Journal of Functional Foods 83 (2021) 104561

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

May bioactive compounds from the olive fruit improve the postprandial insulin response in healthy adults?

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ARTICLE INFO

Keywords:
Gastrointestinal hormones
Insulin sensitivity
Olive oil
Phenolic compounds
Triterpenic acids

ABSTRACT

Scope. The postprandial effects of virgin olive oils (VOOs) enriched with phenolic compounds and triterpenes from the olive fruit on plasma glucose and insulin (primary outcomes), and gastrointestinal hormones responses were evaluated in healthy adults.

Methods and results: Single doses (30 mL) of three oils were evaluated: optimized polyphenols-rich VOO (OVOO); functional olive oil (FOO): OVOO enriched with triterpene acids; and VOO with low content of polyphenols. Postprandial plasma insulin release was lower after the intake of the FOO compared to VOO, while plasma glucose levels were lower after the intake of the VOO compared to OVOO. Matsuda's index of insulin sensitivity improved after the intake of FOO and OVOO, while the insulinogenic index and gastric inhibitory polypeptide (GIP) tended to improve after the intake of OVOO.

Conclusion: The enrichment of VOOs with bioactive compounds from the olive fruit increases its benefits, improving postprandial insulin release and peripheral tissue sensitivity.

1. Introduction

Metabolic syndrome (MetS) and diabetes are chronic metabolic diseases characterized by increased insulin resistance and decreased insulin sensitivity. Elevated postprandial glycemic has been associated with a higher incidence of cardiovascular events in patients with and

Abbreviations: ANOVA, analysis of the variance; iAUC, incremental area under the curve; CVD, cardiovascular disease; FOO, functional olive oil; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; INSI, insulinogenic index; GLM, general linear model; LMM, linear mixed-effects model; MetS, metabolic syndrome; OVOO, optimized virgin olive oil; PP, pancreatic polypeptide; PYY, peptide YY; SPSS, statistical package for the social sciences; T2D, type-2 diabetes; VOO, virgin olive oil.

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https://doi.org/10.1016/j.jff.2021.104561
Received 22 January 2021; Received in revised form 6 May 2021; Accepted 27 May 2021
Available online 2 June 2021

without type-2 diabetes (T2D) (Mannucci, Monami, Lamanna, & Adalsteinsson, 2012; O'Keefe, Gheewala, & O'Keefe, 2008). The American Heart Association considers glycemia to be one of the major controllable risk factors for metabolic diseases (Association, 2020).

Virgin olive oil (VOO), the Mediterranean diet's main fat source, is considered one of the healthiest dietary fats, associated with a low MetS prevalence (Estruch et al., 2018). Indeed, VOO promotes cardiovascular benefits and improves glycemic control, and, consequently, MetS (Yubero-Serrano, Lopez-Moreno, Gomez-Delgado, & Lopez-Miranda, 2019). In 2017, a systematic review and meta-analysis in healthy adults provided evidence that the intake of VOO could be beneficial for the prevention and management of T2D (Schwingshackl et al., 2017).

VOO contains bioactive compounds with recognized beneficial properties, such as polyphenols (Ditano-Vázquez et al., 2019; Peyrol, Riva, & Amiot, 2017), and triterpenic acids (Allouche et al., 2009; Guinda, Rada, Delgado, Gutiérrez-Adánez, & Castellano, 2010). Within them, hydroxytyrosol has demonstrated antioxidant, cardioprotective (Carluccio et al., 2003), anticancer (Fabiani, 2016) and neuroprotective (Rodríguez-Morató et al., 2015) properties. This compound also decreases the risk of MetS (Lemonakis et al., 2017). Triterpenic acids (oleanolic acids and maslinic acid) has demonstrated cardioprotective effects (de la Torre et al., 2020), and improve several factors related to MetS (Sharma, Kumar, Deshmukh, Bishayee, & Kumar, 2018). In humans, they exert beneficial effects against T2D and improve the response to insulin (Castellano, Guinda, Delgado, Rada, & Cayuela, 2013), increasing glycogen synthesis and inhibiting gluconeogenesis and glycogenolysis in mice (Wen et al., 2005).

