Tissue and isoform-selective activation of protein kinase C in insulin-resistant obese Zucker rats – effects of feeding

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Abstract

The mechanisms of insulin resistance in the obese Zucker rat have not been clearly established but increased diacylglycerol-protein kinase C (DAG-PKC) signalling has been associated with decreased glucose utilisation in states of insulin resistance and non-insulin-dependent diabetes mellitus. The purpose of this study was to characterise tissue- and isoform-selective differences in DAG-PKC signalling in insulin-sensitive tissues from obese Zucker rats, and to assess the effects of feeding on DAG-PKC pathways. Groups of male obese (fa/faq, n=24) and lean (fa/−, n=24) Zucker rats were studied after baseline measurements of fasting serum glucose, triglycerides, insulin and oral glucose tolerance tests. Liver, epididymal fat and soleus muscle samples were obtained from fed and overnight-fasted rats for measurements of DAG, PKC activity and individual PKC isoforms in cytosol and membrane fractions. Obese rats were heavier (488 ± 7 vs 315 ± 9 g) with fasting hyperglycaemia (10.5 ± 0.8 vs 7.7 ± 0.1 mM) and hyperinsulinaemia (7167 ± 363 vs 251 ± 62 pM) relative to lean controls. In fasted rats, PKC activity in the membrane fraction of liver was significantly higher in the obese group (174 ± 16 vs 108 ± 12 pmol/min/mg protein, P<0.05) but there were no differences in muscle and fat. The fed state was associated with increased DAG levels and threefold higher PKC activity in muscle tissue of obese rats, and increased expression of the major muscle isoforms, PKC-α and PKC-ε: e.g. PKC activity in the membrane fraction of muscle from obese animals was 283 ± 42 (fed) vs 107 ± 20 pmol/min/mg protein (fasting) compared with 197 ± 27 (fed) and 154 ± 21 pmol/min/mg protein (fasting) in lean rats. In conclusion, hepatic PKC activity is higher in obese rats under basal fasting conditions and feeding-induced activation of DAG-PKC signalling occurs selectively in muscle of obese (fa/faq) rats due to increased DAG-mediated activation and/or synthesis of PKC-α and PKC-ε. These changes in PKC are likely to exacerbate the hyperglycaemia and hypertriglyceridaemia associated with obesity-induced diabetes.

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Introduction

There is a negative linear relationship between body mass index and whole-body insulin sensitivity (Ferrannini et al. 1997). Obese patients and especially those with a high waist-to-hip ratio are insulin resistant and develop a compensatory increase in circulating plasma insulin concentrations. Type 2 (non-insulin-dependent) diabetes mellitus (NIDDM) often supervenes when the pancreatic insulin response is either defective or insufficient to maintain normal glucose tolerance.

The insulin resistance associated with obesity and diabetes affects multiple pathways of glucose and lipid metabolism in peripheral tissues (i.e. muscle/fat) and the liver. The underlying biochemical mechanisms have not been clearly established (Moule & Denton 1997), but most of the available evidence points to defects at or beyond the insulin receptor and in muscle tissue there is a strong link between insulin resistance and increased lipid availability (i.e. triglyceride and diglyceride levels) (Pan et al. 1997). Boneh (1995) has proposed that accumulation of lipid metabolites, via activation of protein kinases such as protein kinase C (PKC), leads to dysregulated substrate phosphorylation and cellular function. Thus, since the reversible addition of phosphate groups by intracellular kinases and phosphatases is an important mechanism for regulating cell-surface receptors and metabolising enzymes, it has been further suggested that changes in the phosphorylation state of key elements in the insulin signalling cascade may contribute to the down-regulation of insulin-mediated responses observed in obesity and other insulin-resistant states (Cortright et al. 1997).

