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Interactive influences of peroxisome proliferator-activated receptor α activation and glucocorticoids on pancreatic beta cell compensation in insulin resistance induced by dietary saturated fat in the rat

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Abstract *Aims/hypothesis:* We sought to elucidate whether excess glucocorticoids and increased dietary lipids act synergistically to impair glucose tolerance and, if so, whether activation of peroxisome proliferator-activated receptor α (PPAR α) has an adverse or beneficial effect on glucose tolerance. *Methods:* Dexamethasone (100 $\mu\text{g kg}^{-1}$ body weight day $^{-1}$; 5 days) was administered to insulin-resistant rats fed a high-saturated-fat (HF) diet for 4 weeks. The PPAR α agonist WY14643 was administered (50 mg kg $^{-1}$ body weight intraperitoneally) 24 h before sampling. Glucose-stimulated insulin secretion (GSIS) was assessed in vivo after an acute glucose bolus injection, and in vitro using step-up and step-down islet perfusions. *Results:* Although neither PPAR α activation nor dexamethasone alone affected fasting glycaemia in the HF group, dexamethasone in combination with PPAR α activation elicited marked postabsorptive hyperglycaemia. Dexamethasone treatment of HF rats had little effect on GSIS after an acute glucose challenge in vivo, but induced glucose intolerance. PPAR α activation augmented GSIS in dexamethasone-treated HF rats in vivo, restoring glucose tolerance. Contrasting with data obtained in vivo, greatly enhanced peak rates of GSIS were observed ex vivo in perfusions of islets from dexamethasone-treated HF rats compared with those from untreated HF rats, an effect attenuated by antecedent PPAR α activation. *Conclusions/interpretation:* The study demonstrates that glucocorticoid excess precipitates the development of glucose intolerance in rats maintained on a high-saturated-fat diet. It does this by interrupting the negative feedback loop between insulin sensitivity and secretion in vivo, such that further enhance-

ment of compensatory insulin secretion is not possible. PPAR α activation restores the coupling between insulin secretion and action.

Keywords Compensatory insulin secretion · Glucocorticoids · Glucose-stimulated insulin secretion · Insulin resistance · Islet perfusions · PPAR α · Saturated fat

Abbreviations AIR: acute insulin response · DEX: dexamethasone · ΔG : incremental blood glucose values integrated over the 30-min period after intravenous injection of glucose · ΔI : incremental plasma insulin values integrated over the 30-min period after intravenous injection of glucose · GSIS: glucose-stimulated insulin secretion · HF: high fat · k : rate of glucose disappearance · PPAR: peroxisome proliferator-activated receptor · WY: WY14643

Introduction

The liver is a major target tissue for the glucocorticoids, which increase hepatic glucose production by stimulating gluconeogenesis (reviewed in [1]). This action is important for maintaining glycaemia in starvation, where increased fatty acid oxidation spares pyruvate for gluconeogenesis via inhibition of the pyruvate dehydrogenase complex, and provides ATP and cofactors for gluconeogenesis. However, glucocorticoids at excessive levels can induce insulin resistance and precipitate glucose intolerance [1–3]. Lowering of circulating fatty acid concentrations reverses insulin resistance in human subjects treated with glucocorticoids [4]. Thus, either lipid oxidation and/or altered lipid signalling is involved in the mechanism by which glucocorticoid excess induces whole-body insulin resistance. The PPAR (peroxisome proliferator-activated receptor) subtypes (α , γ , δ) are lipid-responsive transcription factors that regulate lipid storage and oxidation. PPAR α is highly expressed in liver [5], where its activation promotes hepatic

