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Interaction between specific fatty acids, GLP-1 and insulin secretion in humans

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Abstract

Aims/hypothesis. Fatty acids affect insulin secretion in vivo, but little is known about the effects of specific fatty acids. Our aim was to investigate differential effects of acutely increased plasma monounsaturated, polyunsaturated and saturated fatty acids on glucose-stimulated insulin secretion in healthy humans.
Methods. A new experimental protocol was used to increase plasma monounsaturated (MUFA test), polyunsaturated (PUFA test) or saturated (SFA test) non-esterified fatty acids for 2 h by repeated oral fat feeding and continuous intravenous heparin infusion. This was followed by a hyperglycaemic clamp (10 mmol/l) to test insulin secretion in response to a prior plasma NEFA increase.
Results. Total plasma NEFA concentrations were increased during the fat tests compared to the control visit (1.7-fold increase for MUFA and SFA tests and 1.4-fold increase for PUFA test; $p<0.001$). Exaggerated responses in plasma insulin, C-peptide and proinsulin concentrations were seen during the hyperglycaemic clamp after increasing plasma NEFA concentrations compared with the control ($p<0.01$). The effects were greatest for the MUFA test followed by the PUFA test and SFA test ($p<0.01$). Plasma GLP-1 concentrations increased during fat feeding, with a higher response during the MUFA test compared to PUFA and SFA tests ($p<0.01$).
Conclusion/interpretation. Increasing plasma NEFA concentrations by oral fat feeding with heparin infusion augments glucose-stimulated insulin secretion with the greatest effect for monounsaturated fatty acids and the lowest effect for saturated fatty acids. Monounsaturated fatty acids also increase GLP-1 more than saturated fatty acids. Therefore, the exaggerated insulin concentrations could be due to both NEFA and GLP-1. [Diabetologia (2002) 45:1533–1541]

Keywords Monounsaturated fatty acids, polyunsaturated fatty acids, saturated fatty acids, insulin secretion, GLP-1.

High fat diets lead to obesity, which in turn is one of the main causes of the development of Type II (non-insulin-dependent) diabetes mellitus. Both obesity and Type II diabetes are characterized by increased plasma non-esterified fatty acid concentrations [1] and raised fasting plasma NEFA concentrations are a risk marker for the development of Type II diabetes in Caucasian subjects [2] and in Pima Indians [3]. Increased plasma NEFA concentrations can have adverse effects on various tissues. Excess plasma NEFA concentrations can lead to a reduced skeletal muscle glucose uptake and oxidation [4, 5, 6], increased hepatic glucose output [7] and a decrease in hepatic insulin clearance [8, 9]. These mechanisms could lead to glucose intolerance and insulin resistance leading to Type II diabetes.

Inappropriately high concentrations of plasma NEFA have also been shown to alter glucose-stimulat-

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Abbreviations: GLP-1, Glucagon-like-peptide-1; PI:I, proinsulin to insulin ratio; ISR, insulin secretion rate; MUFA, monounsaturated non-esterified fatty acid; PUFA, polyunsaturated non-esterified fatty acid; SFA, saturated non-esterified fatty acid.

ed insulin secretion in a time-dependent manner. Short-term in vitro incubation of islets with long-chain fatty acids increased insulin release during glucose stimulation [10, 11]. This was also seen in healthy volunteers after a short-term increase of plasma NEFA concentrations by Intralipid and heparin [12, 13, 14]. Contrary findings have been observed after a long-term increase of plasma NEFA concentrations. Studies showed that a prolonged (24–48 h) increase of NEFA concentrations either impaired [15] or increased [14] glucose-stimulated insulin secretion in healthy volunteers.

The type of fat consumed can alter the composition of the fatty acid pool in plasma [16]. However, it is not known how the fatty acid composition of a meal and the plasma pools affect insulin secretion in vivo. At the cellular level, glucose-stimulated insulin release has been shown to be more pronounced with increasing chain length and the degree of saturation of the fatty acids in acute incubations in vitro [17, 18]. In particular, stearic and palmitic acid have marked stimulatory effects on glucose-stimulated insulin secretion from perfused rat pancreas [19]. Results from these in vitro studies cannot necessarily be extrapolated to the situation in vivo. Many factors combine to regulate insulin secretion in vivo, including incretin hormones such as glucagon-like peptide 1 (GLP-1) whose secretion could, in turn, be influenced by fatty acids.

Human studies examining the effect of individual fatty acids on insulin secretion in vivo are lacking, partly because a method has not been available previously to acutely alter the composition of increased plasma NEFA via oral fat administration. We have developed such a method [20] and here we have applied it to investigate whether an increase of plasma monounsaturated, polyunsaturated and saturated NEFA alter islet beta-cell function, and if this is different for specific fatty acids.

