response was not different ($p = 0.299$, drink effect; repeated measures ANOVA), evaluated either at any time point of the curve or as iAUCs [540 ± 16 vs 549 ± 15 mg/dl × 5 h, respectively; $p = 0.903$] (Fig. 2A). Conversely, post-meal insulin levels were lower after RGPD than after CD consumption ($p = 0.025$, drink effect; repeated measures ANOVA), reaching a statistically significant difference at 1 and 2 h after the meal ($p < 0.05$); consistently, the corresponding iAUCs was significantly lower after RGPD than CD [102 ± 10 vs 148 ± 19 μU/ml × 5 h (M±SEM), respectively; $p = 0.036$] (Fig. 2B).

Post-meal plasma triglyceride levels were not different after consuming RGPD or CD ($p = 0.675$, meal effect; repeated measures

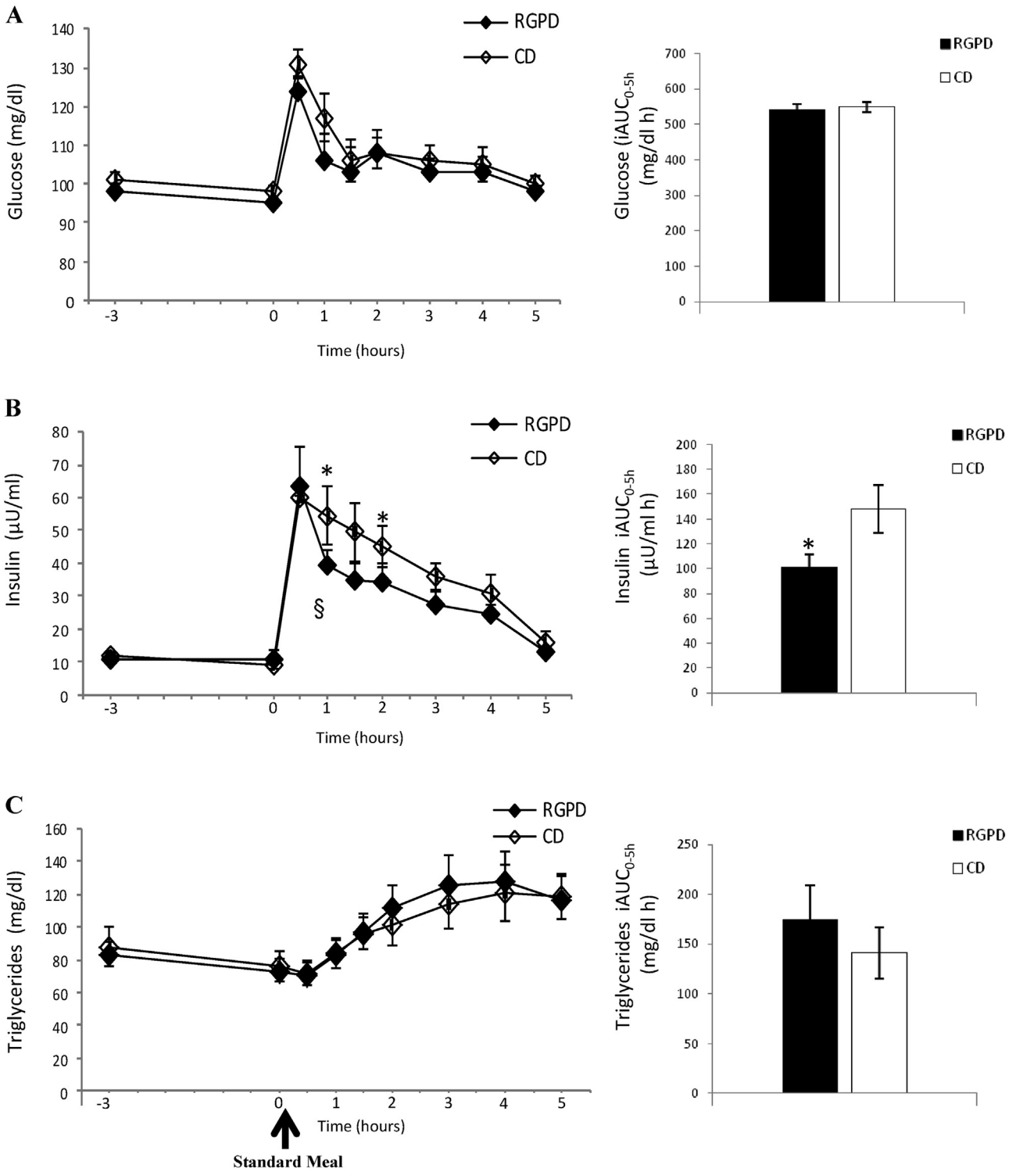

Fig. 2. Postprandial plasma glucose (**A**) insulin (**B**) and triglycerides (**C**) concentrations and corresponding incremental area under the curve (iAUC) (means ± SEM) after the intake of the Red Grape Pomace Drink (RGPD) and Control Drink (CD) in young volunteers. §p < 0.05 for drink effect by Repeated Measures ANOVA; *p < 0.05 Different from Control (Paired sample t-test).

