

Acute (24 h) activation of peroxisome proliferator-activated receptor- α (PPAR α) reverses high-fat feeding-induced insulin hypersecretion *in vivo* and in perfused pancreatic islets

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Abstract

Abnormal depletion or accumulation of islet lipid may be important for the development of pancreatic β cell failure. Long-term lipid sensing by β cells may be co-ordinated via peroxisome proliferator-activated receptors (PPARs). We investigated whether PPAR α activation *in vivo* for 24 h affects basal and glucose-stimulated insulin secretion *in vivo* after intravenous glucose administration and *ex vivo* in isolated perfused islets. Insulin secretion after intravenous glucose challenge was greatly increased by high-fat feeding (4 weeks) but glucose tolerance was minimally perturbed, demonstrating insulin hypersecretion compensated for insulin resistance. The effect of high-fat feeding to enhance glucose-stimulated insulin secretion was retained in perfused islets demonstrating a stable, long-term effect of high-fat feeding to potentiate islet glucose stimulus-secretion coupling. Treatment of high-fat-fed rats with

WY14,643 for 24 h reversed insulin hypersecretion *in vivo* without impairing glucose tolerance, suggesting improved insulin action, and *ex vivo* in perfused islets. PPAR α activation only affected hypersecretion of insulin since glucose-stimulated insulin secretion was unaffected by WY14,643 treatment *in vivo* in control rats or in perfused islets from control rats. Our data demonstrate that activation of PPAR α for 24 h can oppose insulin hypersecretion elicited by high-fat feeding via stable long-term effects exerted on islet function. PPAR α could, therefore, participate in ameliorating abnormal glucose homeostasis and hyperinsulinaemia in dietary insulin resistance via modulation of islet function, extending the established requirement for PPAR α for normal islet lipid homeostasis.

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Introduction

Signalling via peroxisome proliferator-activated receptor- α (PPAR α) appears to influence whole-body insulin sensitivity. Long-term (14 week) administration of fenofibrate as part of a high-fat diet to C57BL/6 mice reverses basal hyperinsulinaemia and hyperglycaemia (Guerre-Millo *et al.* 2000). Similarly, chronic (15 day) administration of ciprofibrate to 5-week-old obese Zucker rats markedly reduces plasma insulin concentrations during an intravenous glucose tolerance test, whereas serum glucose concentrations remain comparable between treated and untreated obese rats, suggesting improved insulin action (Guerre-Millo *et al.* 2000). It was suggested that fibrates, through increasing fatty acid (FA) flux from peripheral tissues to liver and lowering triacylglycerol delivery to peripheral tissues, oppose the development of insulin resistance by relieving FA-mediated inhibition of insulin-stimulated skeletal muscle glucose disposal and decreasing skeletal muscle triacylglycerol storage. Chronic (2 week) WY14,643 treatment enhances insulin-stimulated muscle

glucose uptake in insulin-resistant high-fat-fed rats together with a reduction of muscle long-chain acyl-CoA levels, suggesting that enhanced muscle insulin sensitivity resulted from intramyocellular lipid clearance (Ye *et al.* 2001). Peripheral insulin resistance evoked by increased dietary lipid for 4 weeks is accompanied by compensatory insulin secretion, such that glucose tolerance is maintained (Holness & Sugden 1999). However, PPAR α null mice, unlike wild-type mice, fail to respond to chronic (16–22 week) high-fat feeding with basal hyperinsulinaemia, glucose intolerance and insulin hypersecretion after glucose challenge (Guerre-Millo *et al.* 2001), suggesting a role for PPAR α in the control of peripheral glucose utilisation in response to dietary fat. Although PPAR α deficiency would be predicted to decrease FA oxidation leading to lipid accumulation, insulin secretion by islets from control or high-fat-fed C57BL/6N mice was not significantly affected by PPAR α deficiency (Guerre-Millo *et al.* 2001), suggesting that the effects of PPAR α deficiency on glucose homeostasis do not reflect altered islet function.

However, there is evidence that long-term lipid sensing by the pancreatic β cell could be co-ordinated through the expression and/or activity of the PPARs (Zhou *et al.* 1998, Roduit *et al.* 2000). PPAR α is expressed in islets and islet cell lines and controls genes involved in lipid metabolism, in particular those implicated in mitochondrial and peroxisomal β -oxidation and FA transport (Zhou *et al.* 1998). Pancreatic islets from obese Zucker rats are characterised by β -cell hyperplasia and enhanced basal and glucose-stimulated insulin secretion (Milburn *et al.* 1995). These obesity-related β -cell abnormalities can be reproduced by culturing normal islets in the presence of FA for 7 days (Milburn *et al.* 1995), suggesting that a rise in circulating FA, secondary to increased adipose tissue lipolysis, can signal increased β -cell insulin secretion to compensate for peripheral insulin resistance. PPAR α mRNA expression and enzymes of FA oxidation are induced in normal rat islets by exposure to high FA concentrations (Zhou *et al.* 1998). Similar changes are observed on exposure to PPAR α receptor ligands *in vitro* (Zhou *et al.* 1998) and *in vivo* (Sugden *et al.* 2001). Conversely, islets or INS(832/13) β cells exposed to high glucose show a 60–80% reduction in PPAR α mRNA (Roduit *et al.* 2000), suggesting that prolonged exposure to hyperglycaemia may lead to impaired signalling via PPAR α which, in turn, may impair lipid oxidation leading to lipid accumulation and ultimately β -cell dysfunction.