Subjects and methods

Subjects. Eight healthy volunteers (four women) were studied on four occasions. Their median age was 29 years (range 21–54 years) and median BMI 23 kg/m^2 (range 18–26 kg/m^2). All subjects were non-smokers and no medication was taken which would affect lipid or glucose metabolism. Subjects were asked to have a low fat meal the evening before each study and fasted overnight for 12 h. On separate days, in random order and separated by at least 2 weeks, mainly plasma monounsaturated NEFAs (MUFA test), polyunsaturated NEFAs (PUFA test), saturated NEFAs (SFA test) or no fatty acids (control) were increased before a hyperglycaemic clamp. Baseline characteristics are given in Table 1. All subjects gave informed consent and the study was approved by the Central Oxford Research Ethics Committee.

Study design. On each of the four visits, subjects were weighed and a cannula was inserted retrogradely into a vein draining a hand that was kept in a box with heated air (+65 °C) to obtain arterialized blood samples [21]. An antecubital vein was cannulated for infusion of glucose, heparin or saline. During the first 210 min of the three fat tests (MUFA, PUFA and SFA tests), small boluses of fat were given. The timing and amounts are presented in Figure 1. The fat was given orally as a hot chocolate-flavoured drink (50% (w/w) fat emulsion and virtually carbohydrate and protein free). The drink (100 g) consisted of the following ingredients: 50 g of oil, 0.5 g emulsifier (monoglycerides, HYMONO 8903 K, Quest International, Zwijndrecht, The Netherlands), 1 g of sweetener (Canderel, High Wycombe, UK), 3 g cocoa powder (Cadbury, Premier brands UK, Stafford, UK) and 45.5 g of water. Different types of oils were used during the three fat tests. For the MUFA test, olive oil (Tesco, Oxford, UK) high in oleic acid (69%, w/w) was used. For the PUFA test, refined safflower oil (Anglia Oils Limited, Hull, UK) high in linoleic acid (74%, w/w) was added to the drinks and for the SFA test, palm stearin (Anglia Oils Limited, Hull, UK) high in palmitic acid (59%, w/w) was given. In addition to the fat feeding during the three fat tests, a bolus of heparin (500 IU) was given at 90 min and this was immediately followed by a 0.4 IU·kg^{-1}·min^{-1} heparin infusion (from 90 min until end of experiment) to increase the action of lipoprotein lipase. The combination of continuous oral fat feeding and intravenous heparin infusion was found to result in an acute and steady state increase in total plasma NEFA concentrations and the use of different types of oils altered the composition of the NEFA pool during the three fat tests. During the control test the same protocol was followed but no fat (replaced with water) and no heparin (replaced with saline) were given. This was to prevent a marked increase in total plasma NEFA concentrations. A 90 min hyperglycaemic clamp started at 210 min for all tests until the end of the experiment (300 min) to raise blood glucose concentrations to 10 mmol/l and to test beta-cell function [22]. At this point, plasma NEFA concentrations had been increased for 2 h during the three fat tests. Blood samples were drawn into pre-cooled heparinized syringes (Monovette; Sarstedt, Leicester, UK) and rapidly centrifuged at 2 °C for plasma total and specific NEFA, insulin and proinsulin analysis. Tetrahydrolipstatin dissolved in etha-

Table 1. Fasting characteristics of eight subjects

	MUFA test	PUFA test	SFA test	Control
Plasma glucose (mmol/l)	5.0±0.1	5.0±0.1	4.8±0.1	5.1±0.2
Plasma NEFA (μmol/l)	370±35	378±58	459±58	413±87
Plasma TAG (μmol/l)	992±150	727±59	837±63	895±70
Plasma insulin (pmol/l)	59±5	53±6	49±7	58±7
Plasma C-peptide (nmol/l)	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1
Plasma proinsulin (pmol/l)	8.4±1.6	6.7±0.9	7.9±0.9	8.2±1.5