The serine/threonine-specific protein kinase, PKC, has been particularly implicated in the pathogenesis of insulin resistance (Considine & Caro 1993, Shmueli et al. 1993). For example, increased diacylglycerol (DAG)-mediated
PKC activation has been associated with insulin resistance in humans and animals with diabetes (Considine et al. 1995), and there is evidence of PKC activation in models of obesity (Avignon et al. 1996), ageing (Ishizuka et al. 1993), muscle denervation (Heydrick et al. 1991) and dietary-induced insulin resistance (Donnelly et al. 1994, Schmitz-Peiffer et al. 1997). There is also evidence that hyperglycaemia-induced insulin receptor inhibition is mediated by PKC (Berti et al. 1994). The mechanisms by which PKC activation may down-regulate one or more steps involved in glucose transport and metabolism include decreased autophosphorylation and tyrosine kinase activity of the insulin receptor (Kariski et al. 1990, Kellereit al. 1997), inactivation of glycogen synthase (Blackmore et al. 1986), inhibition of insulin-stimulated Akt1 and Akt3 activity (Barthel et al. 1998), increased insulin receptor degradation (Mostaf et al. 1998) and increased phosphorylation of insulin receptor substrate-1 (Busch et al. 1998).

PKC represents a family of 12 isoenzymes with different biochemical characteristics, substrates and cofactor (calcium and/or phospholipid) requirements (Hug & Sarre 1993), and the novel (group B) PKCs (nPKC-δ, -ε, θ and η) have been particularly implicated in insulin resistance (Donnelly et al. 1994, Kellereit al. 1997, Schmitz-Peiffer et al. 1997). The obese Zucker rat is an excellent model of early-stage NIDDM induced by insulin resistance, overeating and overweight. The model is homozygous for the fatty (fa) mutation in the leptin receptor (the same gene is mutated in the diabetes (db) mouse (Chua et al. 1996)), and the insulin resistance affects primarily muscle with failure of glucose transporter function (King et al. 1992). The purpose of this study was to characterise changes in DAG-PKC signalling in tissues from the obese Zucker rat, and to evaluate whether PKC activity and isoform distribution is influenced by feeding.

Materials and Methods

Animals

Male obese fa/fa Zucker rats (n=24) and lean non-obese fa/- controls (n=24) were obtained from Monash University Animal Supplies (Victoria, Australia) at 9 weeks of age and housed under controlled conditions of temperature (21 °C) and lighting (12 h light–darkness cycle, lights on 0600 h) with free access to water and standard laboratory chow. The experimental protocol was approved by the University of Sydney Animal Care and Ethics Committee.

Metabolic measurements

Blood samples were drawn from the tail vein of unanaesthetised rats for measurements of fasting serum glucose, insulin and triglyceride (TG) concentrations. Fasting glucose levels were measured at weekly intervals until the obese Zucker rats became diabetic at approximately 13 weeks of age. Thereafter, following a 5-h fast, oral glucose tolerance tests (OGTT) were performed on groups of obese (fa/fa) (n=24) and lean (fa/-) (n=24) animals. Tail vein blood samples were collected at baseline and at 30, 60, 90, 120 and 180 min after an oral glucose load (2 g/kg) administered by gavage.

Collection of tissue samples in fed and fasted conditions

Two days after the OGTT, obese and lean Zucker rats were subdivided into two further groups, overnight-fasted (n=12) and fed (n=12) animals, prior to exsanguination at 0900 h. Tissue samples (liver, epididymal fat pad and soleus muscle) were quickly excised following decapitation, snap-frozen in liquid N2 and stored at −70 °C for subsequent biochemical assays of PKC activity, DAG levels and Western blotting in cytosol and membrane (particulate) fractions. Tissue samples were homogenised in a Polytron (Brinkman Instruments, Westbury, NY, USA) in 100 mg/ml buffer A (containing 20 mM Tris–HCl, pH 7·4, 1 mM EDTA, 0·25 mM EGTA, 2 µg/ml leupeptin, 4 µg/ml pepstatin, 4 µg/ml each of calpain I and II inhibitors, 0·2 mM phenylmethylsulphonyl fluoride and 0·25 M sucrose). For subfractionation of the crude homogenate into cytosol and particulate fractions, the samples were first spun at 400 × g for 15 min. The supernatant for liver and muscle, and the infranatant below the fat cake for adipose tissue, were then ultracentrifuged at 105 000 × g for 60 min. The supernatant was removed as the cytosol fraction; the sediment (particulate fraction) was resuspended in 0·5 ml buffer A, then mixed with 0·5 ml buffer B (buffer A without sucrose) containing 2% Triton X–100 and solubilised for 30 s in a sonic dismembrator at 4 °C. An aliquot of each fraction was saved for protein quantification (Bradford 1976), and cytosol and particulate samples were then used for biochemical assays of PKC activity, DAG content and Western blotting.