Insulin and gastrointestinal hormones, namely glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), ghrelin, pancreatic polypeptide (PP), and peptide YY (PYY), are secreted in response to eating to regulate the postprandial glucose response, among other physiological functions. Thus, they contribute to gastric emptying, and satiety and satiation. Therefore, they are crucial in maintaining steady body weight and thus influence overweight, obesity, and associated high-prevalent metabolic and cardiovascular comorbidities. The gastric emptying determines the rate of blood glucose postprandial increase, causing the release of incretins that stimulate insulin secretion (Little et al., 2006). As far as we know, no studies have analyzed the effect of VOO or any of its components on postprandial release of insulin and gastrointestinal hormones in humans.

Therefore, the objective of the present study was to compare the postprandial effect of three olive oils with different bioactive compounds content: phenolics and triterpenic acids, on plasma concentration of glucose, insulin, and gastrointestinal hormones, insulinogenic index and insulin sensitivity in healthy adults.

2. Materials and methods

2.1. Studied olive oils

The present study is part of the NUTRAOLEUM trial (ClinicalTrials.gov ID: NCT02520739). Three olive oils with a different concentration in bioactive compounds and the same fatty acid profile were evaluated: 1) an optimized VOO (OVOO), high in phenolic compounds (490 ppm of phenolic compounds and 86 ppm of triterpenic acids); 2) a functional olive oil (FOO), a VOO high in phenolic compounds and additionally enriched with triterpenic acids, that was prepared by adding maslinic and oleanolic acids to the OVOO (487 ppm of phenolic compounds and 389 ppm of triterpenic acids respectively); and 3) a standard VOO obtained after cold washing of the OVOO to decrease the amount of polyphenols (124 ppm of phenolic compounds and 86 ppm of triterpenic acids). The characteristics and the nutritional composition of experimental oils have been described in detail elsewhere (Biel et al., 2016).

2.2. Study design

The NUTRAOLEUM trial is a randomized, double-blind, crossover and controlled trial conducted in Virgen de las Nieves and San Cecilio General Hospitals of Granada, Spain. The study design, the characteristics of the healthy adults, aspects of their diet, and intervention have been described in detail previously (Biel et al., 2016). In brief, the subjects were randomly assigned to three olive oils administration sequences (sequence 1: OVOO, VOO, and FOO olive oil; sequence 2: VOO, FOO, and OVOO olive oil; and sequence 3: FOO, OVOO, and VOO), paired by gender and age. Olive oils were sequentially administered over three periods of three weeks of intervention, preceded by two weeks of washout periods in which participants were requested to avoid olives and olive oil consumption. A subsample of 18 participants was chosen for a postprandial study, which was carried out the first day of each period of intervention, as specify below (de la Torre et al., 2020).

2.3. Postprandial intervention

Before the postprandial intervention, 10 h-fasting venous blood samples were collected. Then, 30 mL of the corresponding olive oil (VOO, OVOO, or FOO) were administered as a single dose with a piece of bread (80 g) and a glass of water (200 mL) (de la Torre et al., 2020). Blood samples were taken at 30, 45, 60, 120 and 240 min by using EDTA-coated tubes. For the determination of plasma gastrointestinal hormones, Pefabloc SC (AEBSF) (Roche), which is needed for ghrelin determination (1 mg/mL), and dipeptidyl-dipeptidase IV inhibitor (Linco), which is needed for the determination of GLP-1 (50 μM), were added to the whole blood. Blood samples were centrifuged at 1750*g*, 4 °C, during 15 min, and aliquots of plasma were immediately frozen and stored at −80 °C until analysis.

2.4. Analytical methods

Plasma glucose was analyzed using a colorimetric kit (ref. BSIS46-E, Spinreact, Spain) (coefficient of variation (CV): 4.79%). Plasma concentrations of insulin and gastrointestinal hormones were determined using a *MILLIplex*™ kit, with the Luminex 200 multiplex assay system built on xMAP technology with the Human Gut Hormone Panel (Millipore Iberica S.A., Madrid, Spain) as previously described (Gonzalez-Anton et al., 2015) (CV: 6.17% for insulin and 5.97, 8.61, 5.93, 8.38 and 9.85%, respectively for GIP, GLP-1, ghrelin, PP and PYY). The incremental areas under the curves (iAUCs) of postprandial glucose, insulin and gastrointestinal hormones were calculated using a trapezoidal method (Brouns et al., 2005) from baseline to 240 min.

