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

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

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26 **Table of contents entry (highlight)**

27 Postprandial glycaemic control may be at risk by dietary SFA palmitic acid but not stearic

28 acid and may be protected by dietary MUFA oleic acid but not palmitoleic acid.

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30 Note: Please, see a colour image attached.

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51 **Abstract**

52 Dietary fatty acids play a role in glucose homeostasis. The aim of this study was to assess the
53 individual relationship between dietary saturated (SFA), monounsaturated (MUFA) and
54 polyunsaturated (PUFA) fatty acids with postprandial β -cell function and insulin sensitivity
55 in subjects with normal and high fasting triglycerides. We assessed postprandial β -cell
56 function (by the insulinogenic index and the ratio of the insulin to glucose areas under the
57 time-concentration curve) and insulin sensitivity (by the oral glucose and the minimal model
58 insulin sensitivity indices) over four nonconsecutive, randomly assigned, high-fat meals
59 containing a panel of SFA (palmitic and stearic acids), MUFA (palmitoleic and oleic acids)
60 and PUFA (linoleic and α -linolenic acids) in 14 subjects with normal and 14 subjects with
61 high fasting triglycerides. The proportions of each fatty acid in the meals and the values for
62 surrogate measures of postprandial β -cell function and insulin sensitivity were subjected to a
63 Pearson correlation and hierarchical cluster analysis, which revealed two classes of dietary
64 fatty acids in regulating postprandial glucose homeostasis. We successfully discriminated the
65 adverse specific effects of SFA palmitic acid but the beneficial specific effects of MUFA
66 oleic acid on postprandial β -cell function ($r \geq 0.84$ for SFA palmitic acid and $r \geq -0.71$ for
67 MUFA oleic acid; $P < 0.05$) and insulin sensitivity ($r \geq -0.92$ for SFA palmitic acid and $r \geq$
68 0.89 for MUFA oleic acid; $P < 0.001$) both in subjects with normal and high fasting
69 triglycerides. In conclusion, dietary MUFA oleic acid, in contrast to SFA palmitic acid,
70 favours the tuning towards a better postprandial glycaemic control in subjects with normal
71 and high fasting triglycerides.

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73 **Keywords:** fatty acids; β -cell function; insulin sensitivity; postprandial state

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76 Introduction

77 Dietary substitution of saturated fatty acids (SFA) with monounsaturated ones (MUFA)
78 improves glucose homeostasis and influences protection against cardiovascular risk factors
79 and the metabolic syndrome¹. *In vitro* and in animal models, palmitic acid (16:0) is
80 considered the main culprit of SFA impairing pancreatic β -cell function and insulin
81 sensitivity, whereas the major dietary MUFA oleic acid (18:1n-9) is protective for β -cells and
82 insulin target tissues^{2,3}.

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84 In previous studies, we have shown that consumption of meals rich in dietary fats with a high
85 MUFA-to-SFA ratio may decrease exacerbated insulin excursion and increase insulin
86 sensitivity in the postprandial period in subjects with normal fasting triglycerides⁴. Similar
87 findings were observed in subjects with high fasting triglycerides when meals enriched in
88 SFA and MUFA were compared⁵. However, information regarding the individual
89 contribution of each dietary SFA and MUFA to postprandial glucose homeostasis in humans
90 is yet unknown. Therefore, we extended the panel of different dietary fats with a gradual
91 change in the MUFA-to-SFA ratio in subjects with high fasting triglycerides and reanalysed
92 the previously collected data to investigate whether the content of palmitic acid or stearic
93 acid (18:0), among the major dietary SFA in the meals, or the content of oleic acid or
94 palmitoleic acid (16:1n-7), among the major dietary MUFA in the meals, displays linear
95 relationship with surrogate measures of insulin secretion and action in the postprandial period
96 in subjects with normal and high fasting triglycerides. We also extended this reanalysis to the
97 polyunsaturated fatty acids (PUFA), linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3).

