

# Beta cell compensation for insulin resistance in Zucker fatty rats: increased lipolysis and fatty acid signalling

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## Abstract

**Aims/hypothesis** The aim of this study was to determine the role of fatty acid signalling in islet beta cell compensation for insulin resistance in the Zucker fatty *fa/fa* (ZF) rat, a genetic model of severe obesity, hyperlipidaemia and insulin resistance that does not develop diabetes.

**Materials and methods** NEFA augmentation of insulin secretion and fatty acid metabolism were studied in isolated islets from ZF and Zucker lean (ZL) control rats.

**Results** Exogenous palmitate markedly potentiated glucose-stimulated insulin secretion (GSIS) in ZF islets, allowing robust secretion at physiological glucose levels (5–8 mmol/l).

Exogenous palmitate also synergised with glucagon-like peptide-1 and the cyclic AMP-raising agent forskolin to enhance GSIS in ZF islets only. In assessing islet fatty acid metabolism, we found increased glucose-responsive palmitate esterification and lipolysis processes in ZF islets, suggestive of enhanced triglyceride–fatty acid cycling. Interruption of glucose-stimulated lipolysis by the lipase inhibitor Orlistat (tetrahydrolipstatin) blunted palmitate-augmented GSIS in ZF islets. Fatty acid oxidation was also higher at intermediate glucose levels in ZF islets and steatotic triglyceride accumulation was absent.

**Conclusions/interpretation** The results highlight the potential importance of NEFA and glucocretin enhancement of insulin secretion in beta cell compensation for insulin resistance. We propose that coordinated glucose-responsive fatty acid esterification and lipolysis processes, suggestive of triglyceride–fatty acid cycling, play a role in the coupling mechanisms of glucose-induced insulin secretion as well as in beta cell compensation and the hypersecretion of insulin in obesity.

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Triglyceride–fatty acid cycling · Zucker fatty rat

## Abbreviations

ACACA	acetyl-CoA carboxylase $\alpha$
ACACB	acetyl-CoA carboxylase $\beta$
CPT1	carnitine palmitoyltransferase-1
ESM	Electronic Supplementary Material
FFAR1	free fatty acid receptor 1
FASN	fatty acid synthase
GLP-1	glucagon-like peptide-1
Gly-3-P	glycerol-3-phosphate

GPAM	mitochondrial glycerol-3-phosphate acyltransferase
GSIS	glucose-stimulated insulin secretion
HSL	hormone-sensitive lipase
LC-CoA	long chain acyl-CoA
NCEH	neutral cholesteryl ester hydrolase
PKA	cyclic AMP-dependent protein kinase
PPARA	peroxisomal proliferator activator receptor- $\alpha$
SREBF1C	sterol-regulatory element binding factor 1c
TG	triglyceride
TG-FA	triglyceride-fatty acid
TGL	triglyceride lipase
ZF	Zucker fatty
ZL	Zucker lean

## Introduction

Normoglycaemia is maintained in the majority of obese insulin-resistant individuals, as their islet beta cells compensate for insulin resistance with insulin hypersecretion [1, 2]. Obesity-associated type 2 diabetes develops when beta cell compensation processes fail [3, 4]. The mechanisms of islet beta cell compensation, however, are poorly understood. Particularly unclear is the role of excess circulating NEFA, which frequently coexists with insulin resistance. Acutely elevated NEFA are well known to enhance glucose-stimulated insulin secretion (GSIS) [3, 5]; however, some investigators believe that persistently elevated NEFA may be detrimental to beta cell function or viability [6, 7]. We have previously utilised the Zucker *fa/fa* fatty (ZF) rat model of insulin resistance in order to study beta cell compensation [8] and have done so again for the present work, which focuses on the role of NEFA. The ZF rat has a mutated leptin receptor and, as a consequence, is hyperphagic, obese, hyperlipidaemic and severely insulin-resistant [8–10]. Importantly, unlike the Zucker diabetic fatty rat, it does not develop diabetes [8–10], which makes it a good model for the investigation of islet beta cell compensation for insulin resistance.

In earlier work, we reported increased islet glucose utilisation and oxidation in ZF compared with control Zucker lean (ZL) islets [8]. Increased glucose oxidation should contribute to the compensatory hyperinsulinaemia in ZF rats by increasing the activity of the  $K_{ATP}$  channel/ $Ca^{2+}$  pathway of nutrient–insulin secretion coupling [11]. We also showed increased activities of enzymes and increased metabolites of the anaplerotic and malate–pyruvate cycling pathways [8]. As anaplerosis is the pathway by which glucose increases malonyl-CoA levels, these results led us to hypothesise that enhanced glucose-induced increases in malonyl-CoA/long chain acyl-CoA (LC-CoA) signalling may also be involved in ZF islet compensation. This is

based on the malonyl-CoA/LC-CoA model of lipid signalling for insulin secretion that we have described previously [12, 13]. Central to the model is that an increase in glucose, via increasing malonyl-CoA, which inhibits carnitine palmitoyltransferase-1 (CPT1), as was first described by McGarry and Brown [14], switches the partitioning of NEFA in the beta cell away from oxidation towards the accumulation of LC-CoA and fatty acid acylation and esterification products (e.g. diacylglycerol [3, 13–15]), which are proposed to have direct regulatory effects on insulin secretion [3, 12, 13].

Lipolysis is known to be active in the beta cell, and has been implicated in both lipid signalling for insulin secretion [16–18] and, if dysregulated, beta cell failure [19]. However, little is known about its regulation and which lipolytic enzymes are operative. A question addressed in this study was whether activity of the lipolysis arm of beta cell lipid partitioning might be enhanced in situations of chronically increased malonyl-CoA/LC-CoA signalling as a way of protecting islets from excess lipid accumulation [3, 20].