ANOVA), either at any time point of the curve or as iAUCs [173 ± 35 vs 141 ± 26 mg/dl × 5 h, (M±SEM), respectively; p = 0.176] (Fig. 2C).

Insulin sensitivity index (S_I), over 2 h after the meal, was significantly higher (+36%) after RGPD as compared to CD intake [0.34 ± 0.06 vs. 0.25 ± 0.06 mL/min/kg/µU/mL, respectively; p = 0.037] (Fig. 3). Moreover, the insulin secretion index calculated over 5 h after the standard meal was significantly lower (−18%) after RGPD compared to CD consumption [0.29 ± 0.03 vs. 0.35 ± 0.04 µU/mg, respectively; p = 0.016] (Fig. 3).

Twenty-four hours after the test day, fasting glucose, insulin and triglyceride concentrations were similar after consumption of RGPD or CD (glucose: 97 ± 2 vs 98 ± 2 mg/dl; insulin: 11 ± 2 vs

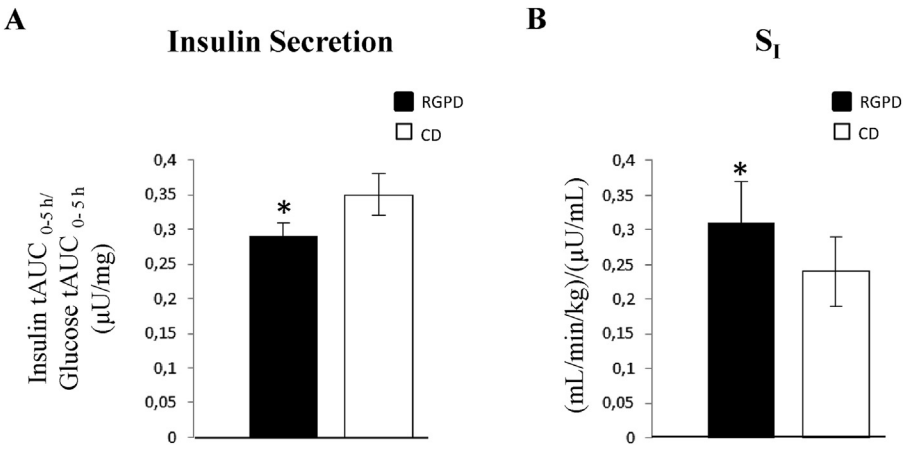

Fig. 3. Insulin Secretion (A) and Insulin Sensitivity (B) Indices (meas±SEM) after the intake of the Red Grape Pomace Drink (RGPD) and Control Drink (CD) in young volunteers. *p < 0.05 Different from Control (Paired sample t-test).

