Clustering effects on postprandial insulin secretion and sensitivity in the response to meals with different fatty acid composition

Running title: Fatty acids on insulin secretion and action in the postprandial state

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Postprandial glycaemic control may be at risk by dietary SFA palmitic acid but not stearic acid and may be protected by dietary MUFA oleic acid but not palmitoleic acid.

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Abstract

Dietary fatty acids play a role in glucose homeostasis. The aim of this study was to assess the individual relationship between dietary saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids with postprandial \( \beta \)-cell function and insulin sensitivity in subjects with normal and high fasting triglycerides. We assessed postprandial \( \beta \)-cell function (by the insulinogenic index and the ratio of the insulin to glucose areas under the time-concentration curve) and insulin sensitivity (by the oral glucose and the minimal model insulin sensitivity indices) over four nonconsecutive, randomly assigned, high-fat meals containing a panel of SFA (palmitic and stearic acids), MUFA (palmitoleic and oleic acids) and PUFA (linoleic and \( \alpha \)-linolenic acids) in 14 subjects with normal and 14 subjects with high fasting triglycerides. The proportions of each fatty acid in the meals and the values for surrogate measures of postprandial \( \beta \)-cell function and insulin sensitivity were subjected to a Pearson correlation and hierarchical cluster analysis, which revealed two classes of dietary fatty acids in regulating postprandial glucose homeostasis. We successfully discriminated the adverse specific effects of SFA palmitic acid but the beneficial specific effects of MUFA oleic acid on postprandial \( \beta \)-cell function \( (r \geq 0.84 \text{ for SFA palmitic acid and } r \geq -0.71 \text{ for MUFA oleic acid}; P<0.05) \) and insulin sensitivity \( (r \geq -0.92 \text{ for SFA palmitic acid and } r \geq 0.89 \text{ for MUFA oleic acid}; P<0.001) \) both in subjects with normal and high fasting triglycerides. In conclusion, dietary MUFA oleic acid, in contrast to SFA palmitic acid, favours the tuning towards a better postprandial glycaemic control in subjects with normal and high fasting triglycerides.

Keywords: fatty acids; \( \beta \)-cell function; insulin sensitivity; postprandial state
Introduction

Dietary substitution of saturated fatty acids (SFA) with monounsaturated ones (MUFA) improves glucose homeostasis and influences protection against cardiovascular risk factors and the metabolic syndrome\(^1\). \textit{In vitro} and in animal models, palmitic acid (16:0) is considered the main culprit of SFA impairing pancreatic \(\beta\)-cell function and insulin sensitivity, whereas the major dietary MUFA oleic acid (18:1n-9) is protective for \(\beta\)-cells and insulin target tissues\(^2,3\).

In previous studies, we have shown that consumption of meals rich in dietary fats with a high MUFA-to-SFA ratio may decrease exacerbated insulin excursion and increase insulin sensitivity in the postprandial period in subjects with normal fasting triglycerides\(^4\). Similar findings were observed in subjects with high fasting triglycerides when meals enriched in SFA and MUFA were compared\(^5\). However, information regarding the individual contribution of each dietary SFA and MUFA to postprandial glucose homeostasis in humans is yet unknown. Therefore, we extended the panel of different dietary fats with a gradual change in the MUFA-to-SFA ratio in subjects with high fasting triglycerides and reanalysed the previously collected data to investigate whether the content of palmitic acid or stearic acid (18:0), among the major dietary SFA in the meals, or the content of oleic acid or palmitoleic acid (16:1n-7), among the major dietary MUFA in the meals, displays linear relationship with surrogate measures of insulin secretion and action in the postprandial period in subjects with normal and high fasting triglycerides. We also extended this reanalysis to the polyunsaturated fatty acids (PUFA), linoleic acid (18:2n-6) and \(\alpha\)-linolenic acid (18:3n-3).