2.5. Pancreas functionality and insulin resistance and sensitivity indices

To describe the metabolic state of volunteers at baseline, pancreatic β-cell function was estimated as the homeostatic model assessment of β-cell functionality index (HOMA-β) as described by Ahren et al. (Ahren, Pratley, Soubt, Dunning, & Foley, 2008) at fasting time: HOMA-β = (20 × plasma insulin (mUI/L))/(plasma glucose (mmol/L)) − 3.5). Fasting peripheral tissue insulin resistance was estimated using the homeostatic model assessment index of insulin resistance (HOMA-IR) that was calculated following the equation HOMA-IR = (plasma insulin (mUI/L) × plasma glucose (mmol/L))/22.5 (Matthews et al., 1985) at fasting time. Fasting peripheral tissue insulin sensitivity was also estimated using the Quantitative Insulin-Sensitivity Check Index (QUICKI), by using the inverse of the sum of the logarithms of the fasting insulin (μU/mL) and fasting glucose (mg/dL) (Katz et al., 2000).

Postprandial peripheral tissue insulin sensitivity was assessed based on the Matsuda index, calculated as (10,000/square root of [fasting glucose × fasting insulin] × [mean glucose × mean insulin during postprandial time] (Matsuda & DeFronzo, 1999). The insulinogenic index (INSI) was calculated as (Insulin (mU/L) at 30 min − fasting

insulin (mU/L))/(Glucose (mg/dL) at 30 min − fasting glucose (mg/dL) as previously described (Aono et al., 2018).

2.6. Statistical analysis

Sample size estimation (n = 18) was carried out considering the specific variance of the main outcome of the postprandial study with a type I error $\alpha = 0.005$ (2-sided), a type II error $\beta = 0.2$ (80% power), and assuming a 10% of dropout rate (de la Torre et al., 2020).

Baseline clinical and biochemical characteristics of the subjects and intervention data are presented as adjusted mean values ± standard error of the mean (SEMs). The normality of variables was assessed using Q-Q graphs. Missing data were imputed using appropriate methods. The outliers for each intervention were removed if kurtosis > 1 and asymmetry > 1 in the distribution of the responses. Variables were analyzed using a linear mixed-effects model (LMM). Post-hoc multiple comparison were analyzed by Sidak test. This statistical model considers the relationship of the responses within the participants to determine differences between interventions (iAUC and at each postprandial time point). In addition, it takes into account all the possible confounders (covariates) which are included on it: age, gender, intervention and period as fixed effects, and for subjects and hospital as random effects, providing an advantage vs. ANOVA in these types of studies and giving a more precise estimation of covariance without mathematical assumptions, unlike the ANOVA (West et al., 2015). All analyses were carried out on an intention-to-treat basis. A p-value of <0.05 was considered significant. Statistical Package for the Social Sciences (SPSS) version 20 software was used to perform the statistical analysis (SPSS Inc, Chicago, IL, USA).

3. Results

Table 1 describes the clinical and biochemical characteristics of the 18 subjects selected for the postprandial study at baseline. This data confirm that selected subjects were healthy, as all values were within the range of normality. There were no differences in nutritional intakes of all volunteers from the study (data not shown). Table 2 shows the postprandial iAUC (from baseline to 240 min) of glucose, insulin and gastrointestinal hormones, Matsuda index of insulin sensitivity, and INSI after consuming a single dose of the three olive oils. Glucose iAUC was higher after the intake of the OVOO compared with the control VOO (p = 0.035). Insulin iAUC was lower after the intake of the FOO compared with VOO (p = 0.049). Matsuda index was higher after the intake of the FOO (p = 0.009) and tended to be higher after the intake of OVOO (p = 0.093) compared with the intake of the standard VOO, while INSI index tended to be higher and the postprandial GIP iAUC tended to be lower, and after the intake of the OVOO compared with the intake of the standard VOO (p = 0.096 and p = 0.083, respectively).

Fig. 1 depicts the postprandial plasma levels of glucose and insulin. At baseline, plasma glucose level was lower before the intake of the OVOO group compared with the intake of VOO (p = 0.017). At 30 min, the plasma glucose level was higher after consuming the OVOO compared with VOO (p = 0.017). At 120 min, plasma glucose level was lower after the consumption of the FOO compared with OVOO (p = 0.014) (Fig. 1a). The plasma insulin level was higher 45 min after consumption of the OVOO compared with the FOO (p = 0.023) (Fig. 1b).