12 ± 1 μU/ml; triglyceride: 81 ± 8 vs 79 ± 12 mg/dl; (M±SEM), respectively).

3.2. Plasma phenolic metabolites

The pharmacokinetic parameters of phenolic metabolites detected in plasma have been reported previously by Castello et al. [21]. In particular, up to 28 compounds have been identified in the plasma samples following RGPD consumption. For each compound, the incremental area under the plasma concentration-time curve (iAUC) over 8 h after RGPD ingestion is reported in Table 2. Phenyl-γ-valerolactones were the most abundant class of phenolic metabolites in plasma, of which the glucuronide- and sulphate-conjugated isomers of 5-(3′,4′-hydroxyphenyl)-γ-valerolactone represented the most abundant compounds (Table 2). A total of 10 hydroxybenzoic acids and simple phenols were detected, being methylpyrogallol-sulphate, protocatechuic acid-3-sulphate followed by vanillic acid-4-sulphate and gallic acid, the most representative compounds (Table 2).

Table 2
Plasma polyphenol metabolites concentrations after red grape pomace drink consumption.

Polyphenolic compounds	$iAUC_{0-8h}$ (nmol h L^{-1})
Simple phenols and hydroxybenzoic acids	
Gallic acid	193.5 ± 43.3[a]
Vanillic acid-4-glucuronide	172.6 ± 25.4
Protocatechuic acid	5.8 ± 2.1
Protocatechuic acid-3-glucuronide	5.2 ± 1.5
Protocatechuic acid-3-sulphate	970.1 ± 134.4
Benzoic acid-4-sulphate	76.8 ± 35.2
Vanillic acid-4-sulphate	205.3 ± 43.8
Catechol-sulphate	4.8 ± 2.4
Methylpyrogallol-sulphate	1394.2 ± 313.7
Methylcatechol-sulphate	136.9 ± 24.5
4-Hydroxyhippuric acid	180.5 ± 62.1
Hydroxyphenylpropionic and hydroxycinnamic acids	
Ferulic acid 4-glucuronide	109.4 ± 24.6
Feruloylglycine	19.4 ± 9.3
Dihydrocaffeic acid-sulphate	8.0 ± 2.0
Dihydroferulic acid-sulphate	4.2 ± 1.2
Ferulic acid-4-sulphate	18.7 ± 4.3
(Epi)catechin derivatives	
(Epi)catechin-glucuronide-sulphate	12.9 ± 3.4
(Epi)catechin-glucuronide	396.8 ± 33.1
(Epi)catechin-sulphate, isomer 1	146.3 ± 32.3
(Epi)catechin-sulphate, isomer 2	241.5 ± 46.4
Methyl(epi)catechin-sulphate, isomer 1	25.8 ± 3.8
Phenyl-γ-valerolactones and phenyl-valeric acids	
5-Phenyl-valeric acid-sulphate-glucuronide	26.0 ± 7.6
5-(3′-Hydroxyphenyl)-γ-valerolactone-4′-glucuronide	712.8 ± 166.8
5-(4′-Hydroxyphenyl)-γ-valerolactone-3′-glucuronide	3788.4 ± 826.5
5-(Hydroxyphenyl)-γ-valerolactone-sulphate isomers	2634.2 ± 536.3
5-Phenyl-γ-valerolactone-3′-glucuronide	206.3 ± 103.7
5-Phenyl-γ-valerolactone-3′-sulphate	141.5 ± 58.6
5-(3′,4′-Dihydroxyphenyl)-γ-valerolactone	42.5 ± 11.2

[a] Means ± SEM (all such values).

Regarding hydroxyphenylpropionic acids and hydroxycinnamic acids, up to five compounds were detected in plasma. Ferulic acid-4-glucuronide was the most abundant compound (Table 2). A total of 5 epicatechin derivatives were detected in plasma, of which the most representative was epicatechin-glucuronide, followed by epicatechin sulphate isomers 1 and 2 (Table 2).