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fatty acid oxidation [6, 7]. Glucocorticoids induce PPAR α expression in cultured hepatocytes and intact liver in rodents [8]. Importantly, PPAR α is required for hepatic insulin resistance to be induced in response to the administration of the synthetic glucocorticoid dexamethasone [9]. Increased dietary saturated fat can also elicit the development of insulin resistance [10–12] (reviewed in [13, 14]). However, contrasting with the facilitatory role of PPAR α in the induction of hepatic insulin resistance in response to glucocorticoids, PPAR α activation improves insulin sensitivity in the high dietary fat model of insulin resistance [15, 16]. The differences in the influence of PPAR α activation on insulin resistance induced by glucocorticoids and that induced by high-saturated-fat feeding may reflect the underlying mechanisms invoking insulin resistance in these two states, namely a more hepatocentric action in glucocorticoid-induced insulin resistance, contrasting with a wider influence of high-fat feeding to induce tissue lipid accumulation with resultant dysregulation of insulin signalling. Importantly, there is increasing evidence that long-term lipid sensing by the pancreatic beta cell could be coordinated through the expression and/or activity of PPAR α [17, 18] (reviewed in [19, 20]). We have recently established that activation of PPAR α can oppose insulin hypersecretion elicited by high-fat feeding via stable long-term effects exerted on islet function [15].

In the present study, we therefore sought to elucidate whether excess glucocorticoids and increased dietary lipid act synergistically to impair glucose tolerance and, if so, whether PPAR α activation has an adverse or beneficial effect on glucose tolerance. We analysed our data in relation to effects of these manipulations on glucose-stimulated insulin secretion (GSIS), the induction of insulin resistance in the intact animal and direct long-term adaptations of islet function. By extending our studies to islet perfusions, we exclude the influence of circulating factors that may signal changes in insulin action in tissues other than the islet.

Materials and methods

Materials

General Laboratory reagents were from Roche Diagnostics (Lewes, UK) or from Sigma (Poole, UK). Kits for determination of insulin (ELISA, rat insulin as standard), glucose (glucose oxidase) and leptin (ELISA, rat leptin as standard) were from Mercodia (Uppsala, Sweden), Roche Diagnostics and Metachem Diagnostics (Northampton, UK). Dexamethasone (sodium phosphate) was obtained from David Bull Laboratories (Warwick, UK). Mini-osmotic pumps were purchased from Charles River (Margate, UK). WY14643 (pirinixic acid) was purchased from Sigma.

Animals

All studies were conducted in adherence to the regulations of the United Kingdom Animal Scientific Procedures Act

(1986). Female Wistar rats (200–250 g) were purchased from Charles River Laboratories. Rats were subjected to a standard light (08:00–20:00 h)–dark (20:00–08:00 h) cycle in a temperature-controlled room ($21 \pm 2^\circ\text{C}$). The rats were housed in individual cages and were given free access to water and to a semi-synthetic diet high in saturated fat for 4 weeks (for details see [11, 12]) (HF group). Dexamethasone ($100 \mu\text{g kg}^{-1}$ body weight day^{-1} ; 5 days) was administered to HF rats via indwelling osmotic pumps [21] for the last 5 days of the 4-week period of high-fat feeding (HF-DEX group). An initial priming dose (0.1 mg) of dexamethasone was given by subcutaneous injection before minipump implantation. This procedure leads to almost total suppression of endogenous corticosterone levels (results not shown). Sham operations involving incision and manipulation under anaesthesia identical to the procedure for implantation of the osmotic minipump were undertaken on a second (control) group of rats. The PPAR α agonist WY14643 (or vehicle) was administered as a single intraperitoneal injection (50 mg kg^{-1} body weight), and rats were sampled after 24 h [15, 22] (HF-WY and HF-DEX-WY groups). We used acute (24 h) exposure to WY14643, rather than longer term repeated administration over several days, since we have previously demonstrated that activation of PPAR α by a single bolus dose of WY14643 opposes insulin hypersecretion elicited by high-fat feeding [15], and therefore the present study can be compared directly with our earlier studies.