The present study focused on the role of lipid signalling in beta cell compensation in ZF rats. The effects of NEFA in augmenting GSIS, alone and in combination with the incretin hormone glucagon-like peptide-1 (GLP-1), and the major pathways of intracellular fatty acid partitioning, including lipolysis, were studied in isolated islets from ZF and ZL control rats.

## Materials and methods

### Animals and measurement of blood and plasma parameters

Zucker fatty (*fa/fa*, ZF) and Zucker lean (*fa/+* or *+/+*, ZL) rats (Harlan, Indianapolis, IN, USA) were fed standard laboratory chow and were all studied at 10 weeks of age. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Vermont. Tail vein blood sampling was performed between 08.00 and 09.00 h from non-anaesthetised, fed rats for measurement of blood glucose using a glucose monitor (Therasense, Alameda, CA, USA), plasma insulin (Insulin RIA kit; Linco Research, St Charles, MS, USA), plasma triglyceride (TG) (GPO Trinder; Sigma-Aldrich, Saint Louis, MS, USA) and plasma NEFA (NEFA C kit; Wako Chemicals, Neuss, Germany).

### Islet isolation and culture

Fed rats were anaesthetised between 08.00 and 09.00 h with sodium pentobarbital and then killed by exsanguination, and pancreatic islets were isolated as described previously

[21]. Prior to initiating experiments or collecting islets for analysis, islets were rested for 1 h in regular RPMI 1640 medium supplemented with 10% fetal calf serum, 10 mmol/l HEPES (pH 7.4), 1 mmol/l sodium pyruvate, 100 U/ml penicillin and 100 µg/ml streptomycin (RPMI complete) at 11 mmol/l glucose at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

#### Measurement of islet insulin secretion

Insulin secretion was measured in freshly isolated islets from ZL and ZF rats at various concentrations of glucose (3–16 mmol/l) and palmitate (0–0.4 mmol/l) in the absence or presence of 10 nmol/l GLP-1, 5 µmol/l forskolin or 0.2 mmol/l Orlistat (tetrahydrolipstatin), as described in detail in the Electronic Supplementary Material (ESM). Total insulin content of the islets was measured following extraction with acid-ethanol (0.2 mmol/l HCl in 75% ethanol).

#### Determination of islet DNA, protein and triglyceride content

Batches of 15 freshly isolated islets were used for determination of DNA and protein as described previously [18], and batches of 75–100 freshly isolated islets were used for determination of TG [18].

#### Oxidation and esterification of islet fatty acids

Fatty acid oxidation and esterification were measured in isolated islets of ZL and ZF rats using radiolabelled tracers, as described in detail in the ESM.

#### Measurement of islet lipolysis

Lipolysis was measured in freshly isolated islets from ZL and ZF rats as the rate of glycerol release at 3 and 16 mmol/l glucose in the presence or absence of 10 nmol/l GLP-1, 5 µmol/l forskolin or 0.2 mmol/l Orlistat, as described in detail in the ESM.

#### Assays for activities of triglyceride lipase and neutral cholesteryl ester hydrolase

Triglyceride lipase (TGL) and neutral cholesteryl ester hydrolase (NCEH) enzyme activities were determined in islet homogenates from ZL and ZF islets, as described in the ESM.

#### RNA extraction and RT-PCR analysis

RT-PCR analyses of ZF and ZL islets were performed according to methods previously described [21], as detailed in the ESM.

#### Statistical analysis

All results are expressed as mean±SEM. Statistical significance was calculated with Student's *t*-test or, for multiple comparisons, two-way ANOVA with Bonferroni post hoc testing as indicated. A *p* value of <0.05 was considered significant. Prism v.4 (GraphPad Software, San Diego, CA, USA) was used to perform the analyses.