Fig. 2 shows postprandial plasma levels of GLP-1, GIP, ghrelin, PP and PYY, after the intake of the three olive oils. GIP was higher 60 min after the intake of VOO compared with OVOO and FOO (p = 0.004 and p = 0.029, respectively) (Fig. 2b). Plasma ghrelin level was higher 45 min after consumption of the FOO compared with VOO and OVOO (p = 0.015 and p = 0.021, respectively), while at 120 min plasma ghrelin was higher after the intake of the OVOO compared with VOO and FOO (p = 0.026 and p = 0.025, respectively) (Fig. 2c). After consumption of the FOO, plasma PP level was higher compared with VOO at 60 min (p = 0.032), and compared with OVOO at 45 and 60 min (p = 0.022 and p = 0.026, respectively) (Fig. 2d). No significant differences were observed in plasma PYY concentrations after the consumption of the three olive oils (Fig. 2e).

Table 1
Baseline clinical and biochemical characteristics of subjects (n = 18) included in the postprandial study.

Age, years	29 ± 1
Gender, (male/female)	9/9
BMI, (kg/m^2)	23.7 ± 0.5
Waist circumference, (cm)	77 ± 2
HDLc, (mg/dL)	62 ± 2
LDLc, (mg/dL)	101 ± 3
Total cholesterol, (mg/dL)	179 ± 4
Triacylglycerides, (mg/dL)	78 ± 5
SBP, (mmHg)	115 ± 2
DBP, (mmHg)	72 ± 1
Total cholesterol/HDL	3 ± 0
LDL/HDL	2 ± 0
Glucose, (mg/dL)	90 ± 1
Insulin, (mUI/L)	6.7 ± 0.3
GIP, (pg/mL)	53.5 ± 3.1
GLP-1, (pg/mL)	4.3 ± 0.4
Ghrelin, (pg/mL)	110.9 ± 10.3
PP, (pg/mL)	29.4 ± 3.0
PYY, (pg/mL)	50.5 ± 3.9
HOMA-β	95.9 ± 6.2
HOMA-IR	1.5 ± 0.8
QUICKI	0.364 ± 0.003

Values are expressed as the means ± SEMs, n = 18 subjects. BMI, body mass index; DBP, diastolic blood pressure; FOO functional olive oil, GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; HOMA-β homeostatic model assessment of beta cell functionality; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; n, number of participants; OVOO optimized virgin olive oil; PP, pancreatic polypeptide; PYY, peptide YY; QUICKI, quantitative insulin-sensitivity check index; SBP, systolic blood pressure; SEM, standard error of the mean; VOO virgin olive oil.

4. Discussion

The NUTRAOLEUM study was designed to assess whether the enrichment of VOO with polyphenol and triterpenic acids from the olive oil may increase the healthy benefits of a standard VOO. Specifically, the

Table 2
Postprandial glucose, insulin and gastrointestinal hormones plasma levels (iAUC from baseline to 240 min), Matsuda index of insulin sensitivity and INSI after the intake of a single dose of three VOO differing on their bioactive compounds.

	VOO	OVOO	FOO
Glucose, (mg/dL·min)	2213 ± 1069^{a}	3213 ± 1068^{b}	2605 ± 1071^{ab}
Insulin, (mUI/L·min)	3546 ± 564^{a}	3191 ± 572^{ab}	2837 ± 578^{b}
GIP (pg/mL·min)	57,371 ± 5748	48,625 ± 5729	55,689 ± 6041
GLP-1, (pg/mL·min)	9062 ± 2844	6864 ± 2842	9672 ± 2833
Ghrelin, (pg/mL·min)	8419 ± 1967	10,654 ± 2082	9845 ± 2150
PP, (pg/mL·min)	19,971 ± 3870	19,443 ± 3869	24,045 ± 4128
PYY, (pg/mL·min)	4606 ± 1113	4215 ± 1220	5933 ± 1079
Matsuda index	9.12 ± 1.25^{a}	10.64 ± 1.31^{ab}	11.60 ± 1.29^{b}
INSI	0.195 ± 0.284	0.875 ± 0.281	0.802 ± 0.333