Intravenous glucose tolerance tests

Glucose was administered as an intravenous bolus ($0.5 \text{ g glucose kg}^{-1}$ body weight; $150 \mu\text{l}$ per 100 g body weight) to conscious, unrestrained rats in the postabsorptive state 6 h after food withdrawal [12, 23]. Glucose was injected via a chronic indwelling jugular cannula and blood samples ($100 \mu\text{l}$) were withdrawn at intervals from the indwelling cannula, which was flushed with saline after the injection of glucose to remove residual glucose. Samples of whole blood ($50 \mu\text{l}$) were deproteinised with $\text{ZnSO}_4/\text{Ba}(\text{OH})_2$ and centrifuged ($10,000 \text{ g}$) at 4°C , and the supernatant was retained for subsequent assay of blood glucose. The remaining blood sample was immediately centrifuged ($10,000 \text{ g}$) at 4°C , and plasma was stored at -20°C until assayed for insulin. The acute insulin response (AIR) was calculated as the mean of suprabasal 2-min and 5-min plasma insulin values. 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 (ΔI) and the corresponding incremental integrated plasma glucose values (ΔG). 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^{-1} in $0.9\% \text{ NaCl}$; 1 ml kg^{-1} body weight intraperitoneally) and, once locomotor activity had ceased, pancreases were excised and islets isolated by collagenase digestion. Free islets were collected under a dissecting microscope with a $20 \mu\text{l}$ pipette into HEPES-buffered Hanks' balanced salt solution containing $5\% \text{ BSA}$. Insulin release from freshly isolated islets was measured in a perfusion system as described [15, 24]. In this system, 50 islets were housed in small chambers on Millicell culture plate inserts (Sigma, Poole, Dorset, UK). Islets were perfused in basal medium (Krebs–Ringer containing 20 mmol l^{-1} HEPES, $\text{pH} 7.4$, 5 mg ml^{-1} BSA and 2 mmol l^{-1} glucose) for 60 min at a flow rate of 1 ml min^{-1} 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 means $\pm$ SEM, with the numbers of rats or islet preparations in parentheses. Statistical analysis was performed by ANOVA followed by Fisher's post hoc tests for individual comparisons or Student's *t*-test as appropriate (Statview; Abacus Concepts, Berkeley, CA, USA). A *p* value of <0.05 was considered to be statistically significant.

Results

PPAR α activation enables dexamethasone to elicit marked postabsorptive hyperglycaemia in rats maintained on a high-fat diet HF rats treated with dexamethasone (HF-DEX group) did not display significantly higher post-absorptive hyperglycaemia compared with the HF group (Fig. 1a). PPAR α activation by treatment with the PPAR α agonist WY14643 did not affect fasting hyperglycaemia in HF rats (Fig. 1b). In marked contrast, PPAR α activation in combination with dexamethasone treatment led to very marked postabsorptive hyperglycaemia in high-fat-fed rats (HF-DEX-WY group) (Fig. 1b). Postabsorptive glucose levels in the HF-DEX-WY group were 57% higher than those of the HF group, 47% higher than those of the HF-WY group and 41% higher than those of the HF-DEX group.

PPAR α activation improves glucose tolerance in dexamethasone-treated high-fat-fed rats

Blood glucose concentrations at intervals after an intravenous glucose bolus are shown in Fig. 1a and b. Values of

Fig. 1 Interactive effects of dexamethasone and PPAR α activation on parameters of glucose tolerance in high-fat-fed rats in vivo. Blood samples were withdrawn from untreated or dexamethasone-treated rats maintained on a high-saturated-fat diet (HF and HF-DEX groups) immediately before (postabsorptive state) and at intervals after an intravenous glucose bolus for measurement of blood glucose concentrations. Subgroups of HF and HF-DEX groups were administered the PPAR α agonist WY14643 as a single intraperitoneal injection (50 mg kg^{-1} body weight), and rats were sampled after a further 24 h (HF-WY and HF-DEX-WY groups). Blood glucose concentrations before and at intervals after an intravenous glucose bolus in the HF (open symbols) and HF-DEX (closed symbols) groups (a) and in the HF-WY (open symbols) and HF-DEX-WY (closed symbols) groups (b). Glucose responses during the glucose tolerance test were used for calculation of the incremental blood glucose values integrated over the 30-min period after the injection of glucose (ΔG). Rates of glucose disappearance (*k*) were calculated from the slopes of the regression lines obtained with log-transformed glucose values from 2 to 15 min after glucose administration and expressed as $\% \text{ min}^{-1}$. ΔG (c) and *k* values (d) are shown for the HF (open bars), HF-DEX (cross-hatched bars), HF-WY (hatched bars) and HF-DEX-WY (closed bars) groups. Results are means $\pm$ SEM for eight HF, five HF-DEX, six HF-WY and five HF-DEX-WY rats. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 for effects of dexamethasone treatment; †*p* < 0.05 for effects of WY14643 treatment

ΔG (the incremental blood glucose values integrated over the 30-min period after the injection of glucose) and *k* (rate of glucose disappearance) after intravenous glucose challenge are shown in Fig. 1c and d respectively. Dexamethasone treatment of HF rats (HF-DEX group) resulted in a significant loss of glucose tolerance, with significantly increased blood glucose concentrations in HF-DEX rats compared with HF rats at 10 (by 30%; *p* < 0.05), 15 (by

72%; $p < 0.05$) and 30 (by 39%; $p < 0.05$) min after the glucose bolus (Fig. 1a), together with a significantly increased ΔG (by 43%; $p < 0.01$) (Fig. 1c) and a significantly decreased k value (by 60%; $p < 0.001$) (Fig. 1d). PPAR α activation in vivo, elicited by the administration of WY14643, did not affect glucose tolerance in HF rats. Thus, blood glucose concentrations, ΔG and k values after an intravenous glucose load were similar in HF and HF-WY rats (Fig. 1). In contrast, PPAR α activation opposed glucose intolerance elicited by dexamethasone treatment of HF rats, as indicated by the decline in ΔG (Fig. 1c) and the increase in the k value for glucose disappearance (Fig. 1d). Thus, although PPAR α activation in dexamethasone-treated HF rats exacerbates hyperglycaemia in the fasted state, it improves glucose tolerance after an acute glucose load.

PPAR α activation exaggerates postabsorptive hyperinsulinaemia induced by dexamethasone treatment of high-fat-fed rats and augments glucose-stimulated insulin secretion in dexamethasone-treated high-fat-fed rats in vivo

Plasma insulin concentrations before and at intervals after an intravenous glucose bolus are shown in Fig. 2a and b. Dexamethasone treatment of HF rats led to increased postabsorptive insulin concentrations to values >5 -fold higher ($p < 0.001$) than those of the HF group (Fig. 2a). PPAR α activation did not affect postabsorptive insulin levels in HF rats, but further increased postabsorptive hyperinsulinaemia in dexamethasone-treated, high-fat-fed rats (HF-DEX-WY group) (by 35%; $p < 0.05$) (Fig. 2b). Insulin responses to acute intravenous glucose challenge in vivo were used for calculation of acute insulin response (AIR) values (means of suprabasal 2-min and 5-min plasma insulin values) (Fig. 2c) and total suprabasal 30-min incremental areas under the curves for insulin (ΔI) (Fig. 2d). Dexamethasone treatment of HF rats significantly impaired the incremental increase in plasma insulin concentrations 2 and 5 min after the glucose bolus such that the AIR value was suppressed by 37% ($p < 0.05$). Nevertheless, ΔI was unaffected due to significantly higher incremental plasma insulin values at later times (10 and 15 min) after glucose administration ($p < 0.05$) (Fig. 2a). PPAR α activation significantly lowered plasma insulin concentrations 2 and 5 min after the glucose bolus in high-fat-fed rats (by 38 and 34%; $p < 0.05$ in both cases) (Fig. 2b). As a consequence, significant decreases in both AIR (by 39%; $p < 0.05$) and ΔI (by 32%; $p < 0.05$) were observed in HF-WY rats compared with HF rats (Fig. 2c,d). In marked contrast, PPAR α activation in combination with dexamethasone treatment dramatically increased plasma insulin concentrations after the glucose bolus (Fig. 2b), resulting in significant increases in AIR (by 1.7-fold; $p < 0.001$) and ΔI (by 1.8-fold; $p < 0.01$) values in HF-DEX-WY rats compared with HF rats (Fig. 2).

Fig. 2 Interactive effects of dexamethasone and PPAR α activation on basal and glucose-stimulated insulin secretion in high-fat-fed rats in vivo. Group designations and further details, see Fig. 1. Plasma insulin concentrations before and at intervals after an intravenous glucose bolus in the HF (open symbols) and HF-DEX (closed symbols) groups (a) and in the HF-WY (open symbols) and HF-DEX-WY (closed symbols) groups (b). The acute insulin response (AIR) was calculated as the mean of suprabasal 2-min and 5-min plasma insulin values. Insulin 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 (ΔI). AIR (c) and ΔI values (d) for the HF (open bars), HF-DEX (cross-hatched bars), HF-WY (hatched bars) and HF-DEX-WY (closed bars) groups are shown. Results are means $\pm$ SEM for eight HF, five HF-DEX, six HF-WY and five HF-DEX-WY rats. * $p < 0.05$ and *** $p < 0.001$ for effects of dexamethasone treatment; † $p < 0.05$, †† $p < 0.01$, and ††† $p < 0.001$ for effects of WY14643

Dexamethasone treatment of high-fat-fed rats enhances GSIS in perfused islets, an effect attenuated by antecedent PPAR α activation

Contrasting with data obtained in vivo, greatly enhanced peak rates of GSIS were observed ex vivo in perfusions of islets from HF-DEX compared with HF rats (by 2.6-fold; $p < 0.05$) (Fig. 3a). Furthermore, whereas PPAR α activation augmented GSIS in vivo in HF-DEX rats, differences in the GSIS profiles between HF and HF-DEX groups at high glucose were greatly attenuated by PPAR α activation (i.e. after WY14643 treatment), indicating partial reversal of the effects of dexamethasone (Fig. 3b). This implies that the direct effect of PPAR α activation on islet function ex vivo is dominant to that of the direct action of glucocorticoids on islet function ex vivo. To allow direct comparison of insulin secretory responses during the step-up and step-down phases of the islet perfusions, areas under the insulin

Fig. 3 Interactive effects of dexamethasone treatment and PPAR α activation on glucose responsiveness of insulin secretion by perfused islets from high-fat-fed rats. Islets isolated from the four groups of rats (designations as in Fig. 1) were perfused in basal medium containing 2 mmol l⁻¹ glucose for 60 min at a flow rate of 1 ml min⁻¹ at 37°C prior to collection of fractions. Perfusate 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. Rates of insulin release by islets isolated from HF (open symbols) and HF-DEX (closed symbols) rats (a). Rates of insulin release by islets isolated from HF-WY (open symbols) and HF-DEX-WY (closed symbols) rats (b). Results are means $\pm$ SEM for five HF, five HF-DEX, six HF-WY and five HF-DEX-WY rats. Statistically significant effects, see text

curves were calculated for discrete 16-min periods during which the perfusate glucose concentrations were rising: $\Delta 1$, during which the perfusate glucose concentration varied between 2 and 8 mmol l⁻¹, and $\Delta 2$, during which the perfusate glucose concentration varied between 8 and 16 mmol l⁻¹. Corresponding areas under the insulin curves over the equivalent 16-min periods of perfusion after switching to basal glucose are referred to as $\Delta 3$ and $\Delta 4$. Data are shown in Fig. 4. Reflecting glucocorticoid-induced increased glucose responsiveness at low extracellular glucose concentrations, $\Delta 1$ values for perfused islets from HF-DEX rats were significantly higher (8.3-fold; $p < 0.001$) than those of HF rats. In addition, $\Delta 2$, $\Delta 3$ and $\Delta 4$ values from HF-DEX islets exceeded corresponding values for the HF group (2.8-fold [$p < 0.001$], 2.9-fold [$p < 0.001$] and 5.3-fold [$p < 0.01$], respectively), indicating increased efficacy of glucose stimulation of insulin secretion in vitro as a consequence of dexamethasone treatment, particularly at low compared with high stimulatory glucose levels. Antecedent PPAR α activation did not significantly affect $\Delta 1$ or $\Delta 4$ values for islets from HF rats, whereas it significantly lowered $\Delta 2$ and $\Delta 3$ values (by 68% [$p < 0.05$] and 55% [$p < 0.05$], respectively), indicating that PPAR α activation predominantly targets GSIS at high stimulatory glucose levels. In contrast, PPAR α activation significantly lowered $\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$ values for GSIS in the HF-DEX group (by 74% [$p < 0.01$], 69% [$p < 0.001$], 60% [$p < 0.001$] and 86% [$p < 0.01$], respectively). Thus, in summary, while the effect of PPAR α activation to suppress GSIS in HF rats is reiterated in isolated perfused islets, an effect of dexamethasone treatment to enhance GSIS is observed only in perfused islets. Furthermore, the effect of

Fig. 4 Effects of dexamethasone and/or WY14643 treatment on individual areas under the insulin curves during perfusion of islets from high-fat-fed rats. To allow direct comparison of insulin secretory responses, areas under the insulin curves were calculated for discrete 16-min periods during which the perfusate glucose concentrations were rising: $\Delta 1$: perfusate glucose concentration between 2 and 8 mmol l⁻¹; $\Delta 2$: perfusate glucose concentration between 8 and 16 mmol l⁻¹. After exposure to 16 mmol l⁻¹ glucose, perfusate glucose was then lowered to basal levels by switching to basal (2 mmol l⁻¹ glucose) medium. Corresponding areas under the insulin curves over the equivalent 16-min periods of perfusion after switching to basal glucose are referred to as $\Delta 3$ and $\Delta 4$. a–d $\Delta 1$, $\Delta 2$, $\Delta 3$ and $\Delta 4$ values, respectively, for the HF (open bars), HF-DEX (cross-hatched bars), HF-WY (hatched bars) and HF-DEX-WY (closed bars) groups. Data are means $\pm$ SEM for five HF rats, five HF-DEX rats, five HF-WY rats and five HF-DEX-WY rats. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ for effects of dexamethasone treatment; † $p < 0.05$, †† $p < 0.01$ and ††† $p < 0.001$ for effects of WY14643 treatment

PPAR α activation to suppress GSIS in HF rats dominates over the effect of dexamethasone to elevate GSIS when GSIS is examined *ex vivo*.

Dexamethasone treatment of high-fat-fed rats does not affect circulating plasma lipid concentrations, but elevates plasma leptin concentrations

To identify potential factors that might suppress GSIS in dexamethasone-treated high-fat-fed rats *in vivo*, we examined effects of dexamethasone treatment on circulating lipids (which can augment or impair GSIS, depending on the period of exposure) and on leptin, which in general suppresses GSIS. Dexamethasone treatment of high-fat-fed rats did not modulate circulating fatty acid concentrations (HF, $0.48 \pm 0.08 \text{ mmol l}^{-1}$, $n=9$; HF-DEX, $0.53 \pm 0.19 \text{ mmol l}^{-1}$, $n=6$) or triacylglycerol concentrations (HF, $0.82 \pm 0.15 \text{ mmol l}^{-1}$, $n=9$; HF-DEX, $0.57 \pm 0.18 \text{ mmol l}^{-1}$, $n=6$). In contrast, dexamethasone treatment elicited a significant and dramatic 4.8-fold increase in plasma leptin levels (HF, $5.8 \pm 1.7 \text{ ng ml}^{-1}$, $n=9$; HF-DEX, $28.1 \pm 3.6 \text{ ng ml}^{-1}$, $n=6$; $p < 0.001$).

Discussion

Normally, in healthy human subjects, changes in insulin sensitivity are compensated by reciprocal changes in GSIS such that the product of insulin sensitivity and insulin secretion remains unchanged (reviewed in [25]). This is reiterated in Wistar rats fed a high level of saturated fats. Although very long-term (10 months) high-saturated-fat feeding of Wistar rats leads to severe insulin resistance, overt diabetes does not develop [26]. In the high-saturated-fat feeding protocol of Wistar rats used in the present study (female Wistar rats; moderate lipid content; 4 weeks of dietary manipulation), euglycaemic-hyperinsulinaemic clamp studies have demonstrated peripheral insulin resistance, but unimpaired hepatic insulin sensitivity [11], and enhanced insulin secretion that allows maintenance of glucose tolerance [15]. Glucocorticoids impair glucose homeostasis by opposing insulin's actions to suppress hepatic glucose production and stimulate peripheral glucose uptake. In the present study, we observed that dexamethasone treatment of rats fed a high level of saturated fats elicited glucose intolerance after an acute intravenous glucose challenge. This response was observed in conjunction with fasting hyperinsulinaemia, but a blunted initial response of insulin secretion to a rise in glycaemia. Overall, the GSIS *in vivo* in response to an acute glucose challenge (ΔI value) was not diminished, but neither was it enhanced sufficiently to prevent glucose intolerance. The finding that GSIS in perfused islets from dexamethasone-treated, high-fat-fed rats exceeds that of high-fat-fed rats not treated with dexamethasone implies a degree of islet adaptation for the additional impairment in insulin action introduced by dexamethasone treatment. Our data imply that

insulin resistance induced by high-fat feeding is exacerbated by glucocorticoid excess and, although our studies with perfused islets demonstrated that dexamethasone treatment exerts a persistent effect on the islet, which allows augmented GSIS *ex vivo*, additional factors must operate in the intact organism to oppose the glucocorticoid-induced enhancement of islet insulin secretion that is identified with perfused islets. At present, the identity of these factors remains to be established, but they presumably relate to interactions between increased dietary lipid supply and glucocorticoid excess, interactions which may interrupt the negative feedback loop between insulin sensitivity and secretion *in vivo*. Although we observed that dexamethasone treatment of high-fat-fed rats did not modulate circulating fatty acid or triacylglycerol concentrations, plasma leptin levels were dramatically increased. Since leptin is known to suppress GSIS [27] it may be involved in uncoupling insulin secretion from insulin action. As the disruption of feedback between insulin sensitivity and secretion *in vivo* in dexamethasone-treated, high-fat-fed rats precipitates the development of glucose intolerance, it reproduces the transition from insulin resistance with islet compensation to type 2 diabetes.

In the present study, neither PPAR α activation alone nor dexamethasone treatment alone exerted any major influence on fasting glycaemia in high-fat-fed rats. However, marked fasting hyperglycaemia was observed in high-fat-fed rats by the combination of dexamethasone treatment and PPAR α activation. Hepatic lipid oxidation is augmented by PPAR α activation, and this provides ATP and cofactors to sustain or enhance flux through the gluconeogenic pathway, the capacity of which is established to be increased in response to glucocorticoids. Like the action of the glucocorticoids, PPAR α activation is an integral component of normal starvation adaptation, as demonstrated from the development of fasting hypoglycaemia in PPAR α -null mice [7]. Hence the hepatic adaptation to starvation, namely enhanced hepatic glucose production, may be reproduced by exposure to excess glucocorticoids in combination with PPAR α activation under conditions of high-fat feeding, even though dietary carbohydrate is available and energy intake is not compromised, with resultant fasting hyperglycaemia.

PPAR α activation enhances insulin sensitivity in peripheral tissues of high-fat-fed rats [16]. This reduces the necessity for compensatory insulin hypersecretion to maintain glucose tolerance [15]. Thus, as shown in the present experiments, PPAR α activation *in vivo* by the administration of WY14643 did not affect glucose tolerance (as reflected by incremental blood glucose values integrated over the 30-min period after the injection of glucose [ΔG] and rates of glucose disappearance [k] values) in HF rats, despite lowered GSIS (which is observed both *in vivo* after a glucose bolus and *ex vivo* in islets perfused at high glucose concentrations). Since antecedent PPAR α activation *in vivo* results in a stable lowering of GSIS in perfused islets isolated from high-fat-fed rats, it is implied that increased GSIS in perfused islets from high-fat-fed rats reflects a stable islet adaptation enabling compensatory insu-

lin secretion. The observation with ex-vivo perfused islets that antecedent PPAR α activation elicits partial reversal of the stable effect of dexamethasone treatment of high-fat-fed rats to augment GSIS is consistent with stable PPAR α -induced modification of islet function that opposes augmentation of islet GSIS, which may be linked to improved peripheral insulin action in vivo as a consequence of PPAR α activation.

PPAR α activation slightly exaggerated the fasting hyperinsulinaemia elicited in high-fat-fed rats in response to dexamethasone treatment. More importantly, in contrast to the data obtained ex vivo using perfused islets, antecedent PPAR α activation greatly augmented GSIS in dexamethasone-treated high-fat-fed rats in vivo, such that glucose tolerance was improved. Thus, within the context of high-fat feeding, PPAR α activation in vivo restores the inter-organ operation of the negative feedback loop between insulin action and secretion that is interrupted by dexamethasone treatment.

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