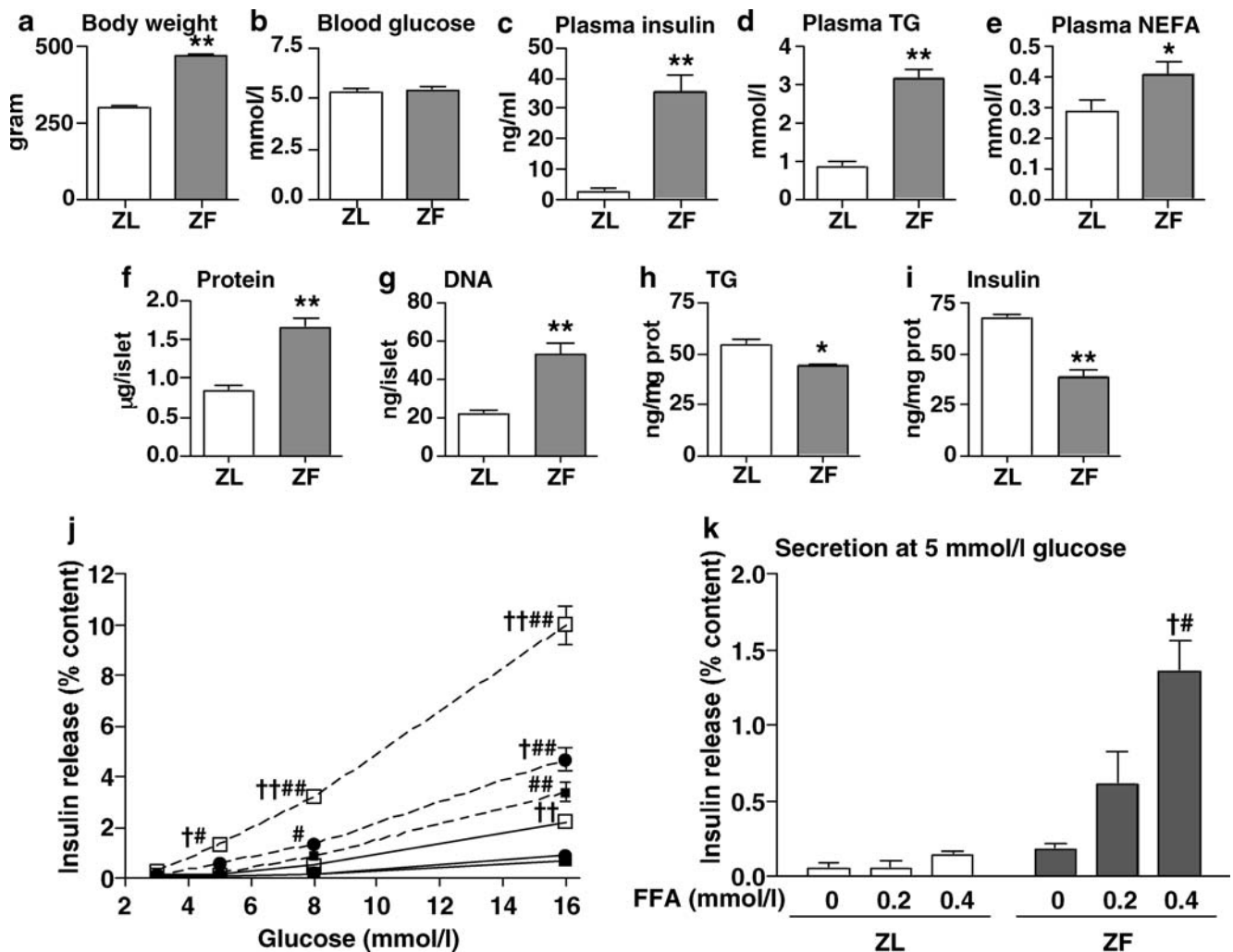
## Results

#### Body weight, fed blood chemistry at 09.00 h and islet parameters

Compared with ZL rats, ZF rats were markedly obese, with severe hypertriglyceridaemia, moderately elevated NEFA levels and a greater than 10-fold increase in insulinaemia (Fig. 1a,c–e). Blood glucose, however, was normal (Fig. 1b). These results are consistent with our previous findings [8] and those of others [9, 10] in the non-diabetic ZF rat, confirming that, in this model, markedly enhanced insulin secretion compensates for the obesity-associated insulin resistance without evidence of beta cell failure. Islets from ZF rats were 2- to 2.5-fold larger than ZL islets according to total protein and DNA contents (Fig. 1f,g). TG content, expressed per mg of islet protein, however, was 19% lower in ZF compared with ZL islets (Fig. 1h). Insulin content, expressed per mg protein, was 43% lower in ZF compared with ZL islets (Fig. 1i).

#### Augmentation of insulin secretion by exogenous NEFA is enhanced in ZF islets

In the absence of exogenous palmitate, GSIS was increased in ZF compared with ZL islets ~5-fold at both 8 and 16 mmol/l glucose (*p*<0.001) (Fig. 1j). Exogenous palmitate at 0.2 mmol/l augmented GSIS at 16 mmol/l glucose by 38% in ZF, but had no effect in ZL islets (Fig. 1j). Exogenous palmitate at the higher concentration of 0.4 mmol/l augmented GSIS at 5, 8 and 16 mmol/l glucose (8-, 4- and 3-fold, respectively) in ZF islets, but in ZL islets increased GSIS by 3-fold at 16 mmol/l glucose only (Fig. 1j). Figure 1k magnifies the insulin secretion data at 5 mmol/l glucose shown in Fig. 1j. Clearly, exogenous palmitate allowed very robust insulin secretion at a physiologically low (5 mmol/l) glucose concentration in ZF islets only. Hence, ZF compared with ZL islets are more sensitive to the effect of exogenous NEFA in augmenting GSIS. Furthermore, the glucose set-point for insulin secretion is lowered by exogenous NEFA in ZF, but not ZL, islets.



**Fig. 1** NEFA augmentation of glucose-stimulated insulin secretion is markedly enhanced in ZF rat islets. **a** Body weight of 10-week-old ZL and ZF rats. Means±SEM of 24 rats per group. \*\* $p<0.001$  compared with ZL. **b–e** Blood glucose, plasma insulin, plasma triglyceride (TG) and plasma NEFA, respectively, in 10-week-old ZL and ZF rats fed at 09.00 h. Means±SEM of 6–20 rats per group. \* $p<0.05$ , \*\* $p<0.001$  compared with ZL. **f–i** ZL and ZF islet protein, DNA, TG and insulin contents. Means±SEM of islets from 5–24 rats per group. \* $p<0.01$ , \*\* $p<0.001$  compared with ZL. **j** Insulin secretion in islets from ZF