In the present study, we investigated whether administration of WY14,643 for 24 h *in vivo*, which increases islet PPAR α expression (Sugden *et al.* 2001), affects basal and glucose-stimulated insulin secretion *in vivo* after intravenous glucose administration. Studies were conducted in insulin-sensitive rats maintained on a high-carbohydrate low-fat diet and in a high-saturated-fat-fed rat model in which the development of peripheral insulin resistance is observed in conjunction with compensatory insulin hypersecretion to maintain glucose tolerance (Holness & Sugden 1999). Because previous studies using INS-1 cells showed that adenovirus-mediated overexpression of PPAR α or treatment with clofibrate directly impairs basal and glucose-stimulated insulin secretion *in vitro* (Tordjman *et al.* 2002), we also analysed effects of antecedent PPAR α activation on insulin secretion by isolated perfused islets. This approach enabled analysis of potential changes in glucose threshold and/or responsiveness of insulin secretion evoked by high-fat feeding and/or activation of PPAR α by treatment with WY14,643 for 24 h.

Materials and Methods

Materials

General laboratory reagents were purchased from Roche Diagnostics (Lewes, East Sussex, UK) or from Sigma (Poole, Dorset, UK). WY14,643 (4-chloro-6-(2,3-xylylidino)-2-pyrimidinylthioacetic acid) was purchased

from Sigma. Kits for measurement of glucose concentrations, determined by the glucose oxidase method, were purchased from Roche Diagnostics. Kits for measurement of insulin concentrations, determined by ELISA using rat insulin as a standard, were purchased from Mercodia (Uppsala, Sweden). Kits for measurement of plasma non-esterified FA and triacylglycerol concentrations, determined spectrophotometrically, were purchased from Alpha Labs (Eastleigh, Hants, UK).

Animals

All studies were conducted in adherence to the regulations of the United Kingdom Animal Scientific Procedures Act (1986). Female albino Wistar rats (200–250 g) were purchased from Charles River (Margate, Kent, UK). Rats were maintained at a temperature of $22 \pm 2^\circ\text{C}$ and subjected to a 12 h light/12 h darkness cycle. Control fed rats (CON) were given free access to a standard, pelleted rodent diet purchased from Special Diet Services (Witham, Essex, UK) (52% carbohydrate, 15% protein, 3% lipid and 30% non-digestible residue (by weight); 2.61 kcal metabolisable energy/g). High-fat-fed rats (HIFAT) were given free access to a semi-synthetic diet high in saturated fat (see Holness & Sugden 1999), henceforth referred to as high-fat diet. The high-fat diet contained 34% carbohydrate, 19% protein and 22% lipid (lard as the major source of lipid, together with corn oil (1.9 g/100 g diet) to prevent essential-FA deficiency) by weight (Holness & Sugden 1999). The lipid component of the high-fat diet comprised 16% saturated FAs (mainly stearic), 16% monounsaturated FAs (mainly oleic) and 7% polyunsaturated FAs (mainly linoleic) by energy. The high-fat diet was prepared at 3-day intervals using components supplied by Special Diet Services, with the exception of the saturated fat component (lard), which was purchased locally. Rats were maintained on the high-saturated-fat diet for 4 weeks. It is already established that exposure to fibrates enhances islet PPAR α expression and upregulates the expression of PPAR α -linked enzymes within 24 h (Sugden *et al.* 2001, Yoshikawa *et al.* 2001). WY14,643 was administered to CON and HIFAT rats with free access to diet as a single i.p. injection (50 mg/kg body weight). Although WY14,643 is specific for PPAR α *in vitro* at 10 μM , all three PPAR subtypes, PPAR α , PPAR δ and PPAR γ , are activated by higher concentrations of WY14,643 (Lehmann *et al.* 1995). Consequently, in the absence of measurements of the concentration of WY14,643 resulting from a single i.p. injection of 50 mg/kg body weight, we cannot categorically exclude the possibility that there may be some additional activation of PPAR δ and/or PPAR γ . Rats were sampled at 24 h after WY14,643 administration (Brun *et al.* 1999), thereby avoiding the long-term effects of PPAR α activation to deplete visceral adipose tissue (Ye *et al.* 2001). Food intake was not influenced by WY14,643

administration in either the CON or HIFAT rats (results not shown). In all experiments, water was available *ad libitum*.