Protein kinase C activity

PKC activity is virtually undetectable in crude subcellular fractions but is expressed following DEAE-cellulose chromatography to remove inhibitors, phosphatases and other protein kinases. Thus, PKC activity was measured in partially purified preparations of cytosolic and particulate (membrane) fractions, as described previously (Azhar 1991, Donnelly et al. 1994). In brief, the supernatant and particulate fractions were applied to DEAE-Sephacryl columns (Pharmacia, NSW, Australia) and eluted in 3-ml fractions from the column using buffer B containing 0·15 M NaCl. Enzyme activity was assayed immediately after completion of the chromatography step by following the incorporation of [32P] from [γ-32P]ATP into glycogen synthase (GS). The standard incubation mixture consisted of the following components in a final volume of 100 µl:
20 μl aliquots of chromatography fractions, 25 mM PIPES-NaOH (pH 6.8), 10 mM magnesium acetate, 20 μM GS peptide, 5 mM 2-mercaptopethanol, 0·1 mM [γ-32P]ATP (200–300 c.p.m./nmol), 1·0 mM CaCl2, 150 μg/ml phosphatidylserine and, and 10 μg/ml diolein. Basal activity was determined in the presence of 0·5 mM EGTA (instead of Ca2+, phosphatidylserine and diolein). The reaction was initiated by the addition of [γ-32P]ATP at 30 °C and terminated after 15 min by spotting 50 μl of the mixture onto 2 × 2 cm phosphocellulose strips (Whatman P81) which were dropped immediately into 75 mM phosphoric acid. The strips were then washed in 75 mM phosphoric acid, dried and counted in a scintillation counter (Beckman LS No. 3801). PKC activity was calculated by subtracting the enzyme activity observed in the presence of 0·5 mM EGTA from that measured in the presence of phosphatidylserine, diolein and calcium. One unit of PKC activity is defined as that amount catalysing the transfer of 1 pmol [32P]phosphate from [γ-32P]ATP to GS peptide per min at 30 °C.

**Western blotting**

Aliquots of the cytosol and particulate fractions were mixed with equal volumes of 2 × sample-loading buffer (4·6% (w/v) sodium dodecyl sulphate (SDS), 10% (v/v) β-mercaptopethanol, 16% (w/v) sucrose, and 0·1 M Tris–HC1, pH 6·8), heated at 95 °C for 5 min, and cooled to room temperature. The mixture was then centrifuged at 5000 × g for 5 min, and the supernatant subjected to sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Laemmli (1970). For each sample, 25 μg total protein were loaded onto the gel together with one lane of protein standards per gel (Bio-Rad Laboratories, Richmond, CA, USA). Following electrophoretic separation, proteins were transferred to Immobilon-P membranes (Millipore, Bedford, MA, USA) in transfer buffer containing 10 mM CAPS (3-[cyclohexylamino]-1-propane sulphonic acid) and 10% methanol, pH 11. The membrane was then incubated for 12 h at 4 °C in phosphate-buffered saline-Tween (PBS-T) containing 5% (w/v) non-fat dried milk, pH 7·4. After the blocking step, membranes were washed in rinsing solution (PBS-T with 1% dried milk, pH 7·4) and then incubated overnight with an affinity-purified polyclonal antibody specific for the θ (Donnelly et al. 1994) and ε (Transduction Laboratories, MA, USA) isozenzyme forms of PKC. After further washings, membranes were incubated with horseradish peroxidase-conjugated immunoglobulin G (IgG) fraction of goat antirabbit IgG, diluted 1:20 000 in PBS-T. The membranes were then washed several times in PBS-T, incubated with alkaline phosphatase-streptavidin–conjugated antirabbit IgG and developed for 60 s using a commercial enhanced chemiluminescence kit (Zymed Laboratories, San Francisco, CA, USA). Each film was exposed for 60 s. The bands obtained from immuno-blotting were scanned by one-dimensional laser densitometry and the areas under the peaks were analysed using the Gelscan XL software package.