A linear correlation analysis showed that plasma gallic acid concentration (Gallic Acid $iAUC_{0-8h}$) was the only phenolic metabolite being inversely correlated with post-meal plasma insulin response (Insulin $iAUC_{0-5h}$) ($r = -0.604$, $p = 0.032$) after RGPD consumption, and positively with the Insulin Sensitivity Index (S_I) ($r = 0.588$, $p = 0.037$) (Fig. 4). No other correlation was statistically significant between any of the phenolic metabolites and insulin secretion and insulin sensitivity indices.

Stepwise linear regression, using the S_I index as dependent variable and plasma phenolic metabolites as independent variables, showed that gallic acid was the best predictor of S_I index ($\beta = 0{,}707$, $p = 0.033$), followed by epicatechin-glucuronide ($\beta = 0{,}274$, $p = 0.001$) and dihydrocaffeic acid-sulphate ($\beta = 0{,}089$; $p = 0.015$).

4. Discussion

The most relevant finding of our study is that the acute consumption of an experimental RGPD rich in polyphenols decreased post-meal insulin response and improved insulin sensitivity. Indeed, $iAUC_{0-5h}$ was 31% lower and the S_I index was 36% higher after the RGPD drink compared to CD. Similarly, insulin secretion index, calculated over 5 h after the standard meal, was 18% lower after RGPD than after CD.

Insulin sensitivity and insulin secretion have long been acknowledged as the main processes regulating glucose homeostasis [23]. They are linked by a close and inverse relationship and, therefore, changes in one process produce adaptation of the other one. Based on this notion, the lower post-meal insulin secretion observed in our study after the consumption of the RGPD drink should be interpreted as a compensatory reciprocal change induced by the improvement in insulin sensitivity following RGPD. The fact that post-meal glucose response was similar after both RGDP and