98

99 Results

100 All of the high-fat meals elicited a comparable postprandial glucose but different insulin and
101 NEFA responses either in subjects with normal (**Figure 1**, left panels) or high (**Figure 1**,
102 right panels) fasting triglycerides. The incremental AUC for triglycerides (from 0 to 480 min)
103 in subjects with normal fasting triglycerides were as follows: 5.27 ± 0.54 , 3.75 ± 0.73 , $4.45 \pm$
104 0.53 and 4.79 ± 0.36 mmol/h $\times$ L after the ingestion of the butter, HPSO, ROO and VEFO
105 meals, respectively (all $P < 0.05$, except the values after the ROO and VEFO meals that did
106 not statistically differ). The incremental AUC for triglycerides (from 0 to 480 min) in
107 subjects with high fasting triglycerides were as follows: 13.43 ± 2.73 , 4.30 ± 1.21 , $6.48 \pm$
108 2.20 and 7.07 ± 1.27 mmol/h $\times$ L after the ingestion of the butter, HPSO, ROO and VEFO
109 meals, respectively (all $P < 0.05$, except the values after the ROO and VEFO meals that did
110 not statistically differ). The suppression of NEFA concentrations in the early postprandial
111 period was mediated by hyperinsulinemia but this association was dependent on the type of
112 dietary fat in the meals [$r = 0.987$ (95% CI: 0.959, 0.995) in subjects with normal fasting
113 triglycerides (**Figure 1**, left panel at the bottom) and $r = 0.983$ (95% CI: 0.946, 0.994) in
114 subjects with high fasting triglycerides; all $P < 0.01$] (**Figure 1**, right panel at the bottom).

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116 A Pearson correlation analysis and hierarchical cluster analysis were performed to determine
117 the detailed relationship between the individual fatty acids in the meals and the surrogate
118 measures of postprandial β -cell function and insulin sensitivity. These analytical results
119 showed two major dietary fatty acid clusters. The SFA palmitic and stearic acids, and the
120 MUFA palmitoleic acid were included in one cluster, which was characterized by a positive
121 response on postprandial β -cell function but a negative response on postprandial insulin
122 sensitivity both in subjects with normal (**Figures 2 and 3A**) and high (**Figures 3B and 4**)
123 fasting triglycerides. Importantly, only the SFA palmitic acid was positively correlated
124 ($P < 0.01$) 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 [$r = -0.92$ for the oral glucose insulin sensitivity index from 0 to 180 min ($OGIS_{0-180}$) and -0.94 for the minimal model insulin sensitivity index from 0 to 480 min (IS_{0-480}) 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 α -linolenic acids that participated in a negative response on postprandial β -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 β -cell function ($r = -0.71$ for IGI and -0.97 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 ($r = 0.89$ for IGI and 0.93 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-workers⁶ 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-lipotoxic and insulin-sensitizing potencies⁷⁻⁹. 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^{1,10}. Consistent with this notion, we have demonstrated that subjects with normal⁴ and high⁵ 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 β -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 β -cell function and insulin sensitivity. Our study also indicates that dietary SFA stearic, MUFA palmitoleic and PUFA linoleic or α -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 meals¹¹. 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/CD36¹²⁻¹⁴. 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.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 β -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 production¹⁶, only long-term SFA palmitic acid accelerates the impairment of the same production rate¹⁷. 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 develops¹⁸. In addition, acute episodes of postprandial hyperinsulinemia, despite insulin-mediated suppression of circulating NEFA, recently have been linked to cardiac steatosis in healthy subjects¹⁹. 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 ($\text{BMI: } 23.9 \pm 1.9 \text{ kg/m}^2$) and newly diagnosed with type IIb or IV hyperlipoproteinemia (fasting triglycerides $>2.24 \text{ mmol/L}$) according to the National Cholesterol Education Program Adult Treatment Panel III ($\text{BMI: } 24.2 \pm 5.1 \text{ kg/m}^2$)²².

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 $4.22 \pm 0.72 \text{ mmol/L}$), nonesterified fatty acids (NEFA) (465 ± 87 and $500 \pm 73 \text{ } \mu\text{mol/L}$), glucose (5.16 ± 0.38 and $5.77 \pm 0.64 \text{ mmol/L}$), insulin (49 ± 9 and $90 \pm 10 \text{ pmol/L}$), homeostatic model assessment (HOMA) for β -cell function ($\text{HOMA-B: } 28 \pm 4$ and $48 \pm 6 \text{ pmol/mmol}$) and for insulin resistance ($\text{HOMA-IR: } 11.2 \pm 1.1$ and $22.9 \pm 2.4 \text{ pmol} \times \text{mmol/L}^2$) 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 described⁴.