Intravenous glucose challenge

Glucose was administered as an intravenous bolus (0.5 g glucose/kg body weight; 150 μ l per 100 g body weight) to conscious, unrestrained rats (see Holness & Sugden 1999). Glucose was injected via a chronic indwelling jugular cannula, which was flushed with saline after the injection of glucose to remove residual glucose, and blood samples (100 μ l) were withdrawn from the indwelling cannula at 2, 5, 10, 15 and 30 min. Samples of whole blood (50 μ l) were deproteinised with ZnSO₄/Ba(OH)₂, centrifuged (10 000 g) at 4 °C, and the supernatant retained for subsequent assay of blood glucose. The remaining blood sample was immediately centrifuged (10 000 g) at 4 °C, and plasma was stored at -20 °C until assayed for insulin. The calculated acute insulin response (AIR) was calculated as the means of mean suprabasal 2- and 5-min plasma insulin values for individual animals. Insulin and glucose responses during the glucose tolerance test were used for calculation of the incremental plasma insulin values integrated over the 30-min period after the injection of glucose (incremental area under curve (IAUC)-insulin; Δ I) and the corresponding incremental blood glucose values integrated over the 30-min period after the injection of glucose (IAUC-glucose; Δ G). The insulin resistance index (IR index) was calculated as the product of the areas under the glucose and insulin curves after glucose challenge (i.e. Δ I \times Δ G). The rate of glucose disappearance (k) was calculated from the slope of the regression line obtained with log-transformed glucose values from 2 to 15 min after glucose administration.

Islet isolation and perfusion

Rats were anaesthetised by injection of sodium pentobarbital (60 mg/ml in 0.9% NaCl; 1 ml/kg body weight i.p.) and, once locomotor activity had ceased, pancreases were excised and islets were isolated by collagenase digestion (Lacy & Kostianovsky 1967). Free islets were collected under a dissecting microscope with a 20 μ l pipette into Hepes-buffered Hanks' balanced salts solution containing 5% BSA. Insulin release from freshly isolated islets was measured in a perfusion system as described by Hughes *et al.* (1992). In this system, 50 islets were housed in small chambers on Millicell culture inserts. Islets were perfused in basal medium (Krebs-Ringer containing 20 mM Hepes pH 7.4, 5 mg/ml BSA and 2 mM glucose) for 60 min at a flow rate of 1 ml/min at 37 °C prior to collection of fractions. Glucose concentrations were then modified as indicated. Fractions (2 ml) were collected at 2-min intervals and stored at -20 °C prior to assay for insulin.

Statistical analysis

Results are presented as the mean \pm standard error of the mean (S.E.M.), with the numbers of rats or islet preparations in parenthesis. Statistical analysis was performed by ANOVA followed by Fisher's post-hoc tests for individual comparisons or Students *t*-test as appropriate (Statview, Abacus Concepts, Inc., Berkeley, CA, USA). A *P* value of <0.05 was considered to be statistically significant.

Results

Glucose-stimulated insulin secretion in vivo is enhanced after high-fat feeding

Plasma insulin concentrations were measured in untreated and WY14,643-treated HIFAT and CON rats at intervals after the administration of an intravenous glucose challenge (0.5 g/kg body weight) (Fig. 1). Plasma insulin concentrations after intravenous glucose challenge were significantly higher in HIFAT rats than in CON rats at 2 min (1.7-fold; *P*<0.01), 5 min (2.3-fold; *P*<0.001) and 15 min (1.8-fold; *P*<0.05). High-fat feeding for 4 weeks elicited a 2.1-fold increase in the calculated acute insulin response (AIR, i.e. the means of suprabasal 2- and 5-min plasma insulin) (CON, 107 \pm 10 μ U/ml; HIFAT, 220 \pm 28 μ U/ml; *P*<0.001) together with a 2.0-fold increase in IAUC-insulin (CON, 249 \pm 28 μ U/min per l; HIFAT, 503 \pm 61 μ U/min per l; *P*<0.001). Obesity-related β -cell abnormalities, including β -cell hyperplasia and enhanced basal and glucose-stimulated insulin secretion, can be reproduced by culturing normal islets in the presence of FA for 7 days (Milburn *et al.* 1995, Hirose *et al.* 1996). However, the protocol of high-fat feeding used in the present study did not significantly affect either circulating non-esterified FA levels (control 0.41 \pm 0.04 mM; high-fat-fed, 0.37 \pm 0.09 mM) or circulating triacylglycerol levels (control 0.53 \pm 0.05 mM; high-fat-fed, 0.33 \pm 0.05 mM). Thus a sustained increase in dietary lipid delivery is also capable of eliciting hypersecretion of insulin in the absence of increased plasma non-esterified FA or triacylglycerol levels. The insulin resistance index (IR index), the product of the areas under the glucose and insulin curves after glucose challenge, was significantly increased by 2.5-fold after high-fat feeding (*P*<0.001) (Fig. 2A). This latter result demonstrates the induction of insulin resistance in this model of high-saturated fat feeding (see also Holness & Sugden 1999).