Values are expressed as the adjusted means ± SEMs, n = 18 for VOO and OVOO and n = 17 for FOO at fasting state, volunteers ingest 30 mL of the corresponding olive oil (VOO, OVOO, or FOO) as a single dose with a standard piece of bread (80 g) and a glass of water (200 mL). LMM was used to compare between groups of intervention. Different superscript letters indicate significant differences between post-interventions ($^{a, b}$). p < 0.05 was considered significant. Abbreviations: iAUC, incremental area under curve; FOO, functional olive oil; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide1; INSI, insulinogenic index; LMM, linear mixed model; OVOO optimized virgin olive oil; PP, pancreatic polypeptide; PYY, peptide YY; SEM, standard error of the mean; VOO virgin olive oil.

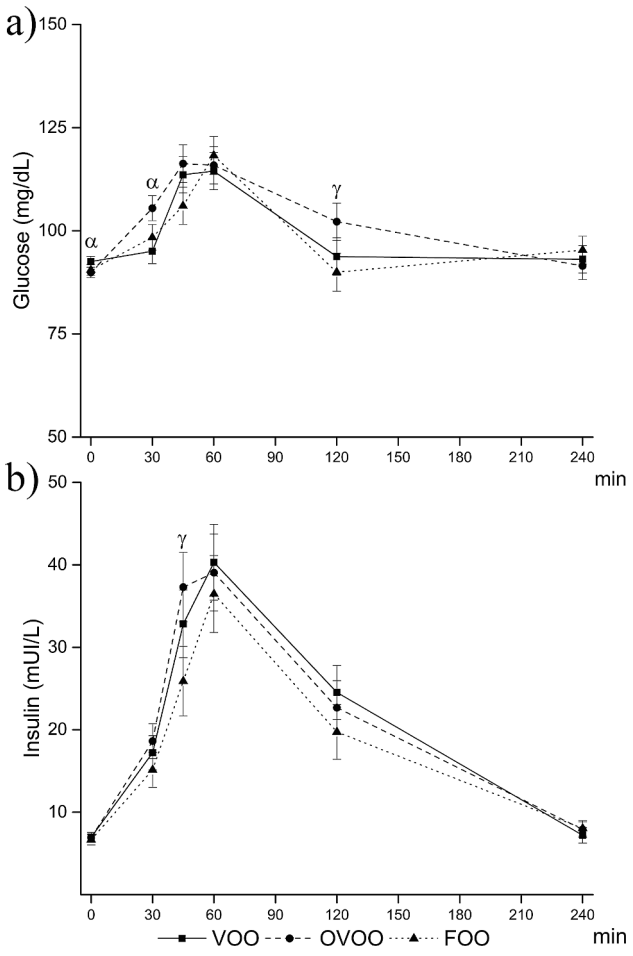

Fig. 1. Postprandial (from baseline to 240 min) plasma levels of insulin (a) and glucose (b) after ingestion of three VOO with different bioactive compounds content. Values are expressed as the adjusted means ± SEM, n = 18 for VOO and OVOO and n = 17 for FOO. At fasting state, volunteers ingested 30 mL of the corresponding olive oil (VOO, OVOO, or FOO) as a single dose with a standard piece of bread (80 g) and a glass of water (200 mL). LMM was used to compare between groups of intervention. α shows significant differences between VOO and OVOO interventions. β shows significant differences between VOO and FOO interventions and γ shows significant differences between OVOO and FOO interventions. $p < 0.05$ was considered significant. FOO, functional olive oil; OVOO, optimized virgin olive oil; VOO, virgin olive oil; SEM, standard error of the mean. Plasma GLP-1 was lower before the VOO intake compared with the OVOO ($p = 0.011$) (Fig. 2a).

present postprandial sub-study aimed to evaluate the metabolic postprandial benefits derived from different amounts of olive oil bioactive compounds. As far as we know, no human randomized controlled trial has previously compared the *in vivo* benefits of VOOs with different content of bioactive compounds, polyphenols and triterpenic acids, on the postprandial glucose, insulin (secretion and sensitivity), and gastrointestinal hormones responses. The most relevant finding of this study was that olive oil enriched whit bioactive compounds from the olive fruit improve insulin functionality by increasing insulin sensitivity and insulin secretion, considered as two independent glucose tolerance risk factor (Häring, 2016). Further studies are needed to evaluate the synergic effect of polyphenols and triterpenes present in EVOO.