Results
All of the high-fat meals elicited a comparable postprandial glucose but different insulin and NEFA responses either in subjects with normal (Figure 1, left panels) or high (Figure 1, right panels) fasting triglycerides. The incremental AUC for triglycerides (from 0 to 480 min) in subjects with normal fasting triglycerides were as follows: 5.27 ± 0.54, 3.75 ± 0.73, 4.45 ± 0.53 and 4.79 ± 0.36 mmol/h × L after the ingestion of the butter, HPSO, ROO and VEFO meals, respectively (all P < 0.05, except the values after the ROO and VEFO meals that did not statistically differ). The incremental AUC for triglycerides (from 0 to 480 min) in subjects with high fasting triglycerides were as follows: 13.43 ± 2.73, 4.30 ± 1.21, 6.48 ± 2.20 and 7.07 ± 1.27 mmol/h × L after the ingestion of the butter, HPSO, ROO and VEFO meals, respectively (all P < 0.05, except the values after the ROO and VEFO meals that did not statistically differ). The suppression of NEFA concentrations in the early postprandial period was mediated by hyperinsulinemia but this association was dependent on the type of dietary fat in the meals [r = 0.987 (95% CI: 0.959, 0.995) in subjects with normal fasting triglycerides (Figure 1, left panel at the bottom) and r = 0.983 (95% CI: 0.946, 0.994) in subjects with high fasting triglycerides; all P < 0.01] (Figure 1, right panel at the bottom).

A Pearson correlation analysis and hierarchical cluster analysis were performed to determine the detailed relationship between the individual fatty acids in the meals and the surrogate measures of postprandial β-cell function and insulin sensitivity. These analytical results showed two major dietary fatty acid clusters. The SFA palmitic and stearic acids, and the MUFA palmitoleic acid were included in one cluster, which was characterized by a positive response on postprandial β-cell function but a negative response on postprandial insulin sensitivity both in subjects with normal (Figures 2 and 3A) and high (Figures 3B and 4) fasting triglycerides. Importantly, only the SFA palmitic acid was positively correlated (P<0.01) with all of the surrogate measures of postprandial β-cell function [r = 0.94 for the
insulinogenic index (IGI) and 0.91 for the ratio of the insulin to glucose areas under the time-concentration curve (AUC_{INS}/AUC_{GLU}) in subjects with normal fasting triglycerides and \( r = 0.91 \) for IGI and 0.84 for AUC_{INS}/AUC_{GLU} in subjects with high fasting triglycerides and negatively correlated (\( P<0.001 \)) with all of the surrogate measures of postprandial insulin sensitivity \( [r = -0.92 \text{ for the oral glucose insulin sensitivity index from 0 to 180 min (OGIS}_{0-180} \text{)} \] and \( -0.94 \text{ for the minimal model insulin sensitivity index from 0 to 480 min (IS}_{0-480} \text{)} \) in subjects with normal fasting triglycerides and \( r = -0.97 \) for OGIS_{0-480} and -0.92 for IS_{0-480} in subjects with high fasting triglycerides]. The other cluster contained the MUFA oleic acid and the PUFA linoleic and \( \alpha \)-linolenic acids that participated in a negative response on postprandial \( \beta \)-cell function but a positive response on postprandial insulin sensitivity both in subjects with normal (Figures 2 and 3A) and high (Figures 3B and 4) fasting triglycerides. However, only the MUFA oleic acid was negatively correlated (\( P<0.05 \)) with all of the surrogate measures of postprandial \( \beta \)-cell function \( (r = -0.71 \text{ for IGI and } -0.97 \text{ for AUC}_{INS}/AUC_{GLU}) \) in subjects with normal fasting triglycerides and \( r = -0.89 \) for IGI and -0.99 for AUC_{INS}/AUC_{GLU} in subjects with high fasting triglycerides and positively correlated \( (P<0.05) \) with all of the surrogate measures of postprandial insulin sensitivity \( (r = 0.89 \text{ for IGI and } 0.93 \text{ for AUC}_{INS}/AUC_{GLU}) \) in subjects with normal fasting triglycerides and \( r = 0.91 \) for IGI and 0.96 for AUC_{INS}/AUC_{GLU} in subjects with high fasting triglycerides).