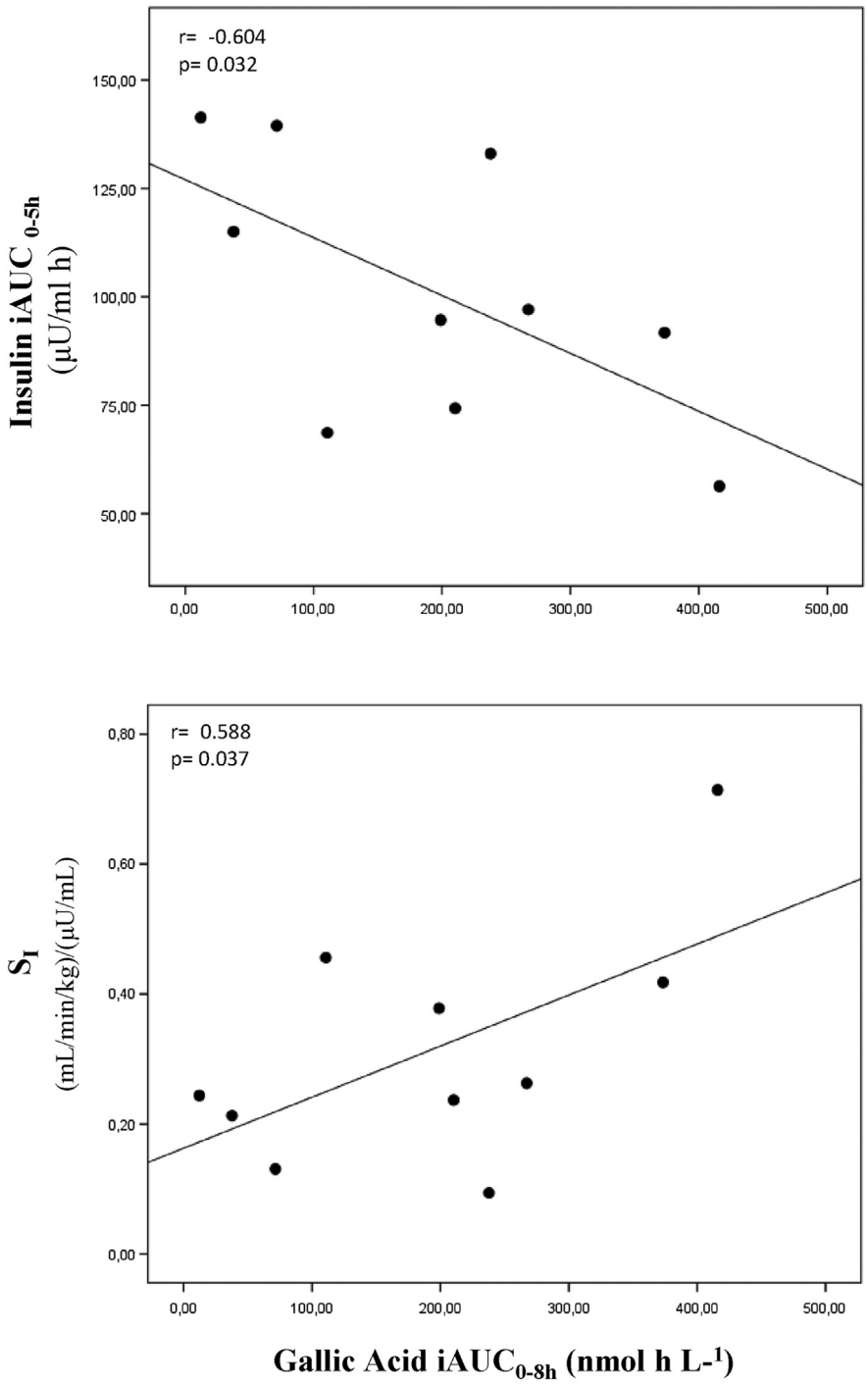

Fig. 4. Correlation between plasma gallic acid concentration ($iAUC_{0-8h}$) and Postprandial Insulin Response ($iAUC_{0-5h}$) and Insulin Sensitivity Index (S_I).

the control drink despite a different insulin response, further supports an improvement of insulin action.

Our findings are in line with previous studies showing a beneficial effect of grape polyphenols on glucose and insulin metabolism. In particular, a randomized controlled trial showed that the intake of grape seed extract for 4 weeks decreased fructosamine levels [19]. Similarly, Banini et al. [20] showed a reduction in fasting glucose, insulin and glycated hemoglobin after red wine and dealcoholized wine consumption for 4-week compared to grape juice. More recently, Urquiaga et al. [18] reported a significant decrease in fasting glucose levels and 2-h OGTT insulin concentrations after a 16-week supplementation with a grape polyphenol flour in patients at high cardiometabolic risk, with at least one component of the metabolic syndrome. Our study provides the novel finding that the beneficial effects of polyphenols and their metabolites on glucose homeostasis seem to be attributable to their ability to improve insulin sensitivity, which is compensated by a reduction, or down regulation, of β-cell function.

The finding that RGPD is associated with a lower post-meal insulin response has important clinical implications in the light of previous studies showing that postprandial hyperinsulinemia is a risk factor for the development of T2D and cardiovascular diseases

[24]. In the medium/long term, an improvement in postprandial insulin and glucose metabolism, through a reduction of β cell stress, might preserve β-cell function and delay the onset of diabetes [25].