Glycemic regulation is mediated in part by incretins, GLP-1 and GIP, that stimulate insulin secretion in response to food intake (Diakogiannaki, Gribble, & Reimann, 2012). If the pancreas can react with compensatory hypersecretion of insulin, in response to food intake, it will maintain plasma glucose and contribute to an adequate metabolic state (Häring, 2016). Our results show that postprandial insulin secretion, as measured as INSI, was enhanced similarly after the intake of the polyphenol-enriched olive oils. In addition, insulin sensitivity, measured as Matsuda index was also higher after the intake of the enriched olive oils. These data indicate a better postprandial metabolic behavior favored by the presence of phenolic compounds in the olive oil. Matsuda index indicates that the presence of phenolic compounds may improve the postprandial insulin response of tissues and contribute to the metabolic control. In addition, increased GIP may explain the higher insulin secretion after polyphenol-rich olive oils intakes. A sub-study of the PREDIMED confirmed a decrease in fasting plasma glucose after the long-term consumption of VOO in T2D subjects (Javier Basterra-Gortari et al., 2019). An interventional study with extra-VOO rich in phenolic compounds (577 mg/kg) during 4 weeks provides evidence of the beneficial effects on the metabolic control in T2D patients that may be mediated by adipokines as visfatin and apelin (Santangelo et al., 2016). In addition, a postprandial study in healthy subjects has shown the beneficial effect after VOO consumption on postprandial glycemia compared with the intake of corn oil or a Mediterranean-type lunch without VOO (Violi et al., 2015). In accordance with our results, those authors found that GIP, but also GLP-1, and subsequently insulin secretions increased more after the intake of the VOO compared with the corn oil, contributing to the decrease of plasma glucose levels (Violi et al., 2015). We did not observed differences in GLP-1 secretion beside other studies have reported an improvement in metabolic conditions regulated by GLP-1 after VOO consumption compared to butter or a low fat diet, in subjects with metabolic diseases (Bozzetto et al., 2019), or when consumed chocolate enriched with 40 g of oleuropein that induced an increase of GLP-1 compare with the same chocolate without the bioactive compound in healthy subjects (Del Ben et al., 2020). These data indicate that the food matrix may interfere and modulate the effect of these bioactive compounds.

Our data suggest a synergist effect of polyphenol and triterpenic acids, since differenced observed were more pronounced after the intake of the FOO. Triterpenic acids (oleanolic acids and maslinic acids) have demonstrated a beneficial effect for the prevention of diabetes and MetS has been reported in an intervention study in pre-diabetic patients (PREDIABOLE: Prevention of Diabetes with Oleanolic Acid) supplemented with an oleanolic acid-enriched olive oil (Santos-Lozano et al., 2019) and in obese mice supplemented with pomace oil (Claro-Cala et al., 2020). In addition, a recent work has demonstrated that the oleanane skeletons of oleanolic and maslinic acid are optimum for the α-glucosidase and α-amylase inhibitory activities, while the hydroxytyrosol moiety may contribute weakly to these activities (Mwakalukwa, Amen, Nagata, & Shimizu, 2020). Those data are in accordance with the improvement of insulin sensitivity observed after the intake of FOO in our study. Evidence has suggested that triterpenic acids would offer interesting alternatives for managing insulin resistance (Castellano et al., 2013). Furthermore, the described bioavailability of triterpenic acids from VOO (de la Torre et al., 2020) confirms that these molecules may be involved in the postprandial response observed after the consumption of the enriched oils compared to the standard VOO. Indeed, triterpenic acids may increase glycogen synthesis and simultaneously inhibit gluconeogenesis and glycogenolysis thus reducing hepatic glucose production that influences insulin release (Wen et al., 2005). We have to stand out that, as expected, all volunteers behave properly since all were metabolically healthy. Nevertheless, the small but significant differences found after the ingestion of the bioactive compounds-rich olive oils lead to the hypothesis that these effects may be more prominent in people with metabolic alterations and after sustained supplementation with the oils. Therefore, more studies are needed in order to evaluate the postprandial response and the mechanisms after a sustained intake of enriches VOOs in subjects with insulin resistance or metabolic diseases.