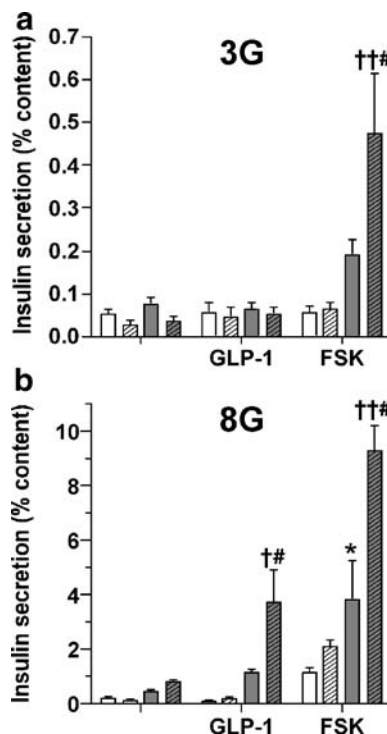
(dashed lines) and ZL (full lines) rats incubated at 3, 5, 8 or 16 mmol/l glucose in the presence of 0 (closed squares), 0.2 (closed circles) or 0.4 (open squares) mmol/l palmitate. **k** Secretion values at 5 mmol/l glucose. Means±SEM (**j**, **k**) of six determinations from islets of six rats of each genotype in two separate experiments. Two-way ANOVA post hoc analyses: # $p<0.01$ , ## $p<0.001$  compared with ZL for the same condition; † $p<0.01$ , †† $p<0.001$  compared with the condition of no palmitate for the same genotype

#### GLP-1 and forskolin synergise with NEFA to augment GSIS in ZF rats

The effects of 10 nmol/l GLP-1 and 5 μmol/l forskolin in augmenting GSIS in the absence and presence of 0.25 mmol/l palmitate were assessed in islets of ZL and ZF rats (Fig. 2). GLP-1, palmitate, and the combination of both, had no effect on insulin secretion at 3 mmol/l glucose in either ZL or ZF islets (Fig. 2a). Lack of effect of GLP-1 and palmitate, alone or in combination, on GSIS was also found at 8 mmol/l glucose in ZL islets (Fig. 2b). In ZF islets at 8 mmol/l glucose, however, palmitate increased GSIS 1.7-fold, GLP-1 increased GSIS 2.6-fold, and

palmitate together with GLP-1 dramatically increased GSIS by 8.8-fold (Fig. 2b). Thus, ZF islets have increased sensitivity to the incretin effect of GLP-1, and this effect is markedly augmented in the presence of palmitate, consistent with synergism in action between GLP-1 and NEFA. Forskolin, an activator of adenylate cyclase that increases islet cyclic AMP levels, was even more potent than GLP-1 in augmenting insulin secretion. Whereas GLP-1 had no effect on insulin secretion at 3 mmol/l glucose in ZL and ZF islets, forskolin did increase insulin secretion in ZF islets (2.5-fold), an effect increased by palmitate (a further 2.5-fold) (Fig. 2a). At 8 mmol/l glucose, forskolin dramatically increased GSIS in ZF islets (8.4-fold), and this



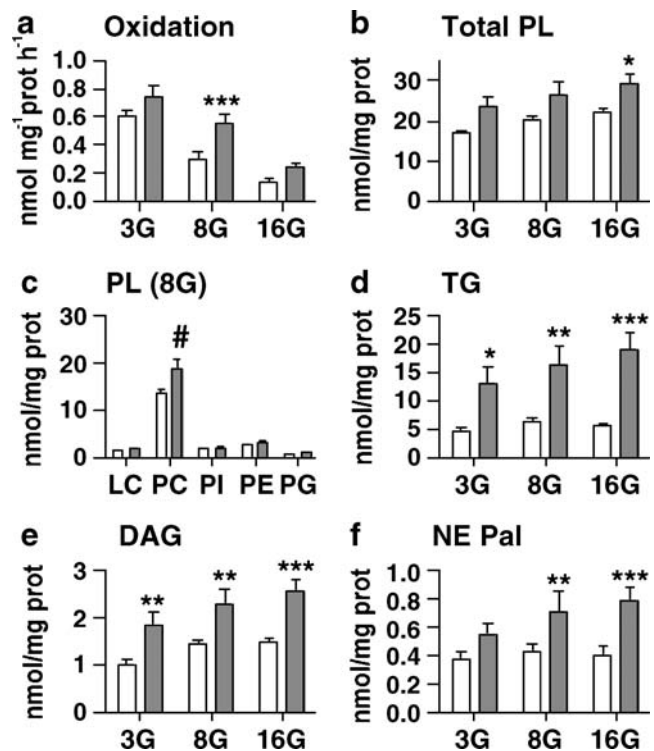


**Fig. 2** NEFA synergise with GLP-1 and forskolin to enhance glucose-stimulated insulin secretion in compensating ZF islets. **a, b** Insulin secretion in islets from ZF (grey bars) and ZL (white bars) islets incubated at **(a)** 3 mmol/l glucose and **(b)** 8 mmol/l glucose in the absence (non-hatched bars) and presence (hatched bars) of 0.25 mmol/l palmitate and 10 nmol/l GLP-1 or 5  $\mu$ mol/l forskolin (FSK). Means $\pm$ SEM of six determinations from islets of six rats of each genotype in two separate experiments. Two-way ANOVA post hoc analyses: \* $p$ <0.01, # $p$ <0.001 compared with ZL for the same condition; † $p$ <0.05, †† $p$ <0.001 compared with ZF for the same condition in the absence of palmitate

effect was increased by palmitate (a further 2.4-fold) (Fig. 2b). Forskolin was also able to augment insulin secretion at 8 mmol/l glucose in ZL islets, albeit to much lower absolute levels than in ZF islets (30 and 21% of secretion in ZF islets in the absence and presence of palmitate, respectively) (Fig. 2b). These results support an enhanced upregulatory effect of NEFA in ZF islets on multiple stimuli for insulin secretion.