An interesting observation of our study is that post-meal levels of gallic acid were inversely correlated with post-meal plasma insulin response and positively with S_I index (Fig. 4). Stepwise linear regression analysis confirmed gallic acid as the best predictor of S_I index. Based on our knowledge, this is a novel finding since until now no clinical trial has shown an association between specific phenolic compounds and outcomes related to type 2 diabetes. The benefit of gallic acid on insulin sensitivity is supported by previous studies showing that the administration of gallic acid together with a high-fat diet in diabetic rats induced lower body weight gain, fasting glucose and insulin levels [26]. In this study, gallic acid significantly enhanced peroxisome proliferator-activated receptor γ (PPARγ) expression and activated glucose transporter protein 4 (GLUT4) in adipose tissue, thus improving adipose tissue insulin sensitivity, modulating adipogenesis and increasing adipose glucose uptake [26]. *In vitro* studies on adipocytes also demonstrated that polyphenols from grapes might improve glucose uptake, directly influencing the expression of PPARγ and the translocation of glucose-transported GLUT-4 [27,28]. Finally, polyphenols might improve glucose metabolism also through their action on carbohydrate digestion and insulin secretion [29].

To our knowledge, our study is the first one demonstrating, in humans, an improvement of insulin sensitivity in acute conditions after polyphenol intake. An improvement in insulin sensitivity has been previously demonstrated by Bozzetto et al. [11] after 8 weeks of a naturally polyphenol-rich diet. A novelty of our study is that the metabolic effects of polyphenols were evaluated 3-h after RGPD ingestion while in previous studies, polyphenols were generally administered together with the meal. We chose this timing since the plasma concentration of different classes of phenolic metabolites peak at different time points [30], as also demonstrated in our previous publication [21]. Therefore, meal consumption closer to the expected maximal peak of phenolic metabolites, achieved 3–4 h after drink consumption, is likely to maximize the potential beneficial effects of these compounds on postprandial metabolic parameters.

The strengths of our study are the well-controlled design and the measurement of plasma phenolic metabolites, which allowed us to explore the relationship between polyphenol consumption and the metabolic responses.

Finally, for its beneficial effects on glucose metabolism, our experimental RGPD could be classified as a functional product to be recommended to the general population to increase their daily polyphenol dietary intake. Notably, the sugar content of our RGPD is very low compared with other fruit juices, especially those based on grapes; this characteristic represents a further health advantage, particularly for individuals with abnormal glucose metabolism.

One limitation of our study is the acute nature and the small sample size, due to the limited availability of RGPD, which was produced on a small scale in a laboratory setting. Nevertheless, RGPD was able to improve insulin sensitivity in subjects with normal glucose homeostasis. Thus, it is conceivable that the beneficial effect of the RGPD consumption could be even greater and more evident with a larger sample size.

In conclusion, polyphenols from a RGPD, consumed away from meal, improve insulin sensitivity and reduce insulin secretion – effects likely mediated by the increase in plasma levels of gallic acid. This drink included in the usual diet could contribute to increase the daily intake of polyphenols with potential health benefits. We have previously demonstrated that consuming regularly a polyphenol rich diet containing 3 g polyphenol/day led to a substantial improvement in glucose tolerance, insulin sensitivity and postprandial triglycerides. During that study, we had to acknowledge the difficulty of the study participants to adhere to the polyphenol rich diet. The possibility to integrate a diet based on natural rich polyphenol products with a drink obtained from grape might allow a reduction in the amount of natural products consumed daily, thus facilitating a better long term compliance to polyphenol rich diets.

Further intervention studies are needed to better clarify the impact of polyphenols and their metabolites on the metabolic parameters related to type 2 diabetes risk.

Statement of authorship

R. Giacco, B. Capaldo, G. Riccardi and AA Rivellese gave a substantial contribution to conception and study design of work; D. Luongo and D. Naviglio produced the experimental drink; G. Costabile and R. Giacco handled drafting of article; G. Costabile and M. Vitale performed the statistical analysis and contributed to the data interpretation; C. Vetrani, P. Ciciola, F. Castello, P. Mena and D. Del Rio performed the biochemical and polyphenols metabolites analysis; A. Tura carried out the assessment of insulin sensitivity and secretion; R. Giacco, G. Costabile and B. Capaldo revised the manuscript. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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