Data are means ± SE. No significant difference seen between visits

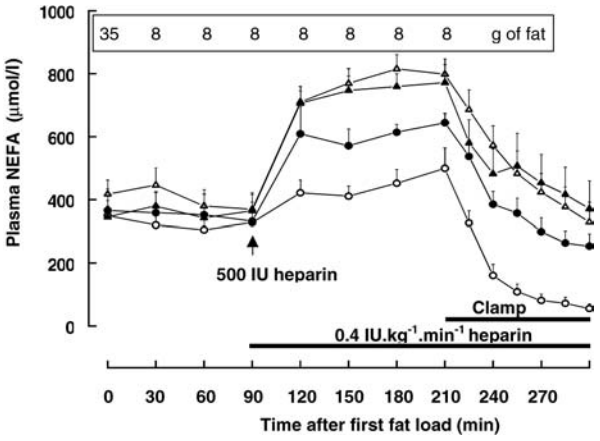

Fig. 1. Plasma NEFA concentrations during the MUFA test (*solid triangle*), the PUFA test (*solid circle*), the SFA test (*open triangle*) and the control visit (*open circle*). Data are means ± SE for eight subjects. Fat was given orally as shown. For the control test, the fat was replaced by water. Intravenous heparin was given as shown. For the control visit, saline was used instead of heparin. A hyperglycaemic clamp was done for all four tests from 210 min until the end of the experiment. Between 90 min and 210 min the concentrations were different for the MUFA test ($p<0.01$), PUFA test ($p<0.05$) and SFA test ($p<0.02$) compared to the control. During the hyperglycaemic clamp (from 210 min to 300 min) the NEFA concentrations were greater for the MUFA test ($p<0.01$), PUFA test ($p<0.01$) and SFA test ($p<0.001$) compared to control. Concentrations were the same for the MUFA test vs the SFA test. Plasma NEFA concentrations were lower during the PUFA test vs MUFA and SFA tests ($p<0.05$)

nol (30 μg/ml plasma) was added to the samples for plasma total and specific NEFA analysis, to prevent in vitro lipolysis. A separate blood sample was taken into tubes containing EDTA and 200 KIU aprotinin/ml blood (Trasylol, Bayer PLC, Newbury, UK) for measurement of C-peptide and GLP-1. Linco DPP-IV inhibitor (10 μl/ml plasma) was added to samples for GLP-1 analysis to prevent Dipeptidyl Peptidase-mediated cleavage. All the samples were frozen immediately at –20 °C (glucose, NEFA, insulin and proinsulin) or –70 °C (C-peptide and GLP-1).

Analysis. Blood glucose concentrations just before and during the hyperglycaemic clamp, were measured at the bedside using a photometer (HemoCue, Sheffield, UK). Plasma NEFA and glucose concentrations were measured using enzymatic methods on an IL Monarch centrifugal analyser (Instrumentation Laboratory, Warrington, Cheshire, UK). For the analysis of specific plasma NEFA, lipids were extracted from plasma using chloroform:methanol (2:1, v:v). After the separation of the plasma NEFA by solid-phase extraction [23] and methylation of fatty acids with methanoic sulphuric acid, gas chromatography was used to analyse the fatty acid composition. Details have been given previously [24]. Plasma insulin concentrations were measured with a Human Insulin-specific radioimmunoassay (RIA) kit (Linco Research, St. Charles, Mo., USA). Plasma C-peptide and proinsulin concentrations were assayed with Linco RIA kits. The active forms (7–36 and 7–37 amide) of plasma GLP-1 were measured by using a Linco GLP-1 active ELISA kit. All the samples from the four experiments for one person were analysed within the same assay run.

Calculations. Beta-cell insulin secretion rate (ISR) was calculated by deconvolution of plasma C-peptide concentrations using a two-compartment mathematical model [25]. Standard parameters for C-peptide clearance were used [26] assuming the experimental protocol had no effect on C-peptide kinetics. The insulin sensitivity index was expressed as the M/I ratio, calculated for every 15 min during the last 30 min of the hyperglycaemic clamp, according to the method described previously [22], where M is the 'glucose metabolized' and I is the average plasma insulin concentration for that period. 'M' was calculated from the mean glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$) for each 15 min period. Corrections were made for glucose that has either been added or removed from the glucose space [22] but not for urinary glucose loss since the blood glucose concentrations in this study were below the threshold for urinary glucose loss. 'I' was the mean plasma insulin concentration (pmol/l) during each 15 min. Area under the curve (AUC) was calculated for insulin secretion rate during the hyperglycaemic clamp (from 210 min until 300 min) and divided by the respective time period (90 min) to give time-averaged AUC.

Statistical analysis. Data are presented as means ± SE. Repeated-measures analysis of variance (ANOVA) was used to look for differences in concentrations between the four visits and between each visit. Comparisons between concentrations were made for the total period of the hyperglycaemic clamp and for the first phase (insulin: from 210 min to 220 min; C-peptide and proinsulin: from 210 min to 225 min) and second phase (insulin: from 220 min to 300 min; C-peptide and proinsulin: from 225 min to 300 min). The p values of less than 0.05 are reported but not corrected for multiple comparisons according to a previous report [27]. All analyses were carried out within-subject, using subject as fixed factor in ANOVA models. The statistical program, SPSS for Windows (SPSS, Chicago, Ill, USA) was used and p values of less than 0.05 were taken as significant.

Results

Plasma total and specific NEFA. No difference of total plasma NEFA concentrations was seen for fasting (0 min) and pre-heparin infusion (from 0 min to 90 min) between the four tests. A mean 1.6-fold increase in total plasma NEFA concentrations was achieved when fat and heparin were given compared to the control test ($p<0.001$) and this was maintained for 2 h (from 90 min to 210 min) (Fig. 1). Throughout the heparin infusion, total plasma NEFA concentrations for the PUFA test were lower than concentrations achieved for the other fat tests (MUFA test and SFA test). During the hyperglycaemic clamp (from 210 min to 300 min), total plasma NEFA concentrations decreased, but remained higher for the three fat tests compared to the control visit ($p<0.01$). The plasma NEFA composition was different for the three fat tests. During the MUFA test, the monounsaturated fatty acid, oleic acid was the predominant fatty acid in plasma while this was the polyunsaturated fatty acid, linoleic acid and the saturated fatty acid, palmitic acid for the PUFA test and the SFA tests, respectively (Fig. 2).

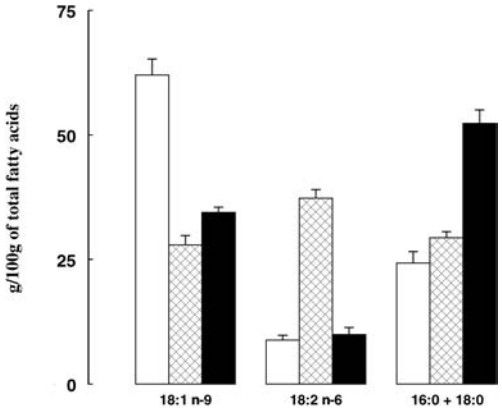

Fig. 2. Plasma NEFA composition for the three fat tests. *Bars* show mean ± SE for eight subjects of samples taken at 180 min and 210 min for the MUFA test (*open*), PUFA test (*hatched*) and SFA test (*solid*). Values are expressed as % (g/100 g) of total fatty acids. Of the 52% saturated fatty acids during the SFA test, 40% was accounted for by palmitic acid (16:0) and 12% by stearic acid (18:0). The fatty acid 18:1 *n*-9 is oleic acid and 18:2 *n*-6 is linoleic acid

Plasma and blood glucose. Mean fasting plasma glucose concentrations (Table 1) and pre-clamp (from 0 min to 210 min) blood glucose concentrations (4.6±0.1 mmol/l) were similar for all four visits (n=8). During the hyperglycaemic clamp, the mean blood glucose concentrations (n=8) for all four tests increased to 10.2±0.2 mmol/l within the first 10 min and remained within 8% of this concentration (Fig. 3A).

Plasma insulin, C-peptide and proinsulin. There were no significant differences in fasting plasma insulin, C-peptide and proinsulin concentrations or between pre-clamp plasma C-peptide and proinsulin concentrations when the four tests were compared (n=8) (Fig. 3B–D). From 90 min to 210 min there was a tendency for a difference in plasma insulin concentrations between the four visits (p=0.054). Since there was no significant difference between the three fat tests during this time period, the mean plasma insulin concentrations for the three fat tests were calculated and compared to the control visit. This resulted in higher plasma insulin concentrations when fat and heparin were given compared to the control visit (p<0.001).

During the glucose infusion (from 210 min to 300 min), differences were seen for all three beta-cell peptides between the four visits (p<0.001) and this was so for the first (p<0.05) and second phase concentrations (p<0.001) with greater concentrations for each

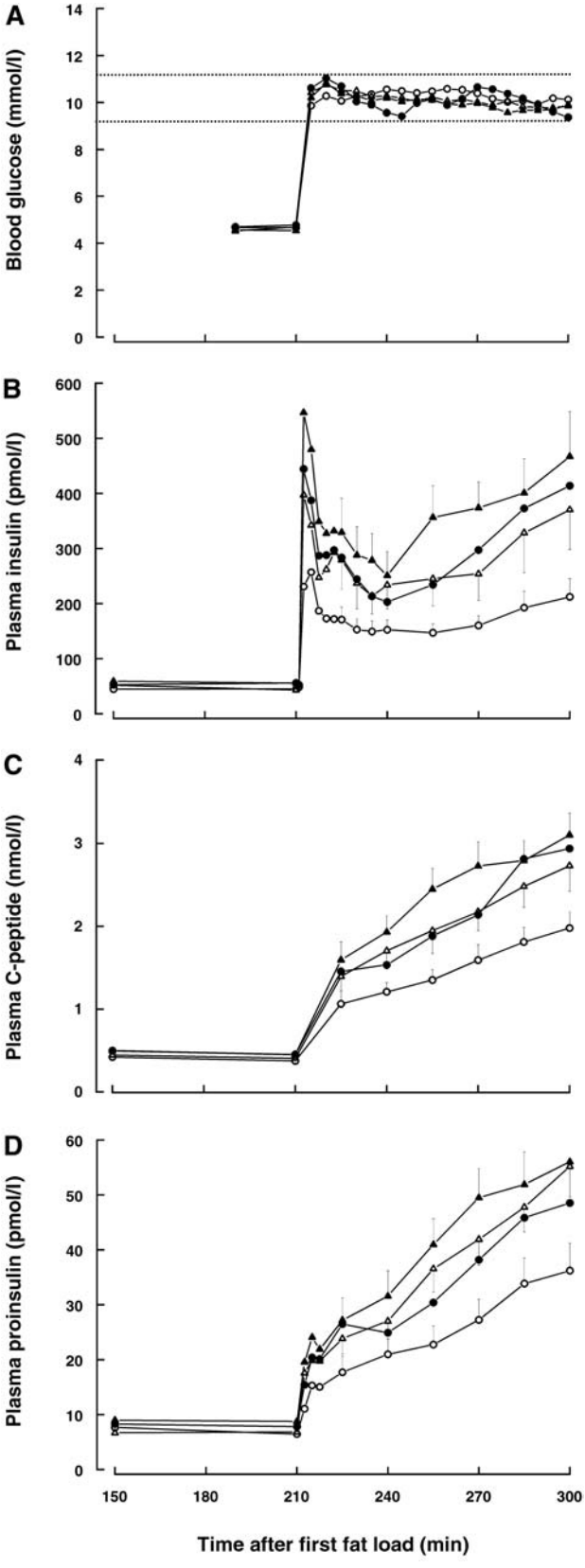

Fig. 3A–D. Blood glucose (**A**), plasma insulin (**B**), plasma C-peptide (**C**) and plasma proinsulin (**D**) concentrations for the MUFA test (*solid triangle*), PUFA test (*solid circle*), SFA test (*open triangle*) and the control visit (*open circle*) before and during the hyperglycaemic clamp (210 to 300 min). Data are means ± SE for eight subjects. During the hyperglycaemic clamp, concentrations were greater for the MUFA test than the PUFA test (for total insulin and second phase C-peptide, p<0.05; total proinsulin, p<0.02), and higher for the MUFA test than the SFA test (for total insulin, p<0.02; total C-peptide, p<0.05; first phase proinsulin, p<0.02). Also the PUFA test was different from the SFA test (for total insulin and proinsulin, p<0.05)

fat test compared to the control visit ($p<0.01$). Also significant differences for beta-cell peptides were seen when the three fat tests were compared during the total period of the hyperglycaemic clamp ($p<0.01$), for the first phase ($p<0.05$) and for the second phase ($p<0.05$) insulin, proinsulin and C-peptide (with the exception of first phase) concentrations. The greatest overall increase in plasma insulin, proinsulin and C-peptide concentrations were seen for the MUFA test. Plasma insulin concentrations were the lowest for the SFA test. Plasma C-peptide concentrations were the lowest for both the PUFA and the SFA tests. Plasma proinsulin concentrations were the lowest during the PUFA test (Fig. 3).

For each test, the ratios of second phase (mean from 220 min to 300 min) to first phase (mean from 210 min to 220 min) plasma insulin concentrations were calculated to investigate if both first and second phase insulin release was affected by a prior increase of different types of plasma NEFA concentrations. No differences were seen between the control visit and the three fat tests or between each fat test (ratios were between 1.1±0.1 and 1.2±0.1).

The ratios between plasma C-peptide and insulin concentrations were calculated for each test at 210 min (at basal steady-state glucose concentrations) as a measure of insulin clearance. The ratios were similar for each fat test and after a 2-h increase of plasma NEFA concentrations, there was no change compared to the control visit.

The efficiency of conversion of proinsulin to insulin with or without a prior increase of plasma NEFA concentrations was estimated by the proinsulin to insulin concentration ratio (PI:I) during the first 10 min of the hyperglycaemic clamp. There was no statistically significant difference in the PI:I ratio after a 2-h increase in plasma NEFA concentrations compared to the control visit. Neither was there a difference after increasing different types of plasma NEFA.

Insulin secretion rate. No difference was observed for the pre-clamp ISR for the four visits (Fig. 4). During glucose infusion, ISR increased for all four experiments, although ISR was more pronounced during the fat tests compared to the control visit ($p<0.01$). The time-averaged AUC for the total duration of the clamp was different between the four tests (MUFA test: 648±61 pmol/min; PUFA test: 585±72 pmol/min; SFA test: 577±76 pmol/min and control test: 389±49 pmol/min) ($p<0.001$).

Plasma GLP-1. No difference was seen for fasting GLP-1 concentrations between the four visits (Fig. 5). Before heparin infusion, plasma GLP-1 concentrations increased while ingesting oil high in monounsaturated (MUFA test) and polyunsaturated fatty acids (PUFA test) ($p<0.01$) but not for oils high in saturated fatty acids (SFA test) compared to the control visit. Plasma

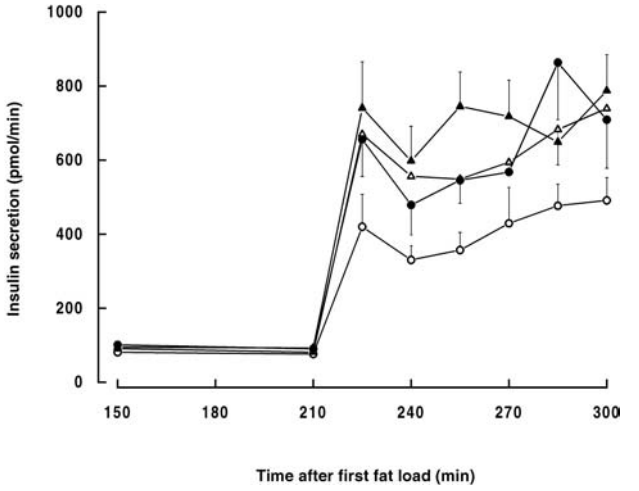

Fig. 4. Mean insulin secretion rate for the MUFA test (*solid triangle*), PUFA test (*solid circle*), SFA test (*open triangle*) and the control visit (*open circle*) before and during the hyperglycaemic clamp (210 to 300 min). Data are means ± SE for eight subjects

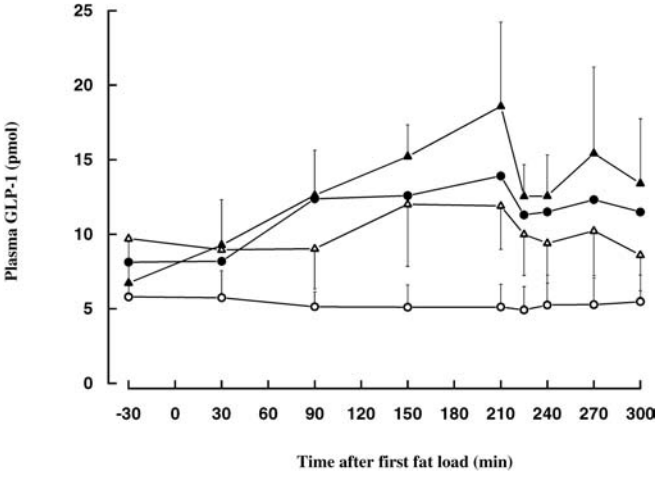

Fig. 5. Plasma GLP-1 concentrations for the MUFA test (*solid triangle*), PUFA test (*solid circle*), SFA test (*open triangle*) and the control visit (*open circle*) before and during the hyperglycaemic clamp (210 min to 300 min). Data are means ± SE for eight subjects

GLP-1 concentrations were different (from 30 min to 300 min) between the four visits ($p<0.001$) with greater concentrations for each fat test compared to the control visit ($p<0.02$). The increase was different for the three types of fat given ($p<0.01$) with the highest increase for the MUFA test followed by the PUFA test and SFA test (MUFA test vs SFA test, $p<0.01$). During the hyperglycaemic clamp, plasma GLP-1 concentrations dropped during the three fat tests, while they remained constant for the control visit.

Insulin sensitivity index. The mean insulin sensitivity index was calculated for each 15 min of the last 30 min of the hyperglycaemic clamp (Fig. 6). There was no change in insulin sensitivity after increasing

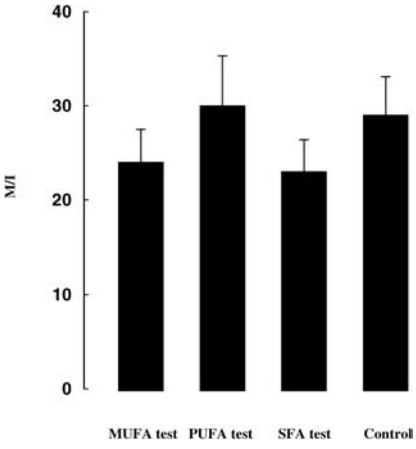

Fig. 6. Insulin sensitivity index calculated for the last 30 min of the hyperglycaemic clamp for the MUFA test, PUFA test, SFA test and the control visit. Data are means ± SE for eight subjects

plasma NEFA concentrations compared to the control visit.

Discussion

The new experimental protocol used in our study, successfully resulted in a short-term increase of monounsaturated, polyunsaturated or saturated NEFA. The prior increase of plasma NEFA concentrations resulted in higher plasma insulin concentrations during basal glucose and during glucose stimulation, with the first and second phase being exaggerated. Other studies also showed enhanced insulin secretion when plasma NEFA concentrations were increased for 20 min, 90 min or 5 h, by Intralipid and heparin in healthy volunteers [12, 13, 15]. A short-term (9-h) plasma NEFA increase induced insulin secretion in humans and this was sufficient to prevent an increase in plasma glucose concentrations [28]. However, no hyperinsulinaemic response has been reported when plasma NEFA concentrations were increased for 5 h to 10 h [29, 30] and this turned into an inhibitory effect after a very long–term increase (>24 h) [13, 15]. In these studies, a wide range of plasma NEFA concentrations were reported, which may partly explain the divergence in insulin response. However, the very high plasma NEFA concentrations reported are likely to be overestimates, due to continued in vitro lipolysis in samples, when no appropriate lipoprotein lipase inhibitor (e.g. tetrahydrolipstatin) is used during sample storage [31].

From numerous isolated islet studies it has become clear that fatty acids have a direct effect on the beta cell and that short-term incubation of fatty acids gives rise to an amplified insulin response, increasing with chain length and degree of saturation [10, 19]. A direct stimulatory effect of fatty acids upon islets provides a plausible explanation for the greater insulinotropic effect with increased NEFA concentrations, as seen in this study, although in vivo, other factors, such as insulin sensitivity, hepatic insulin clearance and other stimulants have to be considered. There is evidence that both insulin sensitivity [13] and hepatic insulin clearance [9] are reduced after a short-term increase of total plasma NEFA concentrations, but it is not clear if there is a difference between the types of fatty acid. It has been reported that the insulin resistance induced by an acute increase (90 min) of plasma NEFA concentrations was precisely countered by a fatty acid-induced increase in insulin secretion [13]. In our study, no change in insulin sensitivity was seen and still an exaggerated insulin response was observed with increased plasma NEFA concentrations. Impairment in hepatic insulin clearance could in part explain the increase of plasma insulin concentrations [8]. This study was not designed to investigate this accurately, but the unaltered C-peptide to insulin ratio after the 2-h plasma NEFA increase suggests that there is no change in insulin clearance. This is in agreement with a recent study, which measured splanchnic insulin extraction directly and showed no effect of increased plasma NEFA concentrations [32]. This provides evidence for a direct role of the increased NEFAs in the hyperinsulinaemic response.

The direct effect of fatty acids upon the pancreatic islets could involve an increase in the conversion of proinsulin to insulin. This was illustrated in vivo in humans by a decrease in the PI:I ratio after acute stimulation with arginine when plasma NEFA concentrations were increased [33]. We did not find a difference between the four tests in the PI:I ratio during the first 10 min of the glucose clamp. This suggests that proinsulin processing was not affected by acutely increasing total or specific plasma NEFA concentrations and did not contribute to the hyperinsulinaemia.

Plasma NEFA composition in humans reflects the fatty acid composition of the diet in the short and in the long-term. In the period after meals, there is considerable entry of dietary fatty acids into the plasma NEFA pool [34, 35]. In the longer-term, the plasma NEFA composition reflects that of adipose tissue, which in turn is an integrated marker of dietary fatty acid intake [36, 37]. Therefore, the composition of plasma NEFA is open to dietary manipulation. It is not clear if individual classes of NEFA have a differential effect on insulin secretion in humans. In one study, a change in the ratio of polyunsaturated to saturated plus monounsaturated NEFA from 0.5 to 0.25 by intravenous lipid infusion (5 h and 24 h) did not change glucose-stimulated insulin secretion [38]. However, medium-chain triacylglycerols were used to increase saturated fatty acid concentrations, so this is not directly comparable. Our findings show a clear differential acute effect of specific plasma NEFA on the stimulation of plasma insulin secretion in vivo, with the

most notable change for monounsaturated NEFA and the most moderate increase for saturated NEFA. Caution has to be taken when comparing the insulin response of the PUFA test with the other fat tests, due to the lower plasma NEFA concentrations during the PUFA test. Therefore, the insulin response and the insulin-mediated action upon NEFA concentrations during glucose infusion could be underestimated. Nevertheless, insulin secretion rate and beta-cell peptide concentrations were still greater in the PUFA and the MUFA tests than in the SFA test and the control study. Slower gastrointestinal handling of safflower oil, decreased selectivity for linoleic acid by LPL during lipolysis and a more rapid turn-over of linoleic acid could have contributed to the lower NEFA concentrations during the PUFA test. Some conflicting data on the type of dietary fatty acids and their effects on insulin secretion are available from meal and dietary intervention studies. In humans, no differences in insulin responses were found after meals [39, 40] or long-term diets [41], which were high in monounsaturated or saturated fatty acids. Some studies have reported greater stimulation of insulin secretion after diets high in saturated fatty acids in subjects with Type II diabetes [42] and normal rats [43] but others have reported a more pronounced effect with polyunsaturated fatty acids [44]. A comparable pattern of glucose-stimulated insulin response to that observed in our study was seen when similar oils were given as a mixed test meal followed by a glucose drink [45]. In rats, a greater hyperinsulinaemic response was seen after a 6-week diet enriched in monounsaturated and polyunsaturated fatty acids compared to a diet high in saturated fatty acids [46].

GLP-1 is secreted by the intestinal endocrine L-cells in response to a mixed meal [47], with both glucose and fatty acids being stimulants [48] but circulating and neural mediators could also be important [49]. Plasma active GLP-1 concentrations potentiate glucose–stimulated insulin secretion [50] in a dose-dependent manner [49] via GLP-1 receptors on the beta cell. In the present study, plasma GLP-1 concentrations increased when oils high in monounsaturated and polyunsaturated fatty acids were ingested but a delayed increase was seen with saturated fatty acids. This could be the result of a slower intestinal absorption of saturated fatty acids [51]. After a mixed meal, the plasma GLP-1 increase has also been shown to be more pronounced with the presence of olive oil than palm oil or butter in healthy humans [39]. Also, in vitro, oleic acid is a stronger stimulator of GLP-1 secretion than saturated fatty acids [52]. The marked increase in GLP-1 at basal glucose concentrations coincided with only a very small increase in plasma insulin concentrations for the three fat tests compared to the control visit. This is in agreement with other studies where no, or only a small, increase in plasma insulin concentrations was seen at low plasma glucose concentrations and plasma GLP-1 concentrations around 16 $pmol \cdot l^{-1}$ [49, 53]. In vitro studies have shown that GLP-1 has a priming effect on glucose-stimulated insulin secretion [50, 54] but in vivo pretreatment with GLP-1 for 60 min did not alter the subsequent beta-cell response to glucose [49]. Therefore, the increase in plasma GLP-1 concentrations during the glucose infusion is probably more physiologically relevant to affect insulin secretion than the high plasma GLP-1 concentrations before the clamp.

During the hyperglycaemic clamp, a decrease in both plasma NEFA and GLP-1 concentrations was seen. An association between plasma GLP-1 and NEFA concentrations has been described in the literature, although this was an inverse relationship [55]. Insulin can act as a mediator for plasma NEFA and GLP-1 concentrations. It is known that insulin stimulates the enzyme lipoprotein lipase and inhibits the enzyme hormone-sensitive lipase, resulting in suppressed plasma NEFA concentrations with high insulin concentrations. It could be speculated that insulin also increases the action of the enzyme dipeptidyl peptidase IV (DPP IV) that is responsible for the degradation of GLP-1 or has a direct inhibitory effect on the L-cells, resulting in decreased circulating GLP-1 concentrations with high insulin concentrations.

Plasma NEFA and GLP-1 are both candidates to have a direct effect on the beta cell, or plasma NEFA could have an indirect effect on glucose-stimulated insulin secretion via plasma GLP-1 concentrations. This study was not designed to distinguish between the effects of NEFA and GLP-1 on insulin secretion. On the other hand, studies in which NEFA concentrations have been increased by intravenous fat infusion also show a marked increase in insulin secretion [13]. In these studies, GLP-1 concentrations are probably not increased (although not usually measured) and therefore favour a direct role for NEFA in the regulation of insulin secretion. Also, the marked stimulatory effect on insulin secretion of saturated fatty acids compared to other fatty acids seen in vitro [19] was completely absent in vivo, again showing the complexity of the in vivo situation.

In conclusion, these results confirm an exaggerated insulin response to glucose after an acute increase of plasma NEFA by oral fat feeding in humans. The degree of hyperinsulinaemia was clearly different for different types of fatty acids; with the highest effect for plasma monounsaturated NEFA and the lowest effect for plasma saturated NEFA. This result is different from the in vitro studies in which the saturated fatty acids have the greatest effect on the islet. It highlights the fact that in vivo, other factors, such as GLP-1, are likely to be involved in the complex hormonal and metabolic milieu that regulates insulin secretion.

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