WY14,643 treatment for 24 h in vivo markedly lowers glucose-stimulated insulin secretion after intravenous glucose challenge in high-fat-fed rats

We analysed the effects of 24-h treatment with WY14,643 *in vivo* on insulin secretion in CON and

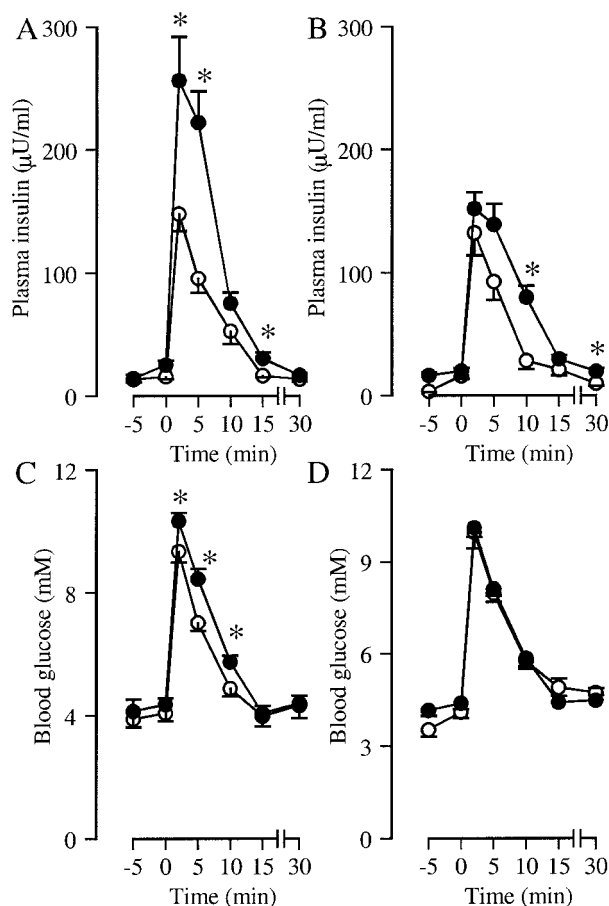


Figure 1 PPAR α activation for 24 h *in vivo* markedly lowers glucose-stimulated hypersecretion of insulin after intravenous glucose challenge in high-fat-fed rats. Plasma insulin (A, B) and blood glucose (C, D) concentrations were measured during an intravenous glucose tolerance test in untreated (A, C) or WY14,643-treated (B, D) CON rats (open symbols) and HIFAT rats (closed symbols). Further details are given in the Materials and Methods section. Results are means \pm S.E.M. for 9 untreated control rats, 10 WY14,643-treated control rats, 8 untreated high-fat-fed rats or 13 WY14,643-treated high-fat-fed rats. * $P < 0.05$, statistically significant effects of high-fat feeding.

HIFAT rats in response to an intravenous glucose challenge. In CON rats, 24-h treatment with WY14,643 did not significantly affect insulin concentrations after an intravenous glucose challenge (Fig. 1). Similarly, neither the IR index (Fig. 2A) nor AIR and IAUC-insulin were significantly modified (results not shown). In contrast, insulin concentrations in the HIFAT group were greatly suppressed by 24-h treatment with WY14,643 at 2 min (by 41%; $P < 0.001$) and 5 min (by 37%; $P < 0.01$) after intravenous glucose challenge (Fig. 1). Thus, WY14,643 treatment reversed the effect of high-fat feeding to elevate insulin concentrations at 2 and 5 min after intravenous glucose challenge by 96% and 66% respectively. Overall,

the insulin response to an intravenous glucose challenge in the HIFAT group was greatly attenuated by 24-h treatment with WY14,643, as reflected by a substantial 42% decrease in AIR (untreated HIFAT, $220 \pm 28 \mu\text{U/ml}$; WY14,643-treated HIFAT, $127 \pm 11 \mu\text{U/ml}$; $P < 0.001$) and 35% decrease in IAUC-insulin (untreated HIFAT, $503 \pm 61 \mu\text{U/min per l}$; WY14,643-treated HIFAT, $328 \pm 28 \mu\text{U/min per l}$; $P < 0.01$). The decline in IAUC-insulin was therefore entirely attributable to a decreased acute insulin response to hyperglycaemia. Although plasma insulin concentrations remained higher at 10 min (2.8-fold; $P < 0.001$) and 30 min (2.0-fold; $P < 0.01$) in HIFAT compared with CON rats after WY14,643 treatment, neither AIR nor IAUC-insulin differed significantly between WY14,643-treated CON rats and WY14,643-treated high-fat-fed rats (results not shown). The present data therefore demonstrate that insulin hypersecretion elicited by high-fat feeding is reversed by WY14,643 administration for only 24 h. Furthermore, WY14,643 administration only affected hypersecretion of insulin since glucose-stimulated insulin secretion was unaffected by WY14,643 treatment in control (low-fat-fed) rats.

