Dysregulated pyruvate dehydrogenase complex in Zucker diabetic fatty rats

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GLUCOSE OXIDATION PLAYS a major role in energy metabolism (33). The first step in the glucose oxidative pathway is the irreversible decarboxylation of pyruvate to acetyl-CoA by the mitochondrial pyruvate dehydrogenase complex (PDC). This reaction links glycolysis to the citric acid cycle and functions as a key reaction in the maintenance of glucose homeostasis in mammals (14). Regulation of PDC by covalent modification is necessary for glucose homeostasis during fed and fasted states (32). The PDC activity is regulated by a phosphorylation/dephosphorylation cycle catalyzed by specific pyruvate dehydrogenase kinases (PDKs) and pyruvate dehydrogenase phosphatases (PDPs) (15). PDK decreases PDC activity by phosphorylation of the pyruvate dehydrogenase (E1) α-subunits (19, 30), whereas PDP1–2 dephosphorylate E1 and thereby enhance PDC activity (20). Four PDK isoenzymes (PDK1–4) are expressed in mammals (6). The products of the PDC reaction, acetyl-CoA and NADH (also products of the β-oxidation of fatty acids), inhibit PDC activity directly and also by increasing PDK activity (indirect allosteric inhibition of PDC activity) (34). Some 30 years ago, Randle (33) proposed that free fatty acids (FFA) compete with glucose as the major energy substrate, leading to decreased glucose oxidation when FFA are elevated. In contrast, the substrates of the PDC reaction, pyruvate and ADP, increase PDC activity and inhibit PDK activity (34). In addition to this allosteric regulation, it has been shown that PDK4, which is of particular importance for the regulation of PDC activity in muscle and liver, is controlled at the level of gene expression (26) with insulin playing a major role in reactivation of PDC (7, 22). Insulin diminishes the levels of PDK4 and PDK2 mRNAs (32). Insulin suppresses PDK4 expression in part through the dissociation of the forkhead transcription factors and peroxisome proliferator-activated receptor-γ coactivator from the PDK4 promoter (44). Insulin increases activity of PDP resulting in dephosphorylation and activation of PDC. The effect of insulin on the activity of PDC is largely caused by stimulation of PDP involving a decrease in $K_m$ for Mg$^{2+}$ (38). More recently, it has been reported that insulin is able to influence the activity of PDC by a combination of two distinct signaling pathways: the Wortmannin-sensitive and the extracellular signal-regulated kinase-1 and -2 pathway (22).

Starvation increases PDK4 expression (15). Because the FFA level increases with starvation and diabetes, FFA has been suggested to stimulate PDK4 expression in skeletal muscle (35). FFA is an endogenous ligand for the peroxisome proliferator-activated receptor-α (PPARα) and may increase PDK4 expression by stimulating PPARα (42). This and the allosteric mechanisms lead to inhibition of PDC and a decrease in pyruvate oxidation, to conserve C3 compounds for gluconeogenesis. During refeeding, PDK expression is decreased, but subsequent reactivation of PDC activity was reported to be delayed (18). In the diabetic state, PDK4 expression is also increased, causing a reduction in PDC activity and contributing to glucose intolerance associated with this disease (19). Under most conditions, the proportion of the “active” form of the enzyme varies without significant changes in the “total” activity (33, 41), while long-term regulation may also involve changes in total amount of PDC present in the cell.

The oral glucose tolerance test (oGTT) is widely used for initial assessment of the presence of insulin resistance in obese and type 2 diabetic patients (13). Animal models of insulin resistance and type 2 diabetes like the Zucker diabetic fatty (ZDF) rat are also widely used for investigations of disturbed...
intermediary metabolism in disease states such as obesity, insulin resistance, and type 2 diabetes (12, 29). Glucose disposal during an oral glucose challenge consists of glucose oxidation and glucose utilization by the nonoxidative pathways, e.g., glycogen formation, predominantly in liver and muscle. Whether impaired glucose tolerance and hyperglycemia in obese ZDF rats are due to impaired glucose oxidation at the level of PDC is not entirely clear.

In the present study, using an integrated approach, we analyzed carbohydrate oxidation (CHO), fat oxidation (FO), PDC activity, and PKD4 protein levels during an OGTT in obese ZDF rats, a model for insulin resistance and type 2 diabetes. To determine the role of elevated FFA on glucose oxidation, the OGTT was reinvestigated during pharmacologically reduced FFA levels using nicotinic acid as an antilipolytic agent.