One limitation of the present study is that we did not have a postprandial control group consuming a control oil without bioactive compounds or different vegetable oil. Thus, we cannot estimate the effect of

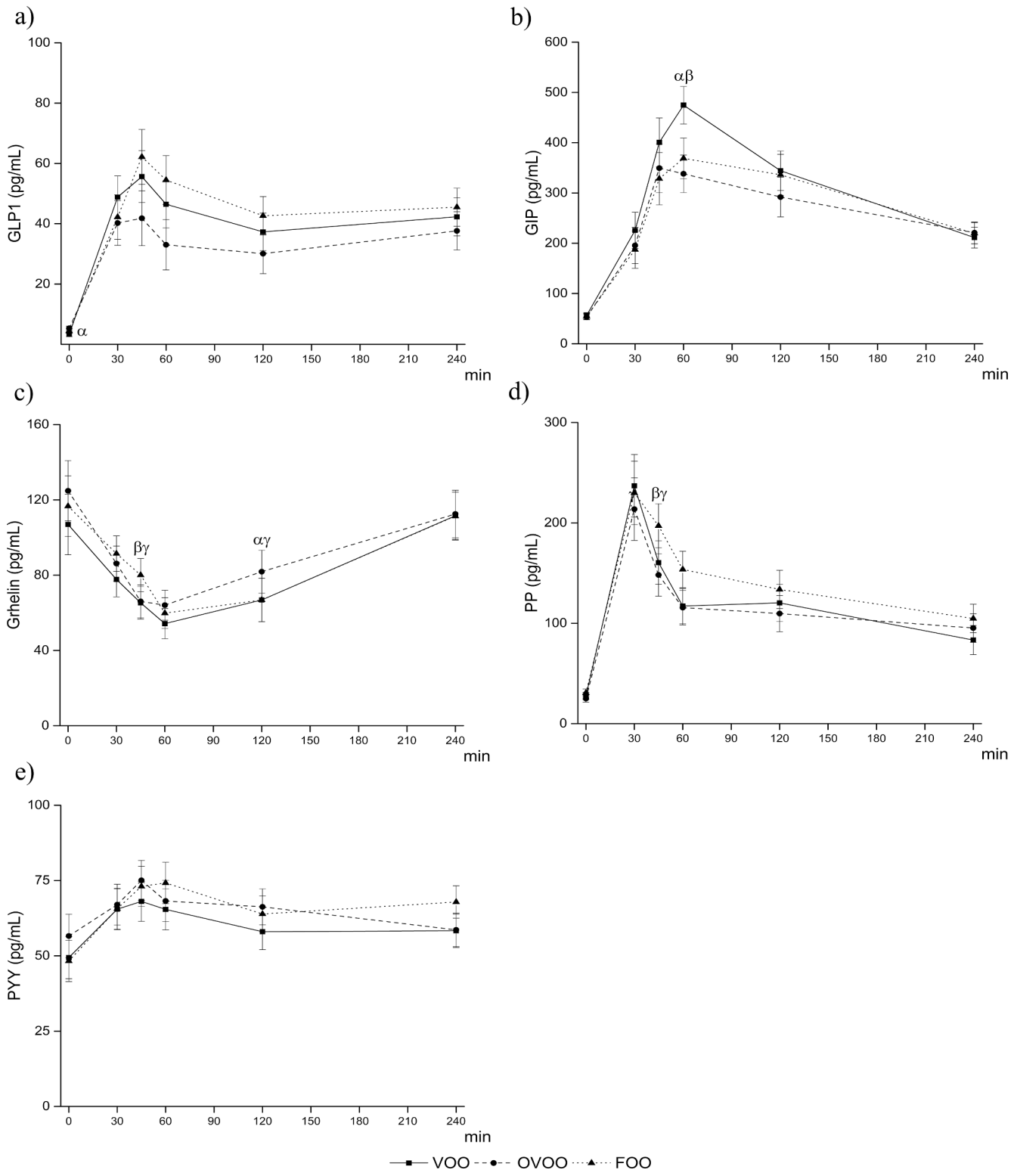

Fig. 2. Postprandial (from baseline to 240 min) plasma levels of gastrointestinal hormones: GIP (a); GLP-1 (b); ghrelin (c); PP (e) and PYY (f). Values are expressed as the adjusted means ± SEM, n = 18 for VOO and OVOO and n = 17 for FOO. At fasting state, volunteers ingested 30 mL of the corresponding olive oil (VOO, OVOO, or FOO) as a single dose with a standard piece of bread (80 g) and a glass of water (200 mL). LMM was used to compare between groups of intervention. α shows significant differences between VOO and OVOO interventions. β shows significant differences between VOO and FOO interventions and γ shows significant differences between OVOO and FOO interventions. $p < 0.05$ was considered significant. FOO, functional virgin olive oil; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; OVOO optimized virgin olive oil; PP, pancreatic polypeptide; PYY peptide YY; SEM, standard error of the mean; VOO, virgin olive oil.

the food matrix on glucose metabolism.

To our best knowledge, no studies have compared the effects of different types and amounts of bioactive VOO compounds on the postprandial gastrointestinal hormone responses. Ghrelin plays a key role in insulin secretion, a decrease of the ghrelin secretion leads to an improvement in glucose tolerance and insulin sensitivity suggesting a strategy for glucose homeostasis (Alamri, Shin, Chappe, & Anini, 2016). Consumption of oleanolic acid during 12 weeks has shown a lower ghrelin secretion (orexigenic hormone) in pre-diabetic animals (Gamede, Mabuza, Ngubane, & Khathi, 2018). It has been described that triterpenes may decrease ghrelin secretion and consequently decrease caloric intake (Luvuno, Mbongwa, & Khathi, 2016). However, we did not observe similar results in the postprandial response after consumption of a single dose of triterpenic-enriched FOO, probably due to the postprandial study design.

Other important polypeptides involved in the regulation of energy homeostasis are PP and PYY. They are released in response to a meal, according to the caloric load. They inhibit appetite and modulate the gastric emptying rate (Katsuura, Asakawa, & Inui, 2002; Wren & Bloom, 2007). As expected, the iAUCs for PP and PYY secretions did not show differences between the groups since the three breakfasts contained the same amount of olive oils, bread, and water and provided the same amounts of energy and macronutrients.