**Discussion**

In 1959, Kinsell and co-workers\(^6\) suggested that dietary fatty acids might have a role in glucose homeostasis. Several decades later, evidence is accumulating from *in vitro* and animal studies that chain-length and double bonds in fatty acids influence its insulinotropic, pro- or anti-litotoxic and insulin-sensitizing potencies\(^7\)\(^9\). In humans, long-term high-MUFA as compared to high-SFA diets appears to be effective for fasting glycaemic control in
healthy subjects and patients with obesity, type 2 diabetes and the metabolic syndrome.\textsuperscript{1,10} Consistent with this notion, we have demonstrated that subjects with normal\textsuperscript{4} and high\textsuperscript{5} fasting triglycerides became less insulin resistant postprandially as the proportion of MUFA vs. SFA in dietary fats increased, which led to a dramatic attenuation of the functional demand on the $\beta$-cell. Here we extend this knowledge and provide evidence of specific associations of dietary MUFA oleic acid, in opposite direction to dietary SFA palmitic acid, with postprandial $\beta$-cell function and insulin sensitivity. Our study also indicates that dietary SFA stearic, MUFA palmitoleic and PUFA linoleic or $\alpha$-linolenic acids remain neutral regarding the antagonism of dietary MUFA oleic and SFA palmitic acids on postprandial glycaemic control.

In this study, each participant served as his own control. The only difference among meals was the type of dietary fat, which provided different fatty acids at different proportions. In the postprandial period, fats absorbed in the small intestine are assembled by enterocytes to produce the triglyceride-rich lipoprotein chylomicron, whose fatty acid composition closely resembles that of fatty acid composition in the meals.\textsuperscript{11} Next, circulating chylomicrons may gain access to target tissues, including pancreas, liver, adipose tissue and skeletal muscle that express lipoprotein lipase, fatty-acid–activated G-protein coupled receptors and fatty acid-translocase FAT/CD36.\textsuperscript{12-14} It should be noted that the molar concentration of circulating fatty acids in chylomicrons is ~30-fold larger than fatty acids bound to albumin. In agreement with Shah et al.\textsuperscript{15} we found postprandial hyperinsulinemia combined with suppressed circulating NEFA after the ingestion of the high-fat meals, suggesting that not the systemic NEFA pool but the abrupt local increase of fatty acids from hydrolysed chylomicrons in the immediate vicinity of the $\beta$-cells, hepatocytes, adipocytes and skeletal muscle cells is likely critical for glycaemic control in the fed state. This conclusion can also be inferred from tight
associations between the content of MUFA oleic and SFA palmitic acids in the meals and the change in surrogate measures of postprandial β-cell function and insulin sensitivity. Our study further shows that the achievement of postprandial glycaemic control may be fine-tuned by adjusting the type and proportion of these fatty acids in the diet, irrespective of the fasting triglyceride concentration.

Although it remains difficult to estimate the relative importance of meals rich in MUFA oleic acid or SFA palmitic acid to the evolution of pancreatic compensation, it seems clear that in pancreatic islets, whereas short-term MUFA and SFA promotes (palmitic acid > oleic acid) the rate of β-cell production\(^{16}\), only long-term SFA palmitic acid accelerates the impairment of the same production rate\(^{17}\). Mathematical models of long-term diabetes progression predict that continued and exacerbated fluctuations of β-cell function exert a fall of β-cell mass and replication that prevents compensation, so that a picture of frank diabetes eventually develops\(^{18}\). In addition, acute episodes of postprandial hyperinsulinemia, despite insulin-mediated suppression of circulating NEFA, recently have been linked to cardiac steatosis in healthy subjects\(^{19}\). Therefore, our results raise the interesting possibility that input of dietary MUFA oleic acid alleviates the needs for glucose disposal during non-fasting periods with minor impact on β-cells and insulin target tissues. This scenario may offer an important additional view to the adipose tissue output of fatty acids with lipokine activity in fasting states\(^{20,21}\).

Experimental

**Design and recruitment**

The design and some of the results on postprandial studies in these subjects with normal and high fasting triglycerides were published\(^{4,5}\). Here the panel of different dietary fats with a
gradual change in the MUFA-to-SFA ratio has been completed in subjects with high fasting triglycerides and all of the data were reanalysed to achieve the aim of the study. Briefly, these were randomized, double-blind, within-subject crossover 4-d feeding studies. All experiments were performed in compliance with the relevant laws and institutional guidelines and the Human Clinical Commission and the Ethics Committee at University Hospital Virgen del Rocio (Seville, Spain) approved protocols before the start of the study, and written consent was obtained from each participant. Caucasian, nonsmoking males were recruited: healthy subjects (BMI: 23.9 ± 1.9 kg/m$^2$) and newly diagnosed with type IIb or IV hyperlipoproteinemia (fasting triglycerides >2.24 mmol/L) according to the National Cholesterol Education Program Adult Treatment Panel III (BMI: 24.2 ± 5.1 kg/m$^2$).