WY14,643 treatment for 24 h in vivo modulates glucose clearance after intravenous glucose challenge in high-fat-fed rats

We examined whether the lowered insulin secretory response to glucose observed in response to WY14,643 treatment for 24 h in HIFAT rats was accompanied by loss of glucose tolerance and/or was compensated for by enhanced whole-body insulin sensitivity. Blood glucose concentrations were measured in untreated and WY14,643-treated HIFAT and CON rats at intervals after the administration of an intravenous glucose challenge (0.5 g/kg body weight) (Fig. 1C, D). Blood glucose concentrations were moderately and significantly higher in HIFAT rats than in CON rats at 2 min, 5 min and 10 min after intravenous glucose challenge (Fig. 1C), but such increases were no longer evident after treatment with WY14,643. Treatment of high-fat-fed rats with WY14,643 for 24 h did not significantly affect either the IAUC-glucose value (HIFAT, $12.7 \pm 0.9 \text{ mmol/min per l}$ ($n=8$); HIFAT+WY14,643, $11.5 \pm 0.7 \text{ mmol/min per l}$ ($n=13$)) or the rate of glucose disappearance calculated over the first 15 min (k value) (HIFAT, $3.1 \pm 0.2\%/ \text{min}$ ($n=8$); HIFAT+WY14,643, $2.8 \pm 0.1\%/ \text{min}$ ($n=13$)), but the IR index was significantly lowered (by 40%; $P < 0.01$) by WY14,643 treatment in HIFAT rats (Fig. 2A), indicating that 24-h PPAR α activation significantly improved insulin sensitivity in the HIFAT group. The present data therefore demonstrate an effect of WY14,643 treatment for 24 h to enhance whole-body insulin sensitivity in high-fat-fed rats in parallel with a decline in glucose-stimulated insulin secretion.

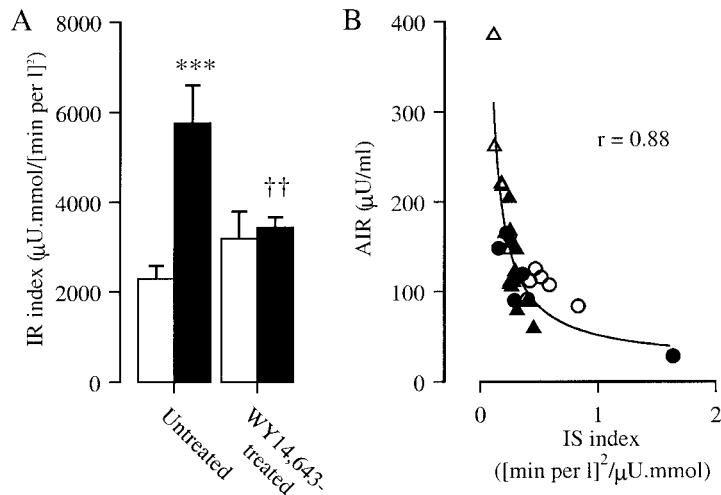


Figure 2 Acute (24 h) WY14,643 treatment *in vivo* reverses insulin resistance elicited by high-fat feeding. The insulin resistance indices (IR index), calculated as the product of the areas under the glucose and insulin curves after glucose challenge in the postabsorptive state, are shown for untreated and WY14,643-treated CON (open bars) or HIFAT (closed bars) rats in (A). The relationship between AIR and insulin sensitivity (IS) index, the reciprocal of the IR index, in untreated (open symbols) or WY14,643-treated (closed symbols) control (circles) or high-fat-fed (triangles) rats in the postabsorptive state is shown in (B). Results are means \pm S.E.M. for 9 untreated control rats, 10 WY14,643-treated control rats, 8 control high-fat-fed rats or 13 WY14,643-treated high-fat-fed rats. *** $P < 0.001$, statistically significant effects of high-fat feeding; †† $P < 0.01$, statistically significant effects of WY14,643 treatment.

The relationship between β -cell function and insulin sensitivity

As recently reviewed (Bergman *et al.* 2002b), increasing evidence suggests that changes in insulin sensitivity in healthy individuals are compensated for by inverse changes in β -cell responsiveness such that the product of insulin sensitivity and insulin secretion, termed the 'disposition index', remains unchanged. Thus, a hyperbolic relationship exists between insulin sensitivity and secretion (Kahn *et al.* 1993). Figure 2B demonstrates that a strong hyperbolic relationship exists ($r = 0.88$) between AIR and insulin sensitivity (assessed as the reciprocal of the IR index) for the various groups in the present study in which changes in insulin secretion and/or sensitivity were achieved in response to high-fat feeding and/or WY14,643 treatment.

Perfused islets from high-fat-fed rats exhibit enhanced insulin secretion, which is reversed by antecedent treatment with WY14,643 for 24 h *in vivo*

The preceding findings raise the question of whether the reversal of insulin hypersecretion in high-fat-fed rats *in vivo* elicited by WY14,643 treatment represents an indirect response of islets to enhanced whole-body insulin sensitivity and/or reflects a long-term modification of islet function that persists in isolated islets. We therefore measured insulin release by perfused islets isolated from

untreated or WY14,643-treated CON or HIFAT rats during stepwise glucose perfusions generating steady rises in perfusate glucose concentrations to target concentrations of 6.8 mM and 13.6 mM, followed by a decline to basal levels over a total 2 h perfusion period. Patterns of insulin release are shown in Fig. 3 for islets from untreated and WY14,643-treated CON and HIFAT groups. Basal insulin release rates by perfused islets isolated from high-fat-fed rats were significantly higher (by 52%; $P < 0.05$) compared with rates by control perfused islets (Fig. 3A). Raising the perfusate glucose concentration to 6.7 ± 0.2 mM and 6.8 ± 0.2 mM did not significantly increase insulin release by perfused islets isolated from either untreated control or high-fat-fed rats. Nevertheless, insulin release rates from islets from high-fat-fed rats remained significantly higher (by 63 to 79%; $P < 0.05$). Raising the perfusate glucose concentration to 13.4 ± 0.2 mM significantly increased insulin release rates to peak rates ($99 \mu\text{U}/\text{min}$) that were 13.8-fold above basal ($P < 0.01$) in islets isolated from untreated control rats. Rates of insulin release by islets isolated from high-fat-fed rats increased more rapidly in response to a mean perfusate glucose concentration of 14.1 ± 0.5 mM and were 1.5-fold higher ($P < 0.01$) than peak rates of insulin release in control islets (Fig. 3A). Reversal of perfusate glucose concentrations to 2 mM for 30 min decreased insulin release by islets isolated

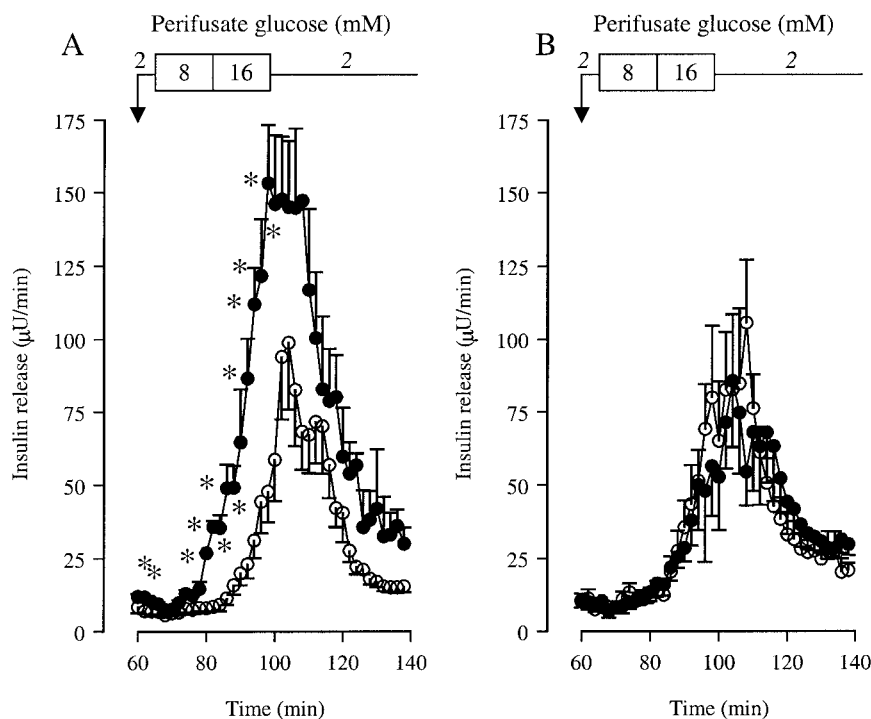


Figure 3 Effects of glucose on insulin release by perfused islets isolated from untreated or WY14,643-treated control or high-fat-fed rats. Islets isolated from untreated (panel A) or WY14,643-treated (panel B) control rats (open circles) or high-fat-fed rats (closed circles) were perfused in basal medium containing 2 mM glucose for 60 min at a flow rate of 1 ml/min at 37 °C prior to collection of fractions. Glucose concentrations were then modified as indicated. Fractions (2 ml) were collected at 2-min intervals and stored at -20 °C prior to assay for insulin and glucose using commercial kits. Assayed perfusate glucose concentrations are provided in the text. Results are means \pm S.E.M. for 9 untreated control rats, 5 WY14,643-treated control rats, 6 control high-fat-fed rats or 6 WY14,643-treated high-fat-fed rats. * P <0.05, statistically significant effects of high-fat feeding.

from untreated control rats and by islets from high-fat-fed rats towards basal rates (Fig. 3A). Increased insulin release by perfused islets from high-fat-fed rats compared with control rats resulted in a 2.1-fold higher IAUC for insulin during the 2-h perfusion period (CON, $890 \pm 143 \mu\text{U}/\text{min per l}$ ($n=9$); HIFAT, $1884 \pm 417 \mu\text{U}/\text{min per l}$ ($n=6$); P <0.01), demonstrating that the effect of high-fat feeding to enhance glucose-stimulated insulin secretion *in vivo* is retained in isolated islets perfused with glucose *ex vivo* (Fig. 3A). Consequently, high-fat feeding elicits a stable, long-term modification of islet function, eliminating the acute influences of circulating factors, including acute changes in systemic lipid delivery to the islet. In contrast to earlier observations obtained using batch islet incubations (Sugden *et al.* 2001), WY14,643 treatment of CON rats for 24 h did not significantly affect insulin release by perfused islets at basal (2 mM) perfusate glucose (compare Fig. 3A and B). Furthermore, insulin release by perfused islets at mid ($6.8 \pm 0.5 \text{ mM}$), or high ($12.6 \pm 0.7 \text{ mM}$) perfusate glucose (compare Fig. 3A and B) and the IAUC-insulin during the 2-h perfusion period

(CON, $890 \pm 143 \mu\text{U}/\text{min per l}$ ($n=9$); CON+WY14,643, $930 \pm 237 \mu\text{U}/\text{min per l}$ ($n=5$)) were unchanged by WY14,643 treatment. WY14,643 treatment of HIFAT rats did not affect insulin release rates by perfused islets at basal (2 mM) or mid ($6.9 \pm 0.3 \text{ mM}$) perfusate glucose concentrations (compare Fig. 3A and B). However, rates of insulin release by islets from WY14,643-treated HIFAT rats perfused at high ($14.1 \pm 0.6 \text{ mM}$) glucose concentrations were significantly lower (by 48–63%; P <0.05) compared with rates in islets from untreated HIFAT rats resulting in a 49% decrease (P <0.05) in the IAUC-insulin during the 2-h perfusion period (HIFAT, $1884 \pm 417 \mu\text{U}/\text{min per l}$ ($n=5$); HIFAT+WY14,643, $965 \pm 211 \mu\text{U}/\text{min per l}$ ($n=6$); P <0.05). Consequently, rates of insulin release did not differ significantly from corresponding rates of insulin release by islets prepared from either untreated or WY14,643-treated CON rats. Thus, our data demonstrate that WY14,643 treatment *in vivo* for 24 h reverses the effects of long-term high-fat feeding to elicit a stable modification of islet function resulting in enhanced

glucose-stimulated insulin secretion by perfused islets *ex vivo* (Fig. 3).

Discussion

The present study investigated whether WY14,643 treatment for 24 h in the intact animal modulates glucose-stimulated insulin secretion in insulin-sensitive control rats maintained on a high-carbohydrate low-fat diet or opposes insulin hypersecretion in insulin-resistant high-fat-fed rats. *Ex vivo* islet perfusions were employed to identify stable, persistent effects of high-fat feeding and/or WY14,643 treatment on islet function. Insulin secretion after intravenous glucose challenge was greatly increased by high-fat feeding but glucose tolerance was only modestly affected, demonstrating that insulin hypersecretion compensated for insulin resistance. Studies using perfused islets demonstrated that the effect of high-fat feeding to enhance glucose-stimulated insulin secretion was retained *ex vivo* indicating that the effect of high-fat feeding is stable to islet isolation and perfusion. Taken together, these findings suggest a stable, long-term effect of high-fat feeding on islet function, such that glucose stimulus-secretion coupling is potentiated. Our data also demonstrate that treatment of HIFAT rats with WY14,643 for 24 h reverses insulin hypersecretion *in vivo*, without impairing glucose tolerance, indicating improved insulin action. WY14,643 treatment only affected hypersecretion of insulin since glucose-stimulated insulin secretion was unaffected by WY14,643 treatment of control rats. Finally, we demonstrate that the effect of WY14,643 treatment for 24 h to reverse insulin hypersecretion elicited by long-term high-fat feeding is retained in perfused islets from high-fat fed rats. Thus, our data suggest a role for PPAR α in the modulation of islet function in insulin resistant states, namely that activation of PPAR α can oppose insulin hypersecretion elicited by high-fat feeding. This may reflect direct activation of islet PPAR α or an indirect, but stable, long-term effect on the islet that reflects the amelioration of peripheral insulin resistance. The studies suggest that PPAR α activation could participate in ameliorating abnormal glucose homeostasis and hyperinsulinaemia in dietary insulin resistance via modulation of islet function, extending the established requirement for PPAR α for normal islet lipid homeostasis.

The mechanism underlying the development of hypersecretion of insulin in response to high-fat feeding may reflect direct persistent effects of fatty acids, or their derivatives, on islet lipid handling. Short-term exposure to elevated fatty acids enhances glucose-stimulated insulin secretion (Dobbins *et al.* 1998a,b), whereas long-term exposure to fatty acids inhibits glucose-induced insulin secretion (Zhou & Grill 1994, 1995). It has been suggested that the accumulation of long-chain acyl-CoA or other lipid-derived metabolites in the islet, possibly secondary to

the suppression of islet carnitine palmitoyltransferase I activity, enhances glucose-stimulated insulin secretion (reviewed in McGarry 2002). Both PPAR α overexpression and clofibrate treatment increase palmitate oxidation in INS-1 cells (Tordjman *et al.* 2002) and adenoviral overexpression of carnitine palmitoyltransferase I in INS1E cells increases carnitine palmitoyltransferase I protein and activity and lowers insulin secretion on stimulation with 15 mM glucose (Rubi *et al.* 2002). Treatment of INS-1 cells with the carnitine palmitoyltransferase I inhibitor etomoxir blocks the effect of clofibrate to suppress glucose-stimulated insulin secretion (Tordjman *et al.* 2002). Since intra-islet concentrations of one or more lipid-derived metabolites may exert a critical influence on glucose-stimulated insulin secretion and PPAR α activation appears to increase islet FA oxidation, it is therefore possible that enhanced glucose-stimulated insulin secretion, secondary to elevated levels of potentiating intra-islet lipids elicited by high-fat feeding, may be reversed by WY14,643 treatment through actions to enhance islet fatty acid oxidation and thereby to decrease the pool of potentiating lipids. Interestingly, in the present study, the enhanced glucose-stimulated insulin secretion evoked by high-fat feeding was observed in conjunction with a significant 1.9-fold increase ($P < 0.05$) in islet PPAR α protein expression (results not shown). The lack of association between increased islet PPAR α protein expression and a lower rate of glucose-stimulated insulin secretion in this model is likely to reflect a lack of endogenous ligand in untreated high-fat-fed rats as treatment of high-fat-fed rats with exogenous ligand, namely WY14,643, markedly decreased glucose-stimulated insulin secretion. Hence, as suggested from our studies *in vivo* and *in vitro*, islets of high-fat-fed rats may be more sensitive to suppression of glucose-stimulated insulin secretion in response to PPAR α activation than rats maintained on high-carbohydrate diet as long as adequate PPAR α ligand is present.

Despite the accumulating evidence for direct effects of islet PPAR α signalling on insulin secretion, it remains possible that amelioration of peripheral insulin resistance is the primary event leading to stable modulation of β -cell function. Long-term treatment with fibrates improves whole-body glucose handling in high-fat-fed mice and obese Zucker rats (Guerre-Millo *et al.* 2000) and WY14,643 treatment enhances muscle insulin sensitivity in insulin-resistant high-fat-fed rats (Ye *et al.* 2001). In addition, chronic PPAR α activation lowers the plasma insulin response to glucose in obese Zucker rats (Guerre-Millo *et al.* 2000). It is increasingly apparent that insulin sensitivity and β -cell responsiveness are linked and that in healthy individuals a hyperbolic relationship exists between insulin sensitivity and secretion (Kahn *et al.* 1993, Bergman *et al.* 2002a). In the present study, insulin resistance induced by increasing dietary saturated fat was compensated for by increased insulin secretion, and

glucose tolerance was maintained. Our data support the concept that it is a failure of β cells to compensate for insulin resistance that may be a critical component in the pathogenesis of type 2 diabetes, such that overt diabetes only occurs when insulin resistance is accompanied by a β -cell defect preventing compensatory upregulation of insulin secretion, the '2-hit' phenomenon (for review see Bergman *et al.* 2002a). Further recent evidence supporting this has been obtained in studies of long-term (10 month) high-fat-fed rats (Chalkley *et al.* 2002). These rats were characterised by severe insulin resistance but only mild glucose intolerance together with a relative deficiency in glucose-stimulated insulin secretion (Chalkley *et al.* 2002). A separate study demonstrated that the development of glucose intolerance in response to long-term (up to 10 months) high-fat feeding in mice was a consequence of insufficient islet compensation to insulin resistance (Ahren & Pacini 2002). Similarly, the UK Prospective Diabetes Study (UKPDS Group 1995) indicated that the progression to type 2 diabetes reflects deteriorating β -cell function in the face of insulin resistance. It has therefore been suggested that anti-diabetic agents (e.g. thiazolidinediones) that target both insulin resistance and β -cell function, may limit the onset and progression of type 2 diabetes (see e.g. Bergman *et al.* 2002b). It is established that chronic hyperinsulinaemia leads to insulin resistance. Thus, the effect of WY14,643 treatment to normalise insulin secretion after high-fat feeding would be predicted to reinforce effects on the periphery to enhance insulin action. The present study provides further support for the potential for PPAR α activation as a means to retard the onset of type 2 diabetes either by relieving peripheral insulin resistance and/or by directly affecting islet function.

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