Fatty acid partitioning into oxidation and esterification pathways is increased in ZF islets

Palmitate oxidation was reduced at high glucose (16 mmol/l) compared to low glucose (3 mmol/l), but for these glucose concentrations was not different between ZF and ZL islets (Fig. 3a). Palmitate oxidation at 8 mmol/l glucose, however, was 89% higher in ZF compared with ZL islets (Fig. 3a). This result is consistent with less suppression of fatty acid oxidation in ZF islets by an increase in glucose concentration within the physiological range. The net

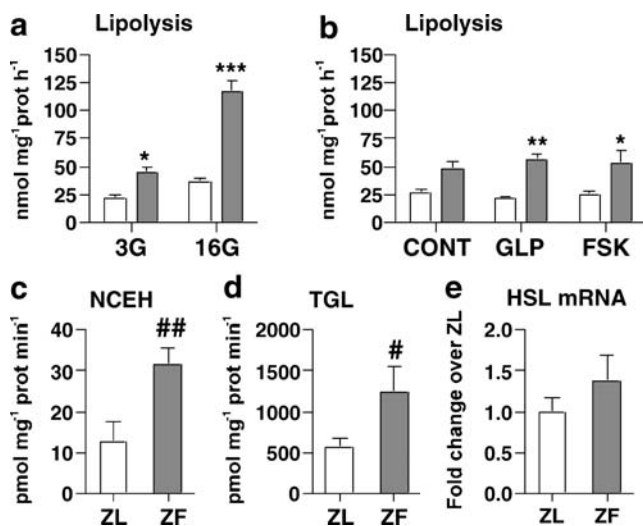


**Fig. 3** Palmitate oxidation at intermediate glucose levels and net palmitate esterification into complex lipids are increased in ZF islets. **a** Palmitate oxidation. Islets from ZF (grey bars) and ZL (white bars) rats were prelabelled for 40 h with [9,10(n)- $^3$ H]palmitate and incubated for 2 h in [9,10(n)- $^3$ H]palmitate oxidation media at 3 (3G), 8 (8G) and 16 (16G) mmol/l glucose. **b–f** Islets were cultured for 40 h in [1- $^{14}$ C]palmitate esterification media at 3, 8 and 16 mmol/l glucose. Esterification into total phospholipids (**b**), phospholipid species lysophosphatidylcholine (LC), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) (**c**), triglyceride (**d**) and diacylglycerol (**e**). **f** Determination of islet non-esterified palmitate content at time 40 h. Means $\pm$ SEM of six determinations from islets of six rats of each genotype in two separate experiments. Two-way ANOVA post hoc analyses: \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001. Student's  $t$ -test: # $p$ <0.05

esterification of palmitate into total complex lipids (total esterification minus lipolysis) was 65, 53 and 71% higher at 3, 8 and 16 mmol/l glucose, respectively (not shown); esterification into TG (169, 164 and 229% higher in ZF) (Fig. 3d) and diacylglycerol (81, 54 and 70% higher in ZF) (Fig. 3e) contributed mostly to this difference. Net esterification into total phospholipids was minimally increased in ZF compared with ZL islets (Fig. 3b) and this was predominantly due to increased esterification into phosphatidylcholine rather than other phospholipid species (Fig. 3c). Interestingly, elevated glucose resulted in higher levels of non-esterified palmitate (an index of cellular LC-CoA [13]) in ZF compared with ZL islets (66 and 98% higher than ZL at 8 and 16 mmol/l glucose, respectively) (Fig. 3f).

## Glucose-stimulated lipolysis is enhanced in ZF islets

Basal lipolysis was 2-fold higher in ZF compared with ZL islets (Fig. 4a,b). Of particular interest, an increase in glucose concentration increased lipolysis by 65% in ZL islets and by 161% in ZF islets, such that at high glucose, lipolysis was 3.2-fold higher in ZF compared with ZL islets (Fig. 4a). GLP-1 and forskolin, both of which increase the activity of cyclic AMP-dependent protein kinase (PKA), failed to increase lipolysis in either ZF or ZL islets (Fig. 4b). In concordance with the increased basal lipolysis in ZF islets, the lipolytic enzyme activities of NCEH and TGL were 148 and 121% higher, respectively, in ZF compared with ZL islets (Fig. 4c,d). Hormone-sensitive lipase (*Hsl*) mRNA was measured in ZL and ZF islets by semiquantitative RT-PCR. There was a trend for increased *Hsl* mRNA expression in ZF islets, but this did not reach statistical significance (Fig. 4e).



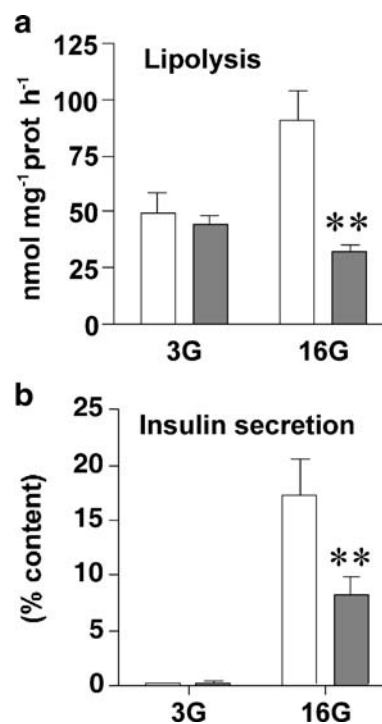
**Fig. 4** Glucose-stimulated lipolysis is increased in islets of ZF rats. **a, b** Lipolysis measured in islets from ZF (grey bars) and ZL (white bars) rats as the rate of glycerol release at **(a)** 3 (3G) and 16 mmol/l (16G) glucose and **(b)** at 3 mmol/l glucose in the absence or presence of 10 nmol/l GLP-1 or 5  $\mu$ mol/l forskolin (FSK). Means $\pm$ SEM of ten determinations from islets of ten rats in five separate experiments **(a)** and six determinations from islets of six rats from three separate experiments **(b)**. Two-way ANOVA post hoc analyses: \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001 compared with ZL for same condition. **c, d** Neutral cholesterol ester hydrolase and triglyceride lipase activities, respectively, measured from islet cell extracts. Means  $\pm$  SEM of four to six determinations from islets from 8–12 rats of each genotype in three separate experiments. Student's *t*-test: # $p$ <0.05, ## $p$ <0.01 compared with ZL. **e** *Hsl* mRNA expression by semiquantitative RT-PCR with correction for  $\beta$ -actin. Means $\pm$ SEM of islets from 11–12 rats of each genotype