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 described²³.

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

- 1 Gillingham LG, Harris-Janz S and Jones PJ, Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. *Lipids* **46**:209-228 (2011).
- 2 Morgan NG and Dhayal S, Unsaturated fatty acids as cytoprotective agents in the pancreatic beta-cell. *Prostaglandins Leukot Essent Fatty Acids* **82**:231-236 (2010).
- 3 Peng G, Li L, Liu Y, Pu J, Zhang S, Yu J *et al.*, Oleate blocks palmitate-induced abnormal lipid distribution, endoplasmic reticulum expansion and stress, and insulin resistance in skeletal muscle. *Endocrinology* **152**:2201-2218 (2011).
- 4 Lopez S, Bermudez B, Pacheco YM, Villar J, Abia R and Muriana FJ, Distinctive postprandial modulation of beta cell function and insulin sensitivity by dietary fats: monounsaturated compared with saturated fatty acids. *Am J Clin Nutr* **88**:638-644 (2008).
- 5 Lopez S, Bermudez B, Ortega A, Varela LM, Pacheco YM *et al.*, Effects of meals rich in either monounsaturated or saturated fat on lipid concentrations and on insulin secretion and action in subjects with high fasting triglyceride concentrations. *Am J Clin Nutr* **93**:494-499 (2011).
- 6 Kinsell LW, Walker G, Michaels GD and Olson FE, Dietary fats and the diabetic patient. *N Engl J Med* **261**:431-434 (1959).
- 7 Kien CL, Dietary interventions for metabolic syndrome: role of modifying dietary fats. *Curr Diab Rep* **9**:43-50 (2009).

300 8 Lopez S, Bermudez B, Abia R and Muriana FJ, The influence of major dietary fatty
301 acids on insulin secretion and action. *Curr Opin Lipidol* **21**:15-20 (2010).

302 9 Giacca A, Xiao C, Oprescu AI, Carpentier AC and Lewis GF, Lipid-induced
303 pancreatic β -cell dysfunction: focus on in vivo studies. *Am J Physiol Endocrinol*
304 *Metab* **300**:E255-E262 (2011).

305 10 Schwingshackl L, Strasser B and Hoffmann G, Effects of monounsaturated fatty acids
306 on glycaemic control in patients with abnormal glucose metabolism: a systematic
307 review and meta-analysis. *Ann Nutr Metab* **58**:290-296 (2011).

308 11 Lopez S, Bermudez B, Pacheco YM, Lopez-Lluch G, Moreda W, Villar J *et al.*,
309 Dietary oleic and palmitic acids modulate the ratio of triacylglycerols to cholesterol in
310 postprandial triacylglycerol-rich lipoproteins in men and cell viability and cycling in
311 human monocytes. *J Nutr* **137**:1999-2005 (2007).

312 12 Wang H and Eckel RH, Lipoprotein lipase: from gene to obesity. *Am J Physiol*
313 *Endocrinol Metab* **297**:E271-E288 (2009).

314 13 Talukdar S, Olefsky JM and Osborn O, Targeting GPR120 and other fatty acid-
315 sensing GPCRs ameliorates insulin resistance and inflammatory diseases. *Trends*
316 *Pharmacol Sci* **32**:543-550 (2011).

317 14 Love-Gregory L and Abumrad NA, CD36 genetics and the metabolic complications
318 of obesity. *Curr Opin Clin Nutr Metab Care* **14**:527-534 (2011).

319 15 Shah M, Adams-Huet B, Brinkley L, Grundy SM and Garg A, Lipid, glycemic, and
320 insulin responses to meals rich in saturated, cis-monounsaturated, and polyunsaturated
321 (n-3 and n-6) fatty acids in subjects with type 2 diabetes. *Diabetes Care* **30**:2993-2998
322 (2007).