**Measurement of 1,2-diacylglycerol (DAG)**

DAG was extracted by the method of Bligh and Dyer (1959) and measured by a slightly modified version of the DAG kinase procedure of Preiss et al. (1987). Dioleoylglycerol was used for the standard curve. Briefly, after extraction the dried lipid extracts were solubilised by sonication in 20 μl of a solution containing octyl–β–d glucoside (7·5%) and cardiolipin (5 μm) in 1 mM diethylenetriamine pentacetic acid (DETAPAC). After 15 min incubation, 50 μl 2 × reaction buffer (100 mM imidazole–HC1, pH 6·8, 100 mM NaCl, 25 mM MgCl2, 2 mM EGTA) and 10 μl 20 mM dithiothreitol were mixed with the solubilised lipid/octylglycoside solution and purified DAG kinase to give a final volume of 90 μl. The reaction was started by addition of 10 μl [γ-32P]ATP (specific activity 5 × 105 c.p.m./nmol) in 10 mM imidazole, 1 mM DETAPAC, pH 6·6, and allowed to proceed at 25 °C for 30 min. The reaction was stopped by adding 3 ml chloroform/methanol (1:2, v/v) and 0·7 ml 1% HClO4. After addition of 1 ml CHCl3 and 1 ml 1% perchloric acid, and brief centrifugation, the lower chloroform phase was washed and then dried under N2. The lipid film was dissolved in 5% methanol in chloroform and 50 μl spotted on to a 20 cm Silica Gel 60 TLC plate activated by prerunning in acetone. Samples of [γ-32P]phosphatidic acid made from dioleoylglycerol were also spotted onto the TLC plates as standards. The plates were then developed with chloroform:methanol:acetic acid (65:15:5, v/v), air dried and subjected to autoradiography. The radioactive spot corresponding to phosphatidic acid (Rf=0·35) was scraped into a scintillation vial, mixed with 6 ml scintillation fluid, and counted.

**Other assays**

Serum glucose and triglyceride levels were measured using enzymatic colorimetric methods (Trinder Glucose kit and GPO–Trinder Tryglyceride kit, Sigma, St Louis, MO, USA). Serum insulin concentrations were measured by double-antibody radioimmunoassay using rat insulin standards (0–8 ng/ml range) and a guinea pig anti-rat insulin primary antibody (Linco Research Inc., St Louis, MO, USA).

**Statistical analysis**

Serum glucose concentration– and insulin concentration–time profiles for the oral glucose tolerance tests were compared by ANOVA. Measurements of fasting serum glucose, insulin and triglyceride concentrations were analysed by one factor ANOVA. All measurements are
Table 1  Comparison of fasting serum glucose, triglyceride and insulin concentrations in obese fa/fa (n=24) and lean fa/- (n=24) rats at 10 and 13 weeks of age. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>10 weeks Lean</th>
<th>Obese</th>
<th>13 weeks Lean</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>266 ± 11</td>
<td>315 ± 9</td>
<td>315 ± 9</td>
<td>488 ± 7*</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>7.1 ± 0.2</td>
<td>7.7 ± 0.1</td>
<td>7.7 ± 0.1</td>
<td>10.5 ± 0.81**</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.57 ± 0.04</td>
<td>1.43 ± 0.05</td>
<td>1.43 ± 0.05</td>
<td>4.08 ± 0.16***</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>208 ± 85</td>
<td>251 ± 62</td>
<td>251 ± 62</td>
<td>7167 ± 363***</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01; ***P<0.001 vs corresponding data from lean control rats.

Expressed as means ± standard error (S.E.M.) and statistical significance was accepted at the 5% level.

