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Effects of acute NEFA manipulation on incretin-induced insulin secretion in participants with and without type 2 diabetes

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Abstract

Aims/hypothesis Incretin effect—the potentiation of glucose-stimulated insulin release induced by the oral vs the i.v. route—is impaired in dysglycaemic states. Despite evidence from human islet studies that NEFA interfere with incretin function, little information is available about the effect in humans. We tested the impact of acute bidirectional NEFA manipulation on the incretin effect in humans.

Methods Thirteen individuals with type 2 diabetes and ten non-diabetic volunteers had a 3 h OGTT, and, a week later, an i.v. isoglycaemic glucose infusion (ISO; OGTT matched). Both pairs of studies were repeated during an exogenous lipid infusion in the non-diabetic volunteers, and following acipimox administration (to inhibit lipolysis) in people with diabetes. Mathematical modelling of insulin secretion dynamics assessed total insulin secretion (TIS), beta cell glucose sensitivity (β-GS), glucose-induced potentiation (P_{GLU}) and incretin-induced potentiation (P_{INCR}); the oral glucose sensitivity index was used to estimate insulin sensitivity.

Results Lipid infusion increased TIS (from 61 [interquartile range 26] to 78 [31] nmol/m^2 on OGTT and from 29 nmol/m^2 [26] to 57 nmol/m^2 [30] on ISO) and induced insulin resistance. P_{INCR} decreased from 1.6 [1.1] to 1.3 [0.1] ($p < 0.05$). β-GS, P_{GLU} and glucagon, glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) responses were unaffected. Acipimox (lowering NEFA by ~55%) reduced plasma glucose and TIS and enhanced insulin sensitivity, but did not change β-GS, P_{INCR}, P_{GLU} or glucagon, GLP-1 or GIP responses. As the per cent difference, incretin effect was decreased in non-diabetic participants and unchanged in those with diabetes.

Conclusions/interpretation Raising NEFA selectively impairs incretin effect and insulin sensitivity in non-diabetic individuals, while acute NEFA reduction lowers plasma glucose and enhances insulin sensitivity in people with diabetes but does not correct the impaired incretin-induced potentiation.

Keywords Acipimox · Beta cell function · Incretin effect · Incretin hormones · Isoglycaemic protocol · NEFA · Potentiation

Brenno Astiarraga and Valéria B. Chueire contributed equally to this study.

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Abbreviations

Acp-OGTT	OGTT following acipimox
Acp-ISO	ISO following acipimox
β-GS	Beta cell glucose sensitivity
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide 1
GSIS	Glucose-stimulated insulin secretion
ISO	Isoglycaemic glucose infusion
ISR	Insulin secretion rate
L-ISO	Lipid infusion/ISO
L-OGTT	Lipid infusion/OGTT
OGIS	OGTT-derived index of insulin sensitivity
P_{GLU}	Glucose-induced potentiation
P_{INCR}	Incretin-induced potentiation
TIS	Total insulin secretion

Research in context

What is already known about this subject?

- The incretin effect is impaired in dysglycaemic states
- In human islets in vitro, NEFA interfere with incretin function

What is the key question?

- How does acute bidirectional manipulation of plasma NEFA impact on the incretin effect in humans?

What are the new findings?

- NEFA elevation selectively impairs the incretin effect, but does not change beta cell function in healthy volunteers
- In participants with type 2 diabetes, acute NEFA reduction does not correct the impairment in incretin-induced potentiation of glucose-stimulated insulin secretion

How might this impact on clinical practice in the foreseeable future?

- The impairment of the incretin effect by an increase in NEFA could be important to the pathophysiology of diabetes. However, the possible therapeutic benefits of NEFA reduction on the incretin effect in type 2 diabetic individuals must be further investigated

Introduction

Previous studies in vitro [1, 2] and in intact animals [3] have consistently shown that acute exposure to NEFA results in a substantial increase in both basal and glucose-stimulated insulin secretion (GSIS). Studies in humans, however, have yielded contrasting results. Acute (3–12 h) lipid infusions in normal participants have been reported to have no effect [4], to increase [5–7] or to inhibit [8] GSIS. In participants with [9] or without [10] family history of type 2 diabetes, a physiological rise in NEFA induced a mild deterioration of beta cell function, which was more evident when insulin secretion was examined in relation to the prevailing insulin resistance. In the same individuals, lowering plasma NEFA with acipimox was associated with an improvement in the acute insulin response to i.v. glucose [10, 11]. Different approaches to assessing GSIS, different types of test, adjustment for the insulin sensitivity and genetic predisposition might influence the controversial results, as reviewed by Giacca et al [12]. Incretin-mediated insulin secretion accounts for about 40–80% [13] of total insulin secretion (TIS) in response to an oral stimulus and is impaired in type 2 diabetic individuals and in those with some degree of glucose intolerance [14–17]. However, surprisingly little information is available on the effect of NEFA on incretin-stimulated insulin secretion in humans, despite recent evidence in human islets that NEFA might interfere with incretin function [18, 19].

We designed this study to test: (1) whether incretin-induced potentiation of insulin secretion is reduced by acute elevation of plasma NEFA levels in healthy volunteers; and (2) whether, in participants with type 2 diabetes, the impaired incretin effect can be improved by an acute reduction in circulating NEFA. The incretin effect was measured as the potentiation of glucose-induced insulin release selectively caused by the oral vs i.v. glucose route of entry. Plasma NEFA levels were acutely raised in non-diabetic volunteers through an i.v. lipid infusion and lowered in type 2 diabetic participants by the administration of acipimox.

Methods

The study was carried out at two centres (University of Pisa, Italy, and State University of Campinas, Brazil). Fifteen individuals with type 2 diabetes treated with diet or stable doses of metformin were recruited from the outpatient clinic of the Catholic University of Campinas and State University of Campinas. Inclusion criteria were: age 18–60 years, HbA_{1c} 47.5–85.8 mmol/mol (6.5–10.0%), BMI <40 kg/m^2, no other endocrine disease, no clinical evidence of neoplastic or chronic inflammatory disease, or major organ (liver, kidney, heart, pulmonary) failure. Two participants with diabetes were excluded: one dropped out after the first test, the other was excluded at the end of the study when it was realised that the participant had an HbA_{1c} of 42.1 mmol/mol [6.0%], lower than the lower limit of the protocol; thus, 13 diabetic individuals were included in the final analysis. Ten healthy volunteers were enrolled at the University of Pisa. Participants gave their informed consent, and the study was approved by the Ethics Committees (Protocol number 23474 at the Pisa centre; Certificate of Presentation for Ethical Appreciation, CAAE, 48181215.2.0000.5404 at the Campinas centre). The

Table 1 Anthropometric and metabolic characteristics of the study participants

Characteristic	Healthy	Type 2 diabetes
n (women/men)	10 (4/6)	13 (10/3)
Age (years)	34 ± 11	55 ± 8
Duration (<1/<10/≥10 years)	-	2/7/4
Body weight (kg)	68 ± 22	88 ± 16
BMI (kg/m^2)	23.8 ± 5.9	32.8 ± 5.6
Fat-free mass (kg)	52 ± 15	51 ± 10
Fat mass (%)	23 ± 3	39 ± 6
Energy expenditure (kJ/24 h)	6592 ± 548	6772 ± 302
HbA$_{1c}$ (mmol/mol)	–	55.2 ± 4.3
HbA$_{1c}$ (%)	–	7.2 ± 0.5
Triacylglycerols (mmol/l)	1.05 ± 0.27	1.77 ± 0.74
Insulin sensitivity (ml min^{-1} m^{-2})	402 ± 22	291 ± 60

Data are mean ± SD

anthropometric and metabolic characteristics of the study participants are summarised in Table 1.

Study protocol All participants had two control tests, a 3 h OGTT (75 g) and, on a separate occasion ~7 days later, a 3 h isoglycaemic glucose infusion (ISO) using an ad hoc algorithm to exactly reproduce the glycaemic curve observed during the OGTT (isoglycaemic protocol). In the healthy volunteers, both tests were repeated during an i.v. infusion of a 20% (vol./vol.) lipid/water emulsion (60 ml/h) together with a primed (200 U) continuous (0.4 U kg^{-1} min^{-1}) heparin infusion, started 2 h before either test (lipid infusion/OGTT [L-OGTT] and lipid infusion/ISO [L-ISO]). In participants with diabetes, the two tests were repeated after the ingestion of acipimox (Olbetam, Pfizer, Rome, Italy) (250 mg) 2 h before glucose administration and 1 h into each study (OGTT following acipimox [Acp-OGTT], ISO following acipimox [Acp-ISO]).

Measurements Fat-free mass was measured by electrical bioimpedance at both sites. Bedside plasma glucose concentrations were measured at 10 min intervals throughout the studies using the glucose oxidase technique (YSI 2300 Stat Plus Glucose Analyser, Yellow Springs, OH, USA, at the University of Campinas; Beckman Glucose Analysers, Fullerton, CA, USA, at the University of Pisa); plasma insulin and C-peptide concentrations were all measured by chemiluminescence (on a COBAS e411 instrument, Roche, Indianapolis, IN, USA). In both centres, total plasma glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) were assayed using the same ELISA kit (Merck
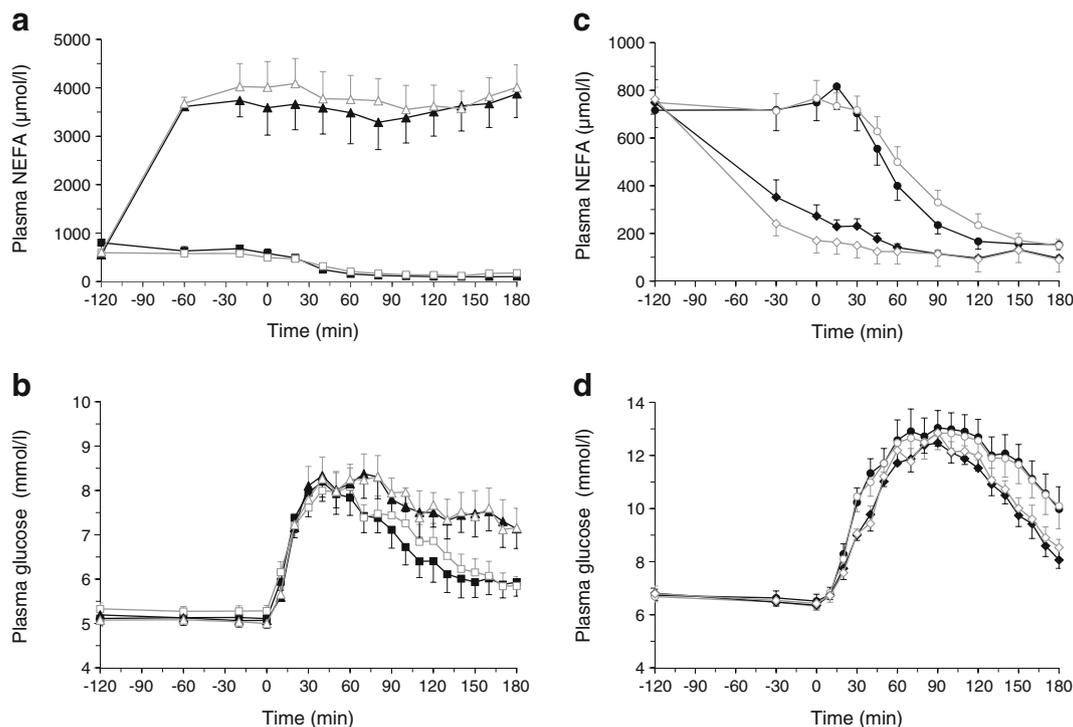

Fig. 1 Plasma NEFA (**a**) and glucose concentrations (**b**) in non-diabetic volunteers in OGTT and ISO control studies and with lipid infusion. Black squares, OGTT; white squares, ISO; black triangles, L-OGTT; white triangles, L-ISO. Plasma NEFA (**c**) and glucose concentrations (**d**) in participants with type 2 diabetes in OGTT and ISO control studies and after acipimox ingestion. Black circles, OGTT; white circles, ISO; black diamonds, Acp-OGTT; white diamonds, Acp-ISO. Data are presented as mean ± SEM for $n = 10$ non-diabetic participants and $n = 13$ type 2 diabetic participants

Fig. 2 TIS (**a,e**), β-GS (**b,f**), P_{INCR} (**c,g**) and rate sensitivity (**d,h**) in non-diabetic volunteers (**a–d**) and participants with type 2 diabetes (**e–h**). *$p < 0.05$ for ISO vs OGTT, L-ISO vs L-OGTT or Acp-ISO vs Acp-OGTT. †$p < 0.05$ for treatment (lipid or acipimox) vs corresponding (OGTT or ISO) control study. Data are presented as mean ± SEM for $n = 10$ non-diabetic participants and $n = 13$ type 2 diabetic participants
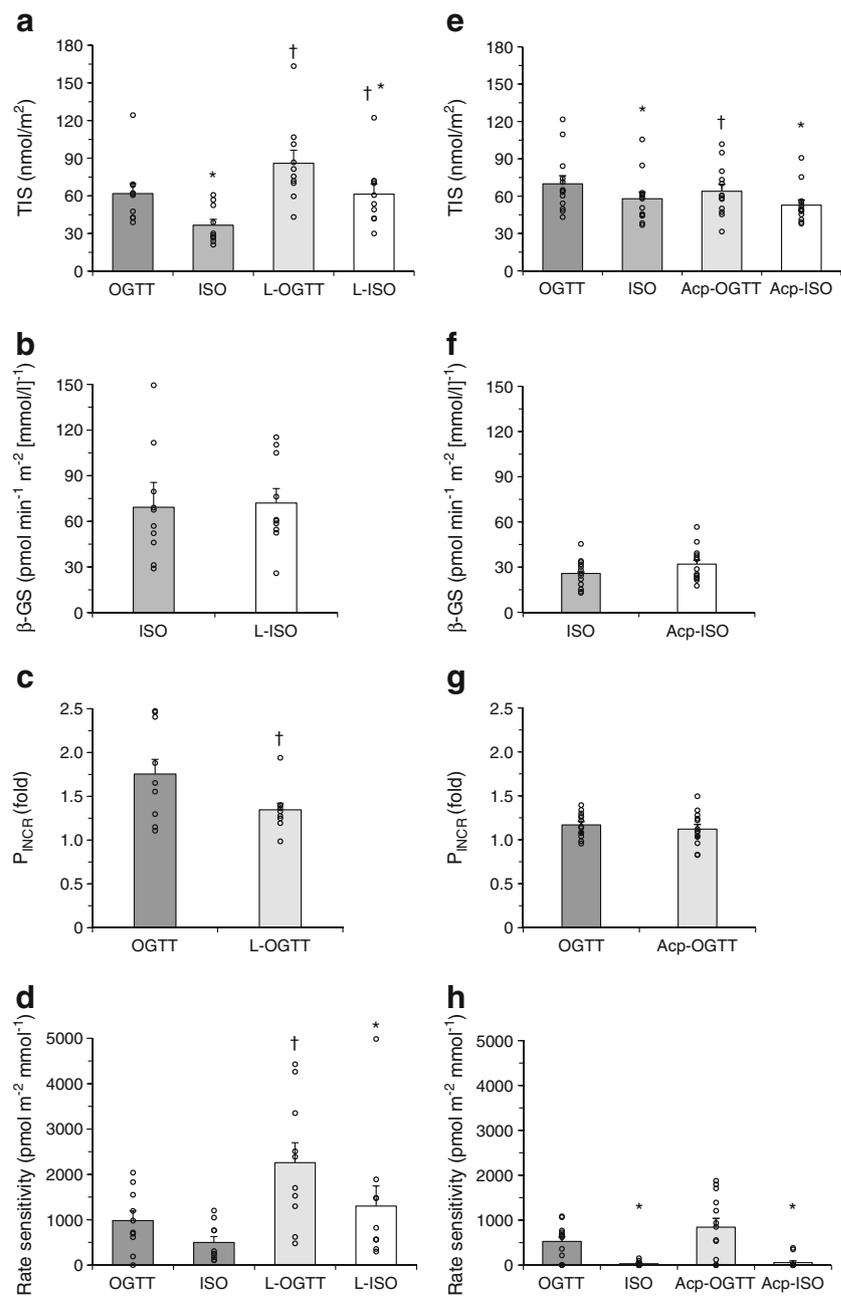
Millipore, Billerica, MA, USA), plasma NEFA by a standard colorimetric method (WAKO Chemicals, Neuss, Germany), and plasma glucagon by ELISA (RD System, Minneapolis, MN, USA; Campinas) and by Mercodia Glucagon ELISA (Mercodia, Uppsala, Sweden; Pisa).

Data analysis Insulin sensitivity was estimated from the plasma glucose and insulin responses to oral glucose using the OGTT-derived index of insulin sensitivity (OGIS) [20]. AUCs were calculated using the trapezoid rule. Plasma C-peptide deconvolution was used to calculate insulin secretion rate (ISR) and TIS, and mathematical modelling to quantify

beta cell function and the incretin effect [21]. The main variables in the beta cell model are glucose sensitivity (β-GS), i.e. the slope of the ISR/glucose dose–response curve obtained during the ISO test. Glucose-induced potentiation (P_{GLU}), a time-dependent modulation of the dose–response calculated during the ISO test, was parameterised as the ratio of the value at 3 h to the fasting value; incretin-induced potentiation (P_{INCR}) was calculated as the time-dependent fold ISR increment during OGTT compared with ISO glucose, and was parameterised as its integral over the 3 h of the tests. The incretin effect was also calculated using conventional model-independent indices (per cent difference ([[OGTT TIS − ISO

TIS)/OGTT TIS] × 100) and the ratio (OGTT TIS/ISO TIS) between oral and i.v. glucose insulin secretory response), as described by Nauck et al [13, 14].

Statistical analysis Data from healthy participants and those with diabetes were analysed separately. Within-group treatment responses were analysed by paired t test or Wilcoxon signed rank test for normally or non-normally distributed variables, respectively. A p value ≤0.05 was considered statistically significant.

Results

Lipid infusion in non-diabetic volunteers While plasma NEFA levels were markedly suppressed during saline (154 mmol/l NaCl) infusion, exogenous lipid infusion increased mean plasma NEFA from 0.20 ± 0.08 to 3.55 ± 1.29 mmol/l during the L-OGTT, and from 0.23 ± 0.08 to 3.77 ± 1.10 mmol/l during the L-ISO study ($p < 0.001$ for both) (Fig. 1a). Correspondingly, mean triacylglycerol rose from 0.7 ± 0.3 to 3.1 ± 1.3 mmol/l and from 0.6 ± 0.2 to 3.2 ± 1.3 mmol/l during the L-OGTT and L-ISO, respectively ($p < 0.005$ for both). Plasma glucose profiles were matched between the oral and i.v. studies. Lipid infusion caused a marked deterioration of glucose tolerance (glucose incremental AUC 0.44 ± 0.04 vs 0.30 ± 0.03 mol/l × h, $p < 0.05$) that was particularly evident

during the second half of the test (Fig. 1b). As expected, total insulin release was greater with oral than i.v. glucose with both saline (Figs 2a, 3a) and lipid (Figs 2a, 3b); in addition, the relative hyperglycaemia induced by lipid infusion produced an increase in ISR and TIS, which was particularly pronounced (and statistically significant) during i.v. glucose administration (Table 2 and Fig. 2a). Neither β-GS (Fig. 2b) nor glucose potentiation (P_{GLU}) (Fig. 4) were significantly affected by the rise in NEFA, while rate sensitivity was approximately doubled (Table 2 and Fig. 2d) in both the oral and i.v. tests. In contrast, lipid infusion induced a marked decrease in incretin potentiation (P_{INCR}) (Table 2; Figs 2c, 4b), as well as in incretin effect calculated by conventional formulae (Table 2).

Response to oral glucose of plasma glucagon (0.76 ± 0.34 vs 0.68 ± 0.25 mol/l × h, $OGTT_{AUC}$ vs $L\text{-}OGTT_{AUC}$, respectively) (data not shown), GLP-1 (7.8 ± 2.2 vs 7.6 ± 2.1 nmol/l × h, respectively) and GIP (9.4 ± 1.1 vs 9.4 ± 1.4 nmol/l × h, respectively) were not different (electronic supplementary material [ESM] Fig. 1). Lipid infusion induced mild insulin resistance (OGIS = 355 ± 28 vs 402 ± 22 ml min^{-1} m^{-2}, L-OGTT vs OGTT, respectively, $p < 0.01$).

Type 2 diabetic participants Plasma glucose profiles were superimposable on the paired oral and the i.v. tests. Acipimox administration was associated with a marked decrease in NEFA AUC ($55 \pm 14\%$, $p < 0.01$), which was similar
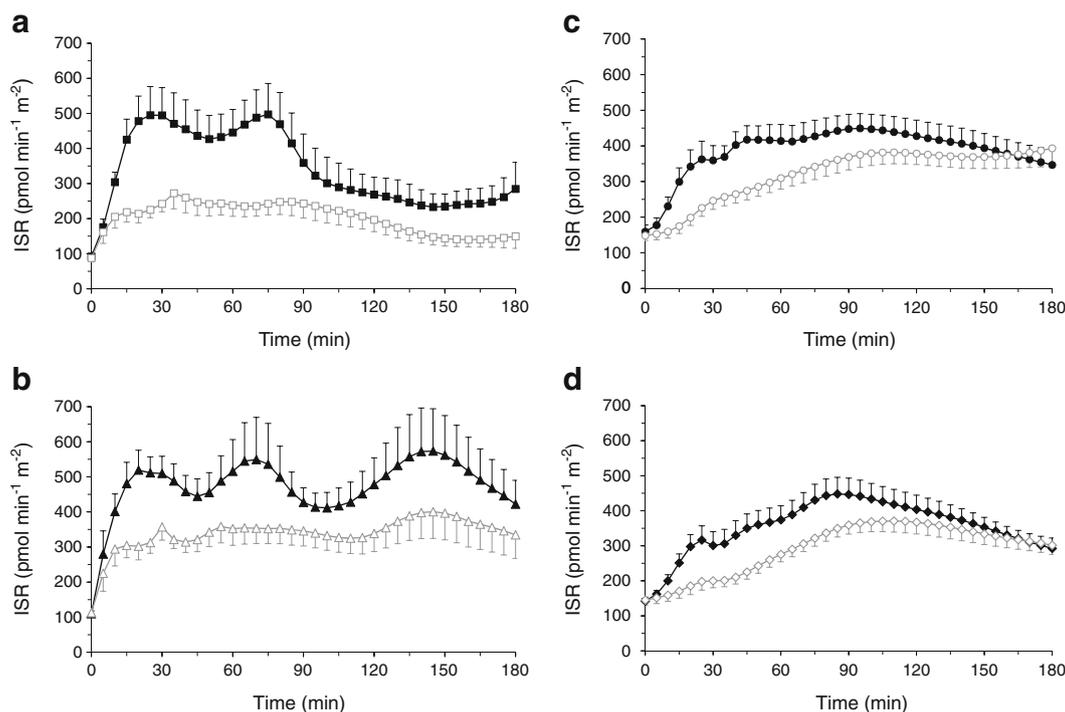

Fig. 3 ISR in non-diabetic volunteers during control studies, OGTT and corresponding ISO test (**a**), and during the lipid infusion studies, L-OGTT and L-ISO (**b**). Black squares, OGTT; white squares, ISO; black triangles, L-OGTT; white triangles, L-ISO. ISR in people with type 2 diabetes during (**c**) control studies and after acipimox ingestion (**d**). Black circles, OGTT; white circles, ISO; black diamonds, Acp-OGTT; white diamonds, Acp-ISO. Data are presented as mean ± SEM for $n = 10$ non-diabetic participants and $n = 13$ type 2 diabetic participants

Table 2 Beta cell function in non-diabetic volunteers and individuals with type 2 diabetes

Variable	OGTT	ISO	L-OGTT	L-ISO
No diabetes				
Fasting plasma glucose (mmol/l)	5.11 ± 0.28	5.33 ± 0.42[*]	5.19 ± 0.50	5.08 ± 0.41[†]
Fasting NEFA (μmol/l)	630 ± 121	595 ± 152[*]	554 ± 109[†]	607 ± 310
Fasting ISR (pmol min^{-1} m^{-2})	81 [19]	73 [43]	103 [25]	105 [24][††]
TIS (nmol/m^2)	61 [26]	29 [26][**]	78 [31][†]	57 [30][**, †]
β-GS (pmol min^{-1} m^{-2} [mmol/l]$^{-1}$)	–	62 [34]	–	61 [50]
Rate sensitivity (nmol m^{-2} mM^{-1})	0.85 [0.94]	0.28 [0.59]	2.02 [2.05][†]	0.70 [0.92][*]
P$_{GLU}$ (fold)	–	1.04 [0.38]	–	1.19 [0.73]
P$_{INCR}$ (fold)[a]	1.61 [1.10]		1.30 [0.10][†]	
Incretin effect (ratio)[a]	1.79 [0.69]		1.41 [0.21][†]	
Incretin effect (%)[a]	44 [19]		29 [12][†]	
Type 2 diabetes	OGTT	ISO	Acp-OGTT	Acp-ISO
Fasting plasma glucose (mmol/l)	6.91 ± 0.97	6.97 ± 0.91	6.78 ± 0.74	6.91 ± 0.86
Fasting NEFA (μmol/l)	716 ± 262	749 ± 231	748 ± 348	763 ± 349
Fasting ISR (pmol min^{-1} m^{-2})	129 [40]	134 [39]	130 [43]	113 [53]
TIS (nmol/m^2)	64 [24]	58 [17][**]	60 [26][†]	49 [10][**]
β-GS (pmol min^{-1} m^{-2} [mmol/l]$^{-1}$)	–	26 [14]	–	29 [15]
Rate sensitivity (nmol m^{-2} mmol^{-1})	0.65 [0.58]	0.23 [0.04][**]	0.85 [1.39]	0.00 [0.00][**]
P$_{GLU}$ (fold)	–	1.61 [0.82]	–	1.38 [0.50]
P$_{INCR}$ (fold)[a]	1.15 [0.21]		1.10 [0.24]	
Incretin effect (ratio)[a]	1.22 [0.26]		1.22 [0.35]	
Incretin effect (%)[a]	18 [18]		18 [24]	

Data are mean ± SD or median [interquartile range]

[a] These values were determined using oral and i.v. glucose results. For a further explanation of these variables, please refer to the 'Data analysis' section of the Methods

[*] $p < 0.05$ and [**] $p < 0.01$ by Wilcoxon test for oral vs i.v. glucose: ISO vs OGTT in non-diabetic and in type 2 diabetic participants; L-ISO vs L-OGTT in non-diabetic and Acp-ISO vs Acp-OGTT in type 2 diabetic participants

[†] $p < 0.05$ and [††] $p < 0.01$ by Wilcoxon test for the comparison of lipid infusion or acipimox vs respective control study

in the oral and i.v. tests (Fig. 1c), and an improved glucose tolerance (glucose AUC = 0.74 ± 0.16 vs 0.90 ± 0.26 mol/l × h, $p = 0.04$) (Fig. 1d). Lowering plasma NEFA with acipimox reduced TIS during the OGTT, without significant changes in β-GS or rate sensitivity (Table 2 and Fig. 2e,h). Similarly, in the control (Fig. 3c) and the acipimox study (Fig. 3d), insulin secretion was lower in response to i.v. than oral glucose, particularly during the first 2 h of the tests. Neither P$_{GLU}$ (Fig. 4c) nor P$_{INCR}$ (Figs 2g,4d) (or the incretin effect indices [Table 2]) were changed by acipimox. P$_{INCR}$ was ~30% lower in these participants with diabetes than in the control group ($p < 0.05$).

Acipimox administration did not change the AUCs of plasma glucagon (4.58 ± 0.92 vs 4.47 ± 0.63 nmol/l × h) (data not shown), GLP-1 (9.0 ± 3.2 vs 9.2 ± 4.3 nmol/l × h) or GIP (16.9 ± 7.4 vs 16.3 ± 5.5 nmol/l × h) (all p = NS) (ESM Fig. 1). Insulin sensitivity was slightly, but significantly, improved by acipimox (OGIS: 326 ± 44 vs 291 ± 60 ml min^{-1} m^{-2}, $p = 0.01$).

In the data from the diabetes group, changes in ISR were directly related to changes in NEFA ($ρ = 0.62$, $p = 0.03$).

Discussion

In this study, we show that the acute effects of NEFA on insulin secretion are asymmetric. While in non-diabetic volunteers NEFA elevation caused a marked impairment of incretin-induced potentiation, in individuals with type 2 diabetes the impaired incretin effect was not improved by acutely lowering plasma NEFA. Of note, in the non-diabetic participants raising NEFA induced all the expected metabolic changes [9, 22]: mild deterioration of glucose tolerance and insulin sensitivity, increased insulin secretion and enhanced early insulin response (i.e. rate sensitivity) without any changes in β-GS. Conversely, acipimox administration in diabetic participants was associated with reduced insulin secretion and plasma glucose excursions and improved insulin sensitivity, but neither beta cell glucose sensitivity nor incretin-induced potentiation was changed. Of interest is that the effects of plasma NEFA modulation were observed in both the oral and i.v. tests; this rules out a role for the entero–insular axis or the
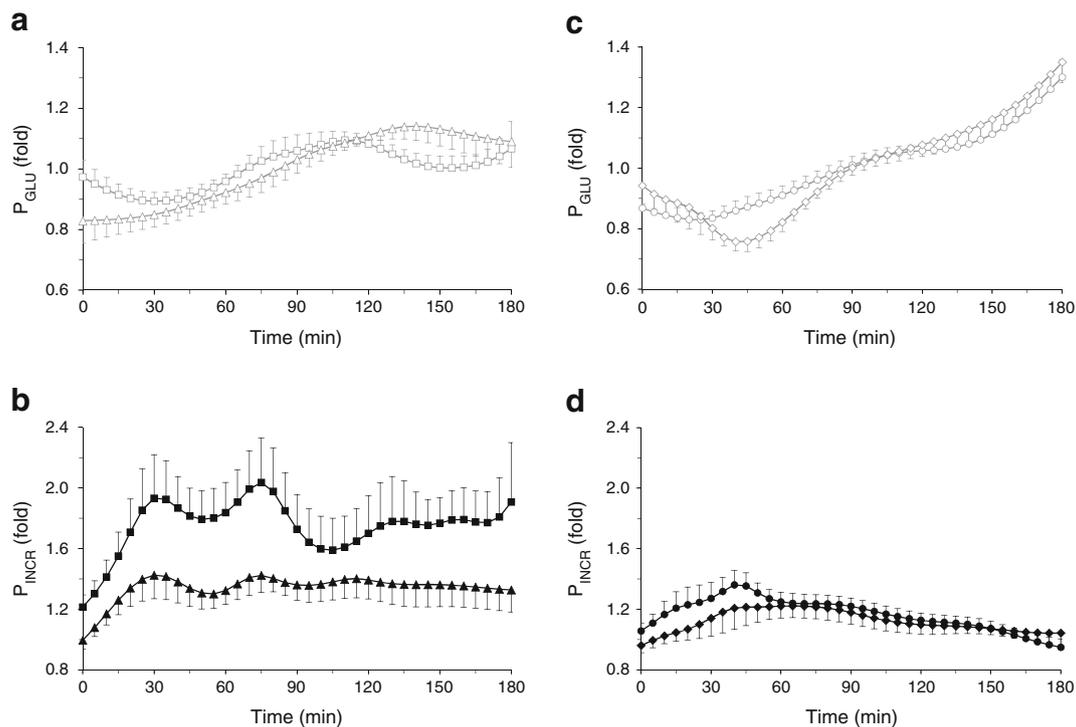
Fig. 4 P_{GLU} during ISO test and L-ISO in non-diabetic volunteers (**a**), and during ISO test and Acp-ISO study in participants with type 2 diabetes (**c**). P_{INCR} during the control studies and during the lipid infusion studies in non-diabetic volunteers (**b**) and during control studies and acipimox studies in participants with type 2 diabetes (**d**). Non-diabetic participants: white squares, ISO; white triangles, L-ISO; black squares, OGTT; black triangles, L-OGTT. Diabetic participants: white circles, ISO; white diamonds, Acp-ISO; black circles, OGTT; black diamonds, Acp-OGTT. Data are presented as mean ± SEM for $n = 10$ non-diabetic participants and $n = 13$ type 2 diabetic participants

taste receptors/cerebral–insular axis. In support of this, neither lipid infusion nor acipimox was associated with any changes in plasma glucagon, GLP-1 or GIP responses in the participants with or without diabetes. The absence of short-term changes in gut hormones agrees with studies in normal volunteers subjected to 1 week of high-fat overeating, in whom glucose tolerance deteriorated, plasma insulin increased but GLP-1 and GIP responses were not modified [23].

Taken together, these findings make it possible to conclude that the direction of the impact of NEFA on incretin effect depends, essentially, on beta cell function: raising NEFA can impair the incretin effect on a normal beta cell but lowering NEFA cannot rescue the incretin effect on a dysfunctional beta cell. In line with the latter result, lipid-induced impairment of beta cell function is not observed in frankly diabetic animals or humans [12]. Obviously, our conclusions apply only to acute changes in NEFA concentration and to the experimental conditions created by these protocols, i.e. they provide proof of concept. In the longer term, the impact of NEFA on beta cell function may be more heterogeneous. For example, Kashyap et al [9] reported that in normal participants a prolonged (4 day) lipid infusion led to mild meal-induced hyperglycaemia and insulin hypersecretion, which was associated with a marked improvement in first-phase insulin secretion. In contrast, opposite responses were observed in

individuals with a family history of diabetes [9]. However, in a similar study, 48 h acipimox treatment improved the acute insulin response to i.v. glucose in individuals with a family history of type 2 diabetes [24]. It must be observed, however, that these studies did not directly assess the incretin effect, which, by definition, requires performing an isoglycaemic protocol. On the other hand, it is pertinent to recall that chronic treatment with a dipeptidyl peptidase IV (DPP-IV) inhibitor, sitagliptin [25] or metformin [26], in diabetic individuals did not improve the incretin effect.

The current results align with those of a previous study carried out in a large cohort of non-diabetic individuals [27], which indicated that relatively higher NEFA levels, such as are typically found in type 2 diabetic individuals, are associated with enhanced absolute ISRs and reduced insulin action, but they do not interfere with β-GS, which is the strongest determinant of glucose tolerance.

Several cellular mechanisms may be involved in the impairment of incretin function caused by NEFA exposure. Kang et al reported that, in rat insulinoma INS-1E cells and in isolated islets of *db/db* mice, palmitate decreases the expression and levels of the GLP-1 receptor; this was associated to impairment of: cAMP production, protein phosphorylation of cAMP-responsive elements binding protein (p-CREB) and insulin secretion [18]. A recent very elegant study investigated

the effect of palmitate on the recruitment, by GLP-1, of the beta cell network, which is essential to synchronise rapid increases in glucose-induced insulin secretion. Gap junctions in human beta cells, through connexin 36 (Cx36), are important for incretin-stimulated but not for GSIS [19]. In these human islet preparations, palmitate reduced Cx36 expression and the coordinated secretory activity in response to GLP-1 and GIP, thereby reducing insulin release. Thus, elevated NEFA might disrupt the GLP-1-sensitive syncytium. Chronic mechanisms may involve accumulation of malonyl-coenzyme A (CoA) and long-chain fatty acyl-CoAs, increased fatty acid esterification and activation of endoplasmic reticulum stress in beta cells (reviewed in Giacca et al [12] and Poitout and Robertson [28]). Decreased free fatty acid receptor 1 (GPR40) expression in the beta cell, or its downregulation by NEFA, may contribute to the impaired incretin action observed in diabetes. It is noteworthy that our diabetic participants had higher fasting glucose and marginally higher fasting NEFA concentrations (700 [352] vs 588 [220], $p = 0.12$), a combination that seems to be synergistically toxic to islets [12], where it downregulates GPR40 expression in all cell types [29]. Similar insulin secretion impairment was observed in human islets exposed to palmitate or oleate for 48 h [30]. GPR40 is highly expressed in islets from young normoglycaemic, hyperlipidaemic and prediabetic Zucker diabetic fatty (ZDF) rats (*fa/fa*) even before diabetes development [29]. Furthermore, in rats maintained under hyperglycaemic conditions for 96 h, GLP-1 receptor expression is reduced, and GIP receptor expression is increased [31]. While these mechanisms, alone or in combination, may underlie the effect of raising NEFA into the millimolar range we observed in our non-diabetic participants, a role for the mild but chronic NEFA elevations, commonly found in individuals with type 2 diabetes, to the loss of incretin effect remains to be conclusively demonstrated. The absence of changes in gut hormones across our NEFA manipulations implies that an impaired incretin effect—whether induced in non-diabetic participants or spontaneous in diabetes—is coupled with a degree of cellular resistance to incretins [32]. Interestingly, in a recent study in type 2 diabetic individuals, the clinical efficacy of liraglutide, a GLP-1 receptor agonist, was related to the baseline plasma triacylglycerols and C-peptide levels [33].

Limitations and strengths These studies were carried out in two centres and the general phenotypes of the two participant groups were different. We chose a very healthy group to test the effects of NEFA, and typical individuals with type 2 diabetes to evaluate a possible improvement in incretin effect after NEFA reduction. The results were not meant to be compared across groups but within groups. Another limitation is the sample size, but the incretin effect, i.e. the main outcome variable of the protocol, was very different after lipid infusion in the non-diabetic participants, and very similar after

acipimox ingestion in the diabetic participants. On the other hand, this is the first study to evaluate the effect of NEFA on the incretin effect in humans with an appropriate protocol. The mathematical model yielded the dynamic characteristics of beta cell function, including the time course of glucose potentiation and the incretin potentiation of insulin secretion.

References

1. Randle PJ, Garland PB, Hales CN, Newsholme EA (1963) The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785–789

2. Boden G, Chen X, Iqbal N (1998) Acute lowering of plasma NEFA lowers basal insulin secretion in diabetic and non diabetic subjects. Diabetes 47:1609–1612

3. Sako Y, Grill VE (1990) A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and β-cell oxidation through a process likely coupled to fatty acid oxidation. Endocrinology 127:1580–1589

4. Amery CM, Round RA, Smith JM, Nattrass M (2000) Elevation of plasma fatty acids by ten-hour intralipid infusion has no effect on basal or glucose-stimulated insulin secretion in normal man. Metabolism 49:450–454

5. Felber JP, Vannotti A (1964) Effects of fat infusion on glucose tolerance and insulin plasma levels. Med Exp Int J Exp Med 10: 153–156

6. Pelkonen R, Miettinen TA, Taskinen MR, Nikkila EA (1968) Effect of acute elevation of plasma glycerol, trygliceride and NEFA levels on glucose utilization and plasma insulin. Diabetes 17:76–82

7. Paolisso G, Gambardella A, Amato L et al (1995) Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. Diabetologia 38:1295–1299

8. Boden G, Chen X, Rosner J, Baron M (1995) Effects of a 48-hour fat infusion on insulin secretion and glucose utilization. Diabetes 44:1239–1242

9. Kashyap S, Belfort R, Gastaldelli A et al (2003) A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop Type 2 diabetes. Diabetes 52:2461–2474

10. Salgin B, Marcovecchio ML, Humphreys SM et al (2009) Effects of prolonged fasting and sustained lipolysis on insulin secretion and insulin sensitivity in normal subjects. Am J Physiol Endocrinol Metab 296:E454–E461

11. Paolisso G, Tagliamonte MR, Rizzo MR et al (1998) Lowering fatty acids potentiates acute insulin response in first degree relatives of people with type II diabetes. Diabetologia 41:1127–1132

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

13. Nauck MA, Homberger E, Siegel E et al (1986) Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 63:492–498

14. Nauck M, Stöckmann F, Ebert R, Creutzfeldt W (1986) Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia 29:46–52

15. Muscelli E, Mari A, Natali A et al (2006) Impact of incretin hormones on β-cell function in subjects with normal or impaired glucose tolerance. Am J Physiol Endocrinol Metab 291:E1144–E1150

16. Muscelli E, Mari A, Casolaro A et al (2008) Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. Diabetes 57:1340–1348

17. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ (2011) Secretion of glucagon-like peptide-1 (GLP-1) in T2D: what is up, what is down? Diabetologia 54:10–18

18. Kang ZF, Deng Y, Zhou Y et al (2013) Pharmacological reduction of NEFA restores the efficacy of incretin-based therapies through GLP-1 receptor signalling in the β-cell in mouse models of diabetes. Diabetologia 56:423–433

19. Hodson DJ, Mitchell RK, Bellomo LA et al (2013) Lipotoxicity disrupts incretin-regulated human β-cell connectivity. J Clin Invest 123:4182–4194

20. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ (2001) A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 24:539–548

21. Tura A, Muscelli E, Gastaldelli A, Ferrannini E, Mari A (2014) Altered pattern of the incretin effect as assessed by modelling in individuals with glucose tolerance ranging from normal to diabetic. Diabetologia 57:1199–1203

22. Ferrannini E, Barrett EJ, Bevilacqua S, DeFronzo RA (1983) Effect of fatty acids on glucose production and utilization in man. J Clin Invest 72:1737–1747

23. Parry SA, Smith JR, Corbett TR, Woods RM, Hulston CJ (2017) Short-term, high-fat overfeeding impairs glycaemic control but does not alter gut hormone responses to a mixed meal tolerance test in healthy, normal-weight individuals. Br J Nutr 117:48–55

24. Cusi K, Kashyap S, Gastaldelli A, Bajaj M, Cersosimo E (2007) Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to T2D. Am J Physiol Endocrinol Metab 292: E1775–E1781

25. Muscelli E, Casolaro A, Gastaldelli A et al (2012) Mechanisms for the antihyperglycemic effect of sitagliptin in patients with type 2 diabetes. J Clin Endocrinol Metab 97:2818–2826

26. Vardarli I, Arndt E, Deacon CF, Holst JJ, Nauck MA (2014) Effects of sitagliptin and metformin treatment on incretin hormone and insulin secretory responses to oral and "isoglycemic" intravenous glucose. Diabetes 63:663–674

27. Rebelos E, Seghieri M, Natali A et al (2015) Influence of endogenous NEFA on beta cell function in humans. Diabetologia 58:2344–2351

28. Poitout V, Robertson RP (2008) Glucolipotoxicity: Fuel excess and β-cell dysfunction. Endocr Rev 29:351–366

29. Meidute Abaraviciene S, Muhammed SJ, Amisten S, Lundquist I, Salehi A (2013) GPR40 protein levels are crucial to the regulation of stimulated hormone secretion in pancreatic islets. Lessons from spontaneous obesity-prone and non-obese type 2 diabetes in rats. Mol Cell Endocrinol 381:150–159

30. Zhou YP, Grill V (1995) Long term exposure to fatty acids and ketones inhibits B cell functions in human pancreatic islets of Langerhans. J Clin Endocrinol Metab 80:1584–1590

31. Xu G, Kaneto H, Laybutt DR et al (2007) Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. Diabetes 56:1551–1558

32. Tura A, Bagger JI, Ferrannini E et al (2017) Impaired beta cell sensitivity to incretins in type 2 diabetes is insufficiently compensated by higher incretin response. Nutr Metab Cardiovasc Dis 27: 1123–1129

33. Tanabe A, Kaneto H, Kamei S et al (2016) Clinical effects of liraglutide are possibly influenced by hypertriglyceridemia and remaining pancreatic β-cell function in subjects with type 2 diabetes mellitus. J Diabetes Complicat 30:1201–1203