In conclusion, our results show that the ingestion of a single dose of extra virgin olive oil enriched with bioactive compounds from the olive fruit (487 ppm of phenolic compounds and 389 ppm of triterpenic acids respectively) improves postprandial insulin release and sensitivity in healthy adults, suggesting that sustained consumption of the enriched VOO may be recommended for subjects with metabolic diseases.

5. Ethics statement

The present study is part of the NUTRAOLEUM trial. The NUTRAOLEUM trial is a randomized, double-blind, crossover and controlled trial conducted in Virgen de las Nieves and San Cecilio General Hospitals of Granada, Spain (Biel et al., 2016). The study protocol and written informed consent were approved by the Institutional Review Board of the Granada Hospitals (Fundación Pública Andaluza para la Investigación Biosanitaria de Andalucía Oriental (FIBAO). Comité de Ética de Investigación de Centro de Granada.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare no conflict of interest. Jose Maria Olmo Peinado, is the owner of the company that finances the project “ACER CAMPESTRES S.L.” and is a producer of technological extracts from the olive tree. Any funding sponsor had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results. This paper will be part of the Laura Alejandra Vazquez Aguilar doctorate that it is being carried out within the context of “Nutrition and Food Sciences Program” at the University of Granada.

Acknowledgements

We are grateful for the support of participating volunteers, the personal of Virgen de las Nieves and San Cecilio general hospitals of Granada: Laura Campaña Martín, Mª Victoria Martín Laguna and Mª Cruz Rico Prados who carried out technical work and sample preparation and Llenalia García Fernández who supervised the statistical analysis. We acknowledge the University of Granada (Spain) and the University of Guadalajara (Mexico) for the predoctoral scholarship of Laura Alejandra Vazquez Aguilar. The “NUTRAOLEUM Study” has been supported by the grant ITC-20131031 from the I + D FEDER-INTERCONNECTA (CDTI) and Junta de Andalucía, Spain”. We thank ACER CAMPESTRES S.L., SAN FRANCISCO DE ASIS Coop and AGRO-INSUR S.L., for the funding financing provided for the development conduct of the clinical trial.

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