Results

Metabolic characteristics of obese fa/fa Zucker rats

Obese rats at 10 weeks of age had fasting serum glucose concentrations within the normal range but fasting triglyceride and insulin levels were significantly higher than in lean (fa/-) controls. At 13 weeks, the genetically obese fa/fa Zucker rats were significantly overweight (488 ± 7 vs 315 ± 9 g) with marked hyperinsulinaemia and hypertriglyceridaemia compared with their lean counterparts (Table 1). Furthermore, by 13 weeks of age the obese rats had become diabetic (Fig. 1). Following an oral glucose tolerance test, the area-under-the-curve (AUC) of the serum glucose concentration-time profile was significantly higher in obese rats; AUC[glu] was 47.4 ± 13.3 mmol.h/l in obese rats vs 25.0 ± 1.6 mmol.h/l in lean controls (P<0.001, Fig. 1). The insulin response to the oral glucose stimulus was also significantly higher: AUC[insulin] was 20 644 ± 1128 mmol.h/l for obese rats compared with 2392 ± 327 pmol.h/l for lean rats (P<0.001, Fig. 1).

PKC activity in insulin target tissues in fed and fasted conditions

PKC activity was generally lower in the cytosol fraction compared with the membrane fraction of all three tissues (liver, soleus muscle and fat) in both obese and lean rats (Table 2). In fasted animals, PKC activity in the membrane fraction of liver was significantly higher in obese compared with lean rats but there were no significant differences in muscle and fat (Table 2).

In obese Zucker rats, feeding was associated with almost a threefold increase in PKC activity in the membrane fraction of skeletal muscle but no increase in liver or fat tissue (Table 2). In lean rats, however, PKC activity in the cytosol and membrane fractions of soleus muscle was similar in fed and fasted conditions.

Discussion

These results show that there are tissue- and isoform-specific differences in PKC signalling in obese (fa/fa) compared with lean (fa/-) Zucker rats under fed and fasted conditions. Obese diabetic animals in the basal fasting state had higher PKC activity in liver but not muscle or fat. Similar 40% increases in membrane-associated PKC were reported in liver biopsy specimens from obese patients with NIDDM (Considine et al. 1995), and in parallel experiments in rats these authors showed that the increase in liver PKC in NIDDM is not secondary to hyperglycaemia. Given that PKC activation in hepatocytes reduces insulin-stimulated glycogen and lipid synthesis (Caro et al. 1994, Schmitz-Peiffer et al. 1997). The results show that in obese rats there is increased expression of PKC-δ and especially PKC-ε in the membrane fraction of soleus muscle in the fed compared with the fasting state (Figs 2 and 3, and Table 3). Under fasted conditions, there were no differences in isoform expression in muscle between obese and lean animals.

To assess whether changes in DAG may be responsible for the increased activation of PKC, and the increased expression of nPKCs in the membrane fraction associated with feeding, DAG content was measured in the cytosol and membrane fractions of soleus muscle from obese and lean rats. In the fasting state, DAG levels were significantly higher in the cytosol fraction of muscle from obese rats, but absolute levels of DAG were higher in the membrane fraction, especially in the fed state (Table 4).

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increase in PKC activity in the membrane fraction of red muscle. In contrast, feeding had no effect on muscle PKC activity in lean animals, and there were no changes in PKC activity in liver or fat in response to food intake. The explanation for the tissue-selective increase in PKC activity after overnight feeding is not entirely clear, and of course this study did not measure PKC activity in situ, but there was a parallel increase in DAG levels and evidence of DAG-mediated activation of the two major isoenzymic forms of PKC in skeletal muscle, PKC-θ and PKC-ε. There was no direct evidence of translocation, since cytosolic PKC enzyme activity was unchanged. However, PKC activation in the membrane fraction can occur without translocation, and these data would also be consistent with increased protein synthesis of PKC-θ and PKC-ε in the fed state.