Lipolysis inhibition by Orlistat abolishes glucose-stimulated lipolysis and markedly reduces insulin secretion in ZF islets

Orlistat binds to the catalytic site of numerous lipases, including HSL [17, 22]. Previously, inhibition of lipolysis by Orlistat in normal rat islets has been shown to inhibit GSIS, suggesting a role for lipolysis in normal insulin secretion [17]. To determine whether the enhanced glucose-stimulated lipolysis observed in ZF islets could be involved in islet compensatory processes for insulin resistance, lipolysis and NEFA-augmented GSIS were measured in ZF islets in the presence and absence of Orlistat. Orlistat had no effect on basal lipolysis, but completely abolished glucose-stimulated lipolysis in the ZF islets (Fig. 5a). Lipolysis inhibition by Orlistat reduced NEFA-augmented GSIS by 53% (Fig. 5b).

## Expression of key transcription factors and enzymes of lipid metabolism in ZL and ZF islets

The expression of the insulin gene at the mRNA level was significantly increased by 49% in ZF compared with ZL



**Fig. 5** Inhibition of lipolysis impairs glucose-stimulated insulin secretion from ZF islets. **a, b** Lipolysis and insulin secretion respectively were measured in media containing 0.25 mmol/l palmitate at 3 (3G) and 16 (16G) mmol/l glucose in the absence (white bars) or presence (grey bars) of 0.2 mmol/l Orlistat. Means $\pm$ SEM of four **(a)** or eight **(b)** determinations from islets of eight animals of each genotype in two separate experiments. Two-way ANOVA: Orlistat effect on lipolysis,  $p$ <0.01; Orlistat effect on insulin secretion,  $p$ <0.05. Bonferroni post hoc test: \*\* $p$ <0.01

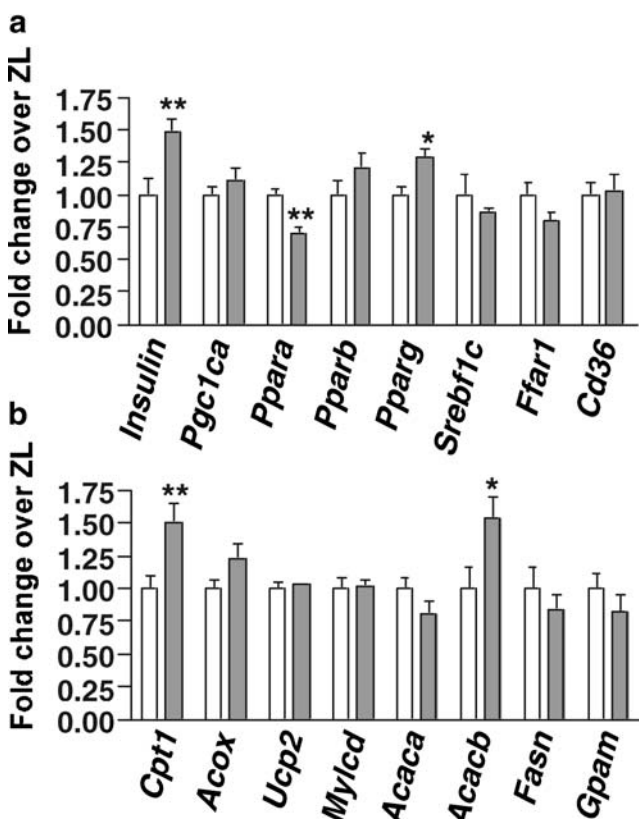
islets (Fig. 6). Of the lipid metabolism transcription factors (peroxisomal proliferator-activated receptors  $\alpha$ ,  $\beta$  and  $\gamma$  [*Ppara*, *Pparb*, *Pparg*] and sterol-regulatory element binding transcription factor 1c [*Srebf1c*]) and the transcription factor coactivator peroxisomal proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (*Ppargc1a*), only *Ppara* (reduced by 31%) and *Pparg* (increased by 28%) mRNAs showed changes in ZF islets (Fig. 6). Expression levels of free fatty acid receptor 1 (*Ffar1*; also known as the G-protein receptor 40 [*Gpr40*]) and *Cd36* (fatty acid transport protein) were not altered (Fig. 6). Of mRNAs for the lipid metabolism enzymes that favour fatty acid oxidation (*Cpt1*, acyl-CoA oxidase, uncoupling protein 2 and malonyl-CoA decarboxylase), only *Cpt1* mRNA was altered in ZF islets (increased by 51%). Of mRNAs for

enzymes favouring fatty acid synthesis and esterification processes (acetyl-CoA carboxylases  $\alpha$  and  $\beta$  [*Acaca* and *Acacb*], fatty acid synthase [*Fasn*] and mitochondrial glycerol-3-phosphate acyltransferase [*Gpam*]), only *Acacb* mRNA (the mitochondrial isoform of acetyl-CoA carboxylase) was increased in ZF compared with ZL islets (increased by 54%) (Fig. 6).