MATERIALS AND METHODS

Animals. Male lean (ZDF/Gmi-Fa/?) and obese (ZDF/Gmi-fa/fa) ZDF rats were obtained from Charles River (Sulzfeld, Germany) and studied at the age of 16 wk. They were housed in groups of up to four per cage in a temperature-controlled room at 20–22°C with a 12:12-h light-dark cycle. All animals had free access to water and to a standard pellet rat chow (ssniff, Soest, Germany) unless otherwise indicated.

Experimental study design. All experimental procedures were conducted according to the German Animal Protection Law. Also according to the German Animal Protection Law, the animal protocol was carefully reviewed by the local authority in South-Hessen and approved under reference no. 4B/19-28. The OGTT in lean and obese ZDF rats (in an intraindividual and an interindividual manner) was performed after an overnight fast of 18 h with a glucose load of 2 g/kg lean body mass; groups of n = 5–8 were used. Blood samples (5 μl) were obtained from tip of the tail for glucose analysis at time points indicated. For the interindividual test, animals were killed under short-term isoflurane anesthesia by terminal blood withdrawal from the abdominal aorta after a glucose load: time 0 (control), 60, and 120 min. In another set of experiments (n = 8), nicotinic acid (50 mg/kg po) was given 10 min before the glucose load. At the end of each period, a portion of the liver and the gastrocnemius muscle were freeze-clamped immediately and were stored at −80°C for subsequent determinations of PDC activity, PKD4, and E1α/β-proteins. Blood glucose and FFA were analyzed using standard methods (4). Plasma insulin concentrations were assayed by an ELISA kit obtained from Mercodia, Uppsala, Sweden.

Body composition analysis by magnetic resonance spectroscopy. In vivo magnetic resonance spectroscopy (MRS) studies in anesthetized rats were performed as described previously (3, 24) to measure total fat mass and thereby to calculate lean body mass. Briefly, under anesthesia (isoflurane in N2/O2/CO2), each rat was secured within a resonator (outer diameter, 255 mm; inner diameter, 197 mm; Bruker, Karlsruhe, Germany). Temperature within the resonator was maintained at 22°C. The resonator was centered within a 4.7 T magnet (Bruker Biospec 47/40). The Paravision 4.0.0 imaging algorithm was used to correct magnetic field inhomogeneities. Spectra were recorded with 16 averages, with 12,800-Hz sweep width, on 1,024 points, and with a 3-s relaxation delay (90° pulse: 100 μs). Before Fourier transformation, zero filling to 8,192 data points was performed. For spectral analysis, the water peak was referenced to 4.7 parts/million (ppm); two fixed integration regions were set: 1) water resonance from 8 ppm to the relative minimum between the water and the lipid peak at −3 ppm and 2) from there to −1.5 ppm for the lipid peak. The relative amount of total fat was calculated in relation to the sum of both integral values; the absolute amount of fat tissue was calculated based on a lean mass-to-water ratio of 1.4 (25).

Substrate oxidation in vivo. CHO and FO were measured by the use of an indirect calorimetry system. The system consists of 16 individually ventilated cages (Tecniplast, Hohenpeissenberg, Germany) localized in a climatic chamber (Weiss Umwelttechnik, Reiskirchen, Germany) with temperature constancy at 22°C. For the individual housing of animals during the study, 15 cages were used, and 1 cage served as reference cage for corrections of O2 and CO2 measurements.

All rats were accustomed to the cages at least 24 h before the start of the experiment. After a fasting period of 18 h with free access to water, the animals were given the glucose load (n = 8) or the vehicle (saline, n = 7). In another experiment, the animals were given either nicotinic acid (n = 8) or vehicle (n = 7) 10 min before glucose load. O2 consumption and CO2 production were measured every 16 min per cage for 1 min (gas analyzers: Magnos 16 and Uras 14; ABB, Frankfurt, Germany) and recorded on a computer. Renal nitrogen (N) excretion was set as a constant value of 0.3 mg/day. CHO and FO were calculated according to Weir (40) as modified by Ferrannini (11) and Boschmann et al. (5) by applying the following formulas: CHO (g) = 2.97 × VO2 (liters) + 4.17 × VCO2 (liters) − 2.44 × N (g), and FO (g) = 1.72 × VCO2 (liters) − 1.96 × N (g) (where VO2 is oxygen consumption, and VCO2 is carbon dioxide production), with both expressed as milligrams per hour normalized for 100 g of lean body mass because of the different body mass of lean and obese ZDF rats.

Preparation of mitochondrial lysates. To analyze the PKD4 and PDC-E1 protein levels from rat liver and muscle mitochondria