PKC-θ is the major isoenzyme of PKC in muscle (Osada et al. 1992); we have previously shown that expression of PKC-θ varies between muscles of different fibre-type composition and insulin sensitivity, and that expression of this isoform is increased in muscle tissue from rats with fructose-induced insulin resistance and dyslipidaemia (Donnelly et al. 1994). It has recently been shown that PKC-θ has an especially strong inhibitory effect on insulin receptor signalling which involves phosphorylation of IRS-1 (Kellerer et al. 1998). Increased expression of PKC-ε and PKC-θ in muscle has also been reported in the high-fat-fed rat (Schmitz-Pfeiffer et al. 1997), which is another (nonobese) dietary model of insulin resistance associated with increased lipid levels in muscle tissue which parallel the decline in whole-body insulin sensitivity (Storlien et al. 1991). The work by Schmitz-Pfeiffer et al. (1997) showed evidence of chronic activation of PKC-ε in high-fat-fed Wistar rats, whereas their data for PKC-θ suggested a combination of chronic activation and down-regulation because PKC-θ expression was reduced in the cytosol fraction. The present study reports slightly different changes in muscle PKC in response to acute feeding under conditions of a normal dietary intake, whereas Schmitz-Pfeiffer et al. (1997) were concerned with chronic feeding of an experimental high-fat diet.

Figure 1 Serum glucose (A) and insulin (B) responses to an oral glucose load (2 g/kg body weight) by gavage in fasted obese (fa/fa) (●, n=12) and lean fa/- (○, n=12) Zucker rats. Values are means ± S.E.M.

The significance of increased expression of PKC-ε in obese compared with lean animals in the present study may be relevant to the role of tumour necrosis factor α (TNFα) in obesity. Secretion of TNFα from adipocytes of obese rats inhibits glucose uptake in skeletal muscle (Hotamisligil et al. 1993) and there is evidence that PKC-ε enhances the inhibitory effect of TNFα on insulin signalling (Kellerer et al. 1997). Thus, enhanced DAG-mediated activation of PKC-θ and PKC-ε in the muscles of obese Zucker rats under postprandial conditions may, via several mechanisms, down-regulate insulin signalling and glucose disposal and worsen the hyperglycaemia observed in these animals.

The notion that DAG-mediated PKC activation contributes to the pathogenesis of obesity-induced insulin
resistance provides an attractive mechanism linking dysregulated lipid metabolism with resistance to insulin-mediated glucose utilisation, especially in muscle (Cortright et al. 1997). Accumulation of naturally occurring lipid metabolites, e.g. unesterified free fatty acids and their coenzyme A esters, activates PKC synergistically with DAG (Shimomura et al. 1991). The substrates phosphorylated at serine and threonine residues in response to PKC activation have largely been identified in vitro, but there is evidence that the insulin receptor tyrosine kinase on the \( \beta \)-subunit is an important target (Karisik et al. 1990, Kellerer et al. 1997), as well as phosphorylation of IRS-1 (De Fea & Roth 1997) and inhibition of insulin-stimulated Akt1 and Akt3 activity (Barthel et al. 1998). Several previous studies have demonstrated impaired tyrosine kinase activity in the Zucker fatty rat insulin receptor (Slieker et al. 1990), but whether PKC activation accounts for this reduction in the tyrosine kinase activity in vivo requires further investigation (Bassemmaier et al. 1997).

Because the majority of whole-body glucose disposal in the fed state is accounted for by skeletal muscle (approximately 85%), PKC-mediated phosphorylation and down-regulation of glycogen synthase (Blackmore et al. 1993) and the PKC isoforms PKC-\( \alpha \) (79 kDa) and PKC-\( \beta \) (95 kDa) (Figure 2 and Figure 3) contribute to insulin resistance.

Table 2: PKC activity (pmol/min/mg protein) in cytosol and membrane (particulate) fractions of three insulin target tissues from obese fa/fa and lean fa/- Zucker rats under fed and overnight-fasted conditions. Values are means ± s.e.m.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Animal</th>
<th>Cytosol</th>
<th>Membrane</th>
<th>Cytosol</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus muscle</td>
<td>Obese</td>
<td>49 ± 9</td>
<td>107 ± 20</td>
<td>46 ± 13</td>
<td>283 ± 42*†</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>47 ± 6</td>
<td>154 ± 21</td>
<td>57 ± 16</td>
<td>179 ± 27</td>
</tr>
<tr>
<td>Liver</td>
<td>Obese</td>
<td>37 ± 5</td>
<td>174 ± 16*</td>
<td>47 ± 4</td>
<td>186 ± 22</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>28 ± 3</td>
<td>108 ± 12</td>
<td>50 ± 5</td>
<td>127 ± 16</td>
</tr>
<tr>
<td>Epididymal fat</td>
<td>Obese</td>
<td>47 ± 11</td>
<td>199 ± 48</td>
<td>99 ± 26</td>
<td>255 ± 42</td>
</tr>
<tr>
<td></td>
<td>Lean</td>
<td>42 ± 9</td>
<td>173 ± 45</td>
<td>92 ± 15</td>
<td>234 ± 35</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \) compared with corresponding value for lean rats; † \( P < 0.02 \) vs membrane of soleus muscle from fasted rats.