- 323 16 Stein DT, Stevenson BE, Chester MW, Basit M, Daniels MB, Turley SD and
324 MacGarry JD, The insulinotropic potency of fatty acids is influenced profoundly by
325 their chain length and degree of saturation. *J Clin Invest* **100**:398-403 (1997).
- 326 17 Morgan NG, Fatty acids and beta-cell toxicity. *Curr Opin Clin Nutr Metab Care*
327 **12**:117-122 (2009).
- 328 18 De Gaetano A, Hardy T, Beck B, Abu-Raddad E, Palumbo P, Bue-Valleskey J and
329 Pørksen N, Mathematical models of diabetes progression. *Am J Physiol Endocrinol*
330 *Metab* **295**:E1462-E1479 (2008).
- 331 19 Winhofer Y, Krssak M, Jankovic D, Anderwald CH, Reiter G, Hofer A *et al.*, Short-
332 term hyperinsulinemia and hyperglycemia increase myocardial lipid content in normal
333 subjects. *Diabetes* **61**:1210-1216 (2012).
- 334 20 Hodson L and Fielding BA, Stearoyl-CoA desaturase: rogue or innocent bystander?
335 *Prog Lipid Res* **52**:15-42 (2013).
- 336 21 Pinnick KE, Neville MJ, Fielding BA, Frayn KN, Karpe F and Hodson L,
337 Gluteofemoral adipose tissue plays a major role in production of the lipokine
338 palmitoleate in humans. *Diabetes* **61**:1399-1403 (2012).
- 339 22 National Cholesterol Education Program (NCEP) Expert Panel on Detection,
340 Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment
341 Panel III). Third Report of the National Cholesterol Education Program (NCEP)
342 Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in
343 Adults (Adult Treatment Panel III) final report, *Circulation* **106**:3143-3421 (2002).
- 344 23 Pacheco YM, Bermudez B, Lopez S, Abia R, Villar J and Muriana FJ. Ratio of oleic
345 to palmitic acid is a dietary determinant of thrombogenic and fibrinolytic factors
346 during the postprandial state in men. *Am J Clin Nutr* **84**:342-349 (2006).
- 347

348 Table 1. Composition of principal fatty acids that are common in the meals^a.

	Butter	HPSO	ROO	VEFO
Fatty acid	<i>g/100 g fatty acids</i>			
16:0	31.8	24.9	11.7	5.4
16:1n-7	2.4	6.8	1.0	0.6
18:0	13.3	2.0	2.8	4.2
18:1n-9	26.2	58.7	79.8	74.4
18:2n-6	3.0	6.9	3.6	10.3
18:3n-3	0.4	0.04	0.5	0.3
SFA	65.3	27.2	14.9	10.7
MUFA	31.3	65.8	81.1	75.4
PUFA	3.4	7.0	4.0	13.9

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350 ^a The butter meal had also short or medium chain SFA [butyric acid (4:0) 2.0, caproic acid
351 (6:0) 1.4, caprylic acid (8:0) 0.9, capric acid (10:0) 2.0, lauric acid (12:0) 2.5, myristic acid
352 (14:0) 9.9, pentadecanoic acid (15:0) 0.8, margaric acid (17:0) 0.6] and MUFA [myristoleic
353 acid (14:1n-7) 0.5, pentadecenoic acid (15:1n-7) 1.1, eicosenoic acid (20:1n-9) 0.6]. Other
354 fatty acids [17:0 < 0.1, heptadecenoic acid (17:1n-7) < 0.1, arachidic acid (20:0) < 0.4, 20:1n-
355 9 < 0.3] were detected in the HPSO, ROO, and VEFO meals. The VEFO meal had
356 eicosapentaenoic acid (20:5n-3) 1.0 and docosahexaenoic acid (22:6n-3) 2.3. All values are in
357 g/100 g fatty acids.

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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 $\times$ L, pmol/h $\times$ L and μ mol/h $\times$ L, respectively. Labeled means in a column without a common letter differ, $P < 0.05$. All values are mean $\pm$ 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

389 sensitivity in subjects with high fasting triglycerides. Data are strength (*r*) and significance
390 (*P*) of correlation derived from Pearson’s correlation analysis.

Figure 1

Figure 2

Figure 3

Figure 4