## Discussion

The findings of this study support an important role for lipid signalling (activation of the NEFA receptor FFAR1 and the production of lipid-derived signals, such as diacylglycerol, signalling phospholipids or fatty acyl-CoA, that are known to modulate biological effectors such as ion channels and enzymes) in beta-cell compensation for insulin resistance in ZF rats. A key observation in this regard was that the normal augmentation of GSIS by exogenous palmitate was markedly enhanced in ZF compared with ZL islets, and this effect was particularly apparent within the physiological range of plasma glucose (5–8 mmol/l). Also, the incretin effect of GLP-1 and the insulin response to the cyclic AMP-enhancing agent forskolin were both further augmented by exogenous palmitate at 8 mmol/l glucose in ZF islets. In investigating the underlying mechanism of this NEFA effect, we found that all pathways of fatty acid partitioning (fatty acid oxidation, esterification and lipolysis) were enhanced in ZF islets. Importantly, the lipase inhibitor Orlistat reversed the enhanced GSIS in ZF islets. Also, our results provide potential insight into the regulation of lipolysis in ZF islets by showing increased activities of NCEH and TGL. Together, these findings support enhancement in ZF islets of the normal cycle of TG production and lipolysis as a mechanism of their insulin hypersecretion. In addition, enhanced lipolysis may prevent lipid-induced islet damage by minimising the accumulation of toxic lipids. Our results thus support a beneficial role for the in vivo hyperlipidaemia in ZF rats in the beta cell compensation for insulin resistance, as opposed to the belief of some that chronic excess of NEFA, even in the absence of hyperglycaemia, invariably causes beta cell dysfunction or death [6, 7].

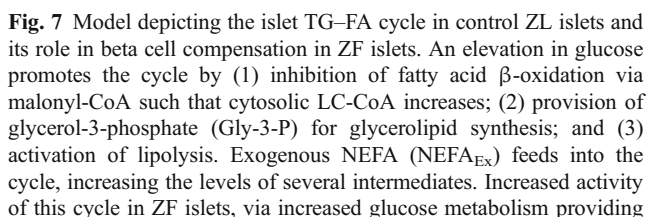
The finding that NEFA dramatically synergised with GLP-1 and the cyclic AMP-increasing agent forskolin in augmenting GSIS in ZF islets is interesting with respect to beta cell compensation. This finding is in keeping with the previous observation that NEFA augment insulin secretion in response not only to glucose but also to all fuel and non-fuel secretagogues, including GLP-1 [13]. The present findings reinforce the concept that NEFA are not only essential for insulin secretion [23] but are also involved in enhancement of secretion in response to a wide variety of



**Fig. 6** Expression of genes related to lipid metabolism and signalling at the mRNA level in ZF and ZL islets. Insulin, peroxisomal proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (*Ppargc1a*), peroxisomal proliferator-activated receptors  $\alpha$  (*Ppara*),  $\beta$  (*Pparb*) and  $\gamma$  (*Pparg*), sterol regulatory element binding transcription factor 1c (*Srebf1c*), free fatty acid receptor 1 (*Ffar1*), CD 36 (**a**), carnitine palmitoyltransferase 1 (*Cpt1*), acyl-CoA oxidase (*Acox*), uncoupling protein 2 (*Ucp2*), malonyl-CoA decarboxylase (*Mlycd*), acetyl-CoA carboxylases  $\alpha$  (*Acaca*) and  $\beta$  (*Acacb*), fatty acid synthase (*Fasn*) and mitochondrial glycerol-3-phosphate acyltransferase (*Gpam*) (**b**) mRNA levels were measured in ZF (grey bars) and ZL (white bars) islets by RT-PCR analysis followed by Southern blot hybridisation and autoradiography. Expression was corrected for  $\beta$ -actin mRNA levels. Means $\pm$ SEM of islets from 5–11 rats of each genotype. Student's *t*-test: \**p*<0.05, \*\**p*<0.01

Even though we have shown coordinately increased fatty acid esterification and lipolysis processes in response to glucose in ZF islets, we have not proven that TG-FA cycling exists as we have not shown that the NEFA resulting from lipolysis are in fact re-esterified. However, it seems likely that at least some of the cytosolic NEFA from lipolysis would be re-activated to acyl-CoA, followed by either re-esterification (cycling) or oxidation. Intracellular TG-FA cycling is known to occur in multiple other tissues, including adipose tissue [27], muscle [28] and liver (reviewed in [29]). It is a new concept to suggest a role for TG-FA cycling in beta cell metabolic signal transduction and compensation for insulin resistance. We propose a model (Fig. 7) in which glucose promotes activity in a beta cell TG-FA cycle by (1) the malonyl-CoA/LC-CoA switch of fatty metabolism from oxidation to esterification [3, 13]; (2) providing glycerol-3-phosphate (Gly-3-P) for synthesis of complex glycerolipids; and (3) activation of lipolysis, as demonstrated in this study. NEFA also contribute to activation of the cycle by providing the other substrate