Figure 2: Western blots of PKC-\( \alpha \) (79 kDa) in cytosol and membrane fractions of soleus muscle from obese (O) and lean (L) Zucker rats under fed and fasting conditions. Typical blots (A) and summary of densitometric data (B): lean (open bars, \( n = 12 \)) and obese (solid bars, \( n = 12 \)) groups are shown. *, \( P < 0.01 \) vs lean.

Figure 3: Western blots of PKC-\( \varepsilon \) (95 kDa) in cytosol and membrane fractions of soleus muscle from obese (O) and lean (L) Zucker rats under fed and fasting conditions. Typical blots (A) and summary of densitometric data (B): lean (open bars, \( n = 12 \)) and obese (solid bars, \( n = 12 \)) groups are shown. *, \( P < 0.01 \) vs lean.
1986) may also be an important consequence of the PKC activation observed in muscles of obese rats under fed conditions. In addition, local formation of nitric oxide (NO) stimulates glucose uptake in isolated rat skeletal muscle and isoform-specific upregulation of DAG-PKC signalling in experimental obesity. In conclusion, the obese Zucker rat is associated with abnormalities associated with obesity and diabetes.

### Acknowledgements

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### References


Bartel A, Nakatani K, Dandekar AA & Roth RA 1998 Protein kinase C modulates the insulin-stimulated increase in Akt1 and Akt3 activity in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications* 243 509–513.


### Table 4 DAG levels (nmol/mg protein) in cytosol and membrane fractions of soleus muscle tissue from obese fa/fa and lean fa/- Zucker rats under fed and fasted conditions. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Lean fa/-</th>
<th>Obese fa/fa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>1·42 ± 0·12</td>
<td>2·30 ± 0·20*</td>
</tr>
<tr>
<td>Membrane</td>
<td>9·07 ± 0·48</td>
<td>10·54 ± 1·55</td>
</tr>
</tbody>
</table>

*P<0·05 compared with corresponding data from lean control.

### Table 3 Comparison of densitometric data for two nPKC isoforms, PKC-θ and PKC-ε, from Western blots of cytosol and membrane fractions of soleus muscle from obese fa/fa rats relative to lean fa/- controls. Data are means ± S.E.M. The ratio is calculated using the lean sample as control for each obese sample. The samples were analysed in parallel and subjected to SDS-PAGE on the same gel.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Fraction</th>
<th>Fasted (n=12)</th>
<th>Fed (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKC-θ</td>
<td>Cytosol</td>
<td>1·01 ± 0·18</td>
<td>1·55 ± 0·39</td>
</tr>
<tr>
<td>Membrane</td>
<td>1·03 ± 0·18</td>
<td>1·45 ± 0·17*</td>
<td></td>
</tr>
<tr>
<td>PKC-ε</td>
<td>Cytosol</td>
<td>0·89 ± 0·57</td>
<td>1·54 ± 0·61*</td>
</tr>
<tr>
<td>Membrane</td>
<td>1·02 ± 0·10</td>
<td>2·64 ± 0·69**</td>
<td></td>
</tr>
</tbody>
</table>

*P<0·05, **P<0·01 compared with corresponding data in the fasted state.


Kellerer M, Mushack J, Seffer E, Mischak H, Ullrich A & Haring HU 1998 Protein kinase C isoforms α, δ and θ require insulin receptor substrate-1 to inhibit the tyrosine kinase activity of the insulin receptor in human kidney embryonic cells (HEK 293 cells).


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