Four separate visits at intervals of 1-2 wk were designed to compare responses to high-fat meals containing different fatty acids at different proportions (Table 1). Subjects presented at 0800 h after a 12 h fast. Baseline values for triglycerides (0.86 ± 0.27 and 4.22 ± 0.72 mmol/L), nonesterified fatty acids (NEFA) (465 ± 87 and 500 ± 73 µmol/L), glucose (5.16 ± 0.38 and 5.77 ± 0.64 mmol/L), insulin (49 ± 9 and 90 ± 10 pmol/L), homeostatic model assessment (HOMA) for β-cell function (HOMA-B: 28 ± 4 and 48 ± 6 pmol/mmol) and for insulin resistance (HOMA-IR: 11.2 ± 1.1 and 22.9 ± 2.4 pmol × mmol/L$^2$) were obtained in subjects with normal and high fasting triglycerides. Subjects were provided with a standardized high-fat meal that consisted of a dietary fat [50 g/m$^2$ body surface area of butter, high-palmitic sunflower oil (HPSO), refined olive oil (ROO) or a mixture of vegetable and fish oils (VEFO)], along with a portion of plain pasta (30 g/m$^2$ body surface area), one slice of brown bread and one skimmed yogurt. Following the ingestion of the meals, blood samples were collected each 60 min in appropriate tubes for determination of NEFA, glucose and insulin levels over 480 min.
Calculations

A sample size of 14 subjects per group was calculated to provide 80% power, assuming a comparable effect on the postprandial excursion of plasma insulin levels and considering a two-sided test with a significance level of 5%. Postprandial β-cell function was estimated by two methods: 1) the insulinogenic index (IGI), which is a surrogate measure of first-phase insulin secretion and was calculated using the difference between the postprandial insulin peak (t = 60 min) and the fasting insulin level in relation to the difference in glucose levels (IGI = \( \Delta I_{0:60}/\Delta G_{0:60} \)); and 2) the ratio of the insulin to glucose areas under the time-concentration curve (AUC_{INS}/AUC_{GLU}), which significantly correlates with glucose sensitivity and early-phase insulin secretion, calculated using the trapezoidal method from 0 to 120 min. Postprandial insulin sensitivity was estimated by two methods: 1) the oral glucose insulin sensitivity index from 0 to 180 min (OGIS_{0:180}); and 2) the minimal model insulin sensitivity index from 0 to 480 min (IS_{0:480}). The details of the equations and references using this multisampling protocol have been previously described\(^4\).

Laboratory methods

Plasma glucose and triglycerides were measured on a Hitachi Modular Analytics D-2400 analyser (Roche Diagnostics, Basel, Switzerland) using commercially available reagents and enzyme-based kit. Plasma insulin was measured using a specific enzyme-linked immunosorbant assay (Dako, Cambridge, UK) on a Hitachi Modular Analytics E-170 analyser. Plasma NEFA were measured using an ACS-ACOD assay (Wako Chemicals GmbH, Germany) on a COBAS Mira-Plus analyser. Fatty acid composition in the meals was determined as described\(^23\).
Statistical analyses

Comparisons of quantitative variables were performed using ANOVA, and a Bonferroni correction was applied for the post hoc detection of significant pairwise differences. A Pearson correlation was used to explore the strength of the association between the content of each fatty acid in the meals with postprandial estimates of β-cell function and insulin sensitivity. Thereafter, a hierarchical cluster analysis applying average linkage method of Euclidean distances and a heatmap visualization of the correlation coefficients were performed. Statistical significance was set at \( P \) values < 0.05. Data were analysed using StatView and MeV for Windows.

Conclusion

We provide evidence demonstrating the individual contribution of major dietary SFA, MUFA and PUFA to postprandial β-cell function and insulin sensitivity both in subjects with normal and high fasting triglycerides. This study shows that postprandial glycaemic control may be at risk by dietary SFA palmitic acid but not SFA stearic acid and may be protected by dietary MUFA oleic acid but not MUFA palmitoleic acid. The clinical significance of our findings may benefit nutrition management for prevention of acute insulin disorders by customized ranges of dietary fatty acids.

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BB, RA, FJGM and SL were involved in the conception of the present study. SL performed the statistical analyses. BB, AOG, LMV, JV, RA, FJGM and SL interpreted the data. FJGM and SL drafted the manuscript. All authors were involved in the subsequent edits of the manuscript, and have read and approved the final manuscript. This study was supported by grants (AGL2004-04958 and AGL2011-29008) from the Spanish Ministry of Economy and
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References


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Table 1. Composition of principal fatty acids that are common in the meals. The butter meal had short or medium chain SFA [butyric acid (4:0) 2.0, caproic acid (6:0) 1.4, caprylic acid (8:0) 0.9, capric acid (10:0) 2.0, lauric acid (12:0) 2.5, myristic acid (14:0) 9.9, pentadecanoic acid (15:0) 0.8, margaric acid (17:0) 0.6] and MUFA [myristoleic acid (14:1n-7) 0.5, pentadecenoic acid (15:1n-7) 1.1, eicosenoic acid (20:1n-9) 0.6]. Other fatty acids [17:0 < 0.1, heptadecenoic acid (17:1n-7) < 0.1, arachidic acid (20:0) < 0.4, 20:1n-9 < 0.3] were detected in the HPSO, ROO, and VEFO meals. The VEFO meal had eicosapentaenoic acid (20:5n-3) 1.0 and docosahexaenoic acid (22:6n-3) 2.3. All values are in g/100 g fatty acids.
**FIGURE LEGENDS**

Fig. 1. Time-course of plasma concentrations (after subtracting baseline values) and incremental AUC (iAUC) (inserts) of glucose, insulin and NEFA during the postprandial period from 0 to 480 min, and correlations between iAUC of insulin and NEFA during the early postprandial period (from 0 to 180 min) (top) in response to the meals (butter: red, HPSO: orange, ROO: green, VEFO: blue) in subjects with normal (left panels) and high (right panels) fasting triglycerides. Units for iAUC of glucose, insulin and NEFA: mmol/h × L, pmol/h × L and µmol/h × L, respectively. Labeled means in a column without a common letter differ, *P*<0.05. All values are mean ± SD (*n* = 14).

Fig. 2. Pearson correlation analysis between the proportions of principal fatty acids in the meals and the values for surrogate measures of postprandial β-cell function and insulin sensitivity in subjects with normal fasting triglycerides. Data are strength (*r*) and significance (*P*) of correlation derived from Pearson’s correlation analysis.

Fig. 3. Hierarchical cluster analysis and heatmap of the results obtained from the effects of principal fatty acids in the meals on surrogate measures of insulin secretion and action in the postprandial period in subjects with normal (A) and high (B) fasting triglycerides. Each square indicates the Pearson correlation coefficient of a pair of fatty acid vs. surrogate measure, and the value for the correlation coefficient is represented by the intensity of the red or green colour, as indicated on the colour scale. Hierarchical clusters are represented by a cluster tree.

Fig. 4. Pearson correlation analysis between the proportions of principal fatty acids in the meals and the values for surrogate measures of postprandial β-cell function and insulin sensitivity.
sensitivity in subjects with high fasting triglycerides. Data are strength ($r$) and significance ($P$) of correlation derived from Pearson’s correlation analysis.
Glucose (mmol/L) iAUC$_{0-180}$ of NEFA

-1.07 ± 0.67$^a$
-0.45 ± 0.30$^a$
-0.54 ± 0.21$^a$
-0.72 ± 0.60$^a$

Minutes

Insulin (pmol/L) iAUC$_{0-180}$ of insulin

-0.26 ± 0.10$^a$
-0.32 ± 0.21$^a$
-0.14 ± 0.05$^a$
-0.24 ± 0.15$^a$

Minutes

NEFA (μmol/L) iAUC$_{0-480}$ of NEFA

236 ± 44$^a$
120 ± 14$^b$
73 ± 16$^c$
38 ± 10$^d$

Minutes

iAUC$_{0-480}$ of NEFA

450 ± 51$^a$
-181 ± 40$^b$
-420 ± 54$^d$
-340 ± 37$^c$

Minutes

iAUC$_{0-180}$ of NEFA

957 ± 140$^a$
197 ± 37$^b$
-345 ± 86$^d$
-226 ± 52$^c$

Minutes

iAUC$_{0-180}$ of insulin

Figure 1

Food & Function
Figure 2