Increased glucose metabolism and increased availability of NEFA, resulting in increased TG synthesis, are almost certainly contributors to the proposed TG-FA cycle in ZF islets (Fig. 7). The regulatory factors of cycling at the level of lipase enzymes in both normal and compensating islets, however, are yet to be determined. ZF islet extracts were shown to have greater than 2-fold increased TGL activity, which could be a result of increased expression of lipase enzymes or increased lipase intrinsic activity. There was a non-significant 38% increase in *Hsl* mRNA levels. Interestingly, we have now analysed islet *Hsl* mRNA levels by real-time PCR in a new series of ZL and ZF animals that had a sham laparotomy for another study protocol. In that study, *Hsl* mRNA was significantly increased in ZF islets ( $1.49 \pm 0.08$  compared with  $0.78 \pm 0.11$  relative units; ZF vs ZL,



more Gly-3-P and higher malonyl-CoA levels, a greater supply of exogenous NEFA and higher activity of lipases, contributes to beta cell compensation for insulin resistance through the provision of increased lipid signalling molecules for exocytosis, such as LC-CoA and diacylglycerol DAG. The cycle also allows the detoxification of lipids via lipolysis and the enhanced  $\beta$ -oxidation of fatty acids. The TG-FA cycle may also protect the beta cell due to the fact that it is a futile cycle resulting in the consumption of ATP



$p < 0.01$ ). Therefore, it seems likely that the expression of *Hsl* is higher in ZF islets, which is consistent with the enzyme activity data. The role of HSL in islet beta cell function is unclear, however, as insulin secretion has been shown to be little or not affected in *Hsl*  $-/-$  animals [18, 31]. We showed impaired GSIS in only male fasted *Hsl*  $-/-$  mice, and this was completely reversed by the provision of exogenous NEFA [18]. The expression of HSL in beta cells, however, has been shown to be upregulated by a prolonged high glucose concentration [32] and HSL has been shown to be co-localised with insulin granules [33]. There has been particular interest in the role of non-HSL lipases in adipose tissue metabolism [34, 35]. The roles of these alternative lipases in the islet also warrant investigation.

There is increasing evidence that islet beta cell lipolysis is increased by elevated glucose, as shown previously in normal rat islets [17] and in both wild-type and *Hsl*  $-/-$  mouse islets [18]. We have also shown glucose-stimulated lipolysis to be tightly correlated with GSIS in INS832/13 beta cells (C. J. Nolan and M. Prentki, unpublished observations). It may be that the effect of glucose on lipolysis results from its effect in increasing TG synthesis, perhaps into a labile intracellular pool, thus providing more substrate for lipase enzymes. Another possibility is that elevated glucose might activate lipase enzyme activity. Of relevance, we recently showed that LC-CoA, which is thought to increase in the cytoplasm in response to elevated glucose [3, 13], activates lipolysis in vitro in islet extracts from control and *Hsl*  $-/-$  mice [36]. It is an attractive hypothesis, therefore, that activation of lipolysis by LC-CoA could also be involved in the enhanced TG-FA cycling that was observed in ZF islets.

In fat cells, hormonal stimulation of lipolysis is by activation of HSL via PKA phosphorylation. While GLP-1 and forskolin, both activators of PKA, have been shown to increase lipolysis in clonal pancreatic beta cells (HIT cells) [37], neither agent enhanced lipolysis in ZF or ZL islets. This finding is in agreement with our study of *Hsl* wild-type and knockout mice [18]. Glucose activation of lipolysis was noted in the HSL-deficient mice but no significant effect of GLP-1 on lipolysis was observed [18]. Thus, it seems that glucose acts via a mechanism other than PKA, possibly via an enzyme other than HSL [18]. The results also indicate that the synergistic effect of GLP-1 or forskolin with NEFA in augmenting GSIS is not due to an effect of GLP-1 or forskolin in accelerating lipolysis.

At first glance, it seems odd that increased malonyl-CoA/LC-CoA signalling and increased fatty acid oxidation at a physiologically relevant glucose level should occur together in ZF islets. Interestingly, the expression of important enzymes of both pathways, ACACB (synthesises malonyl-CoA) and CPT1 (the rate-limiting step in mitochondrial fatty acid oxidation), were increased in ZF islets.

We suspect that the increased fatty acid oxidation is maintained, despite more inhibition of CPT1 by malonyl-CoA, because of the maintenance of higher levels of LC-CoA (substrate for fatty acid oxidation) by accelerated TG-FA cycling. Previously it has been shown that inhibition of fatty acid oxidation by an inhibitor of CPT1 reduces basal hypersecretion in ZDF rats, such that the maintenance of fatty acid oxidation may also be important in compensation processes [38]. Islets of the ZF rat displayed none of the well-described features of lipotoxicity [7, 39–43]. The TG content was mildly decreased rather than dramatically increased [7, 40]. The mRNA expression of genes that promote lipogenesis and fatty acid esterification (*Srebf1c*, *Acaca*, *Fasn* and *Gpam*) was not altered in ZF islets. Thus, the question arises as to how ZF islets escape lipotoxicity. As illustrated in Fig. 7, enhanced TG-FA cycling provides an attractive mechanism of protection from lipid toxicity in the face of obesity-associated hyperlipidaemia. The cycle prevents steatosis due to the high rates of TG lipolysis and by allowing the maintenance of LC-CoA as a substrate for detoxification via fatty acid oxidation. In addition, TG-FA cycling is futile, in that fatty acid activation to LC-CoA and its metabolism back to NEFA via TG expend ATP energy.

In conclusion, our results document very active fatty acid glucose-responsive esterification/lipolysis processes in pancreatic beta cells, which are further enhanced in the islets of ZF rats. We [16, 18] and co-workers [17] have provided genetic (*Hsl* knockout studies) and pharmacological (Orlistat, dimethylpyrazole) evidence that lipolysis plays a role in GSIS. This study expands that concept by providing evidence that enhanced lipolysis and, most probably, TG-FA cycling in ZF islets appear to be a mechanism for the enhanced insulin secretion that characterises beta cell compensation for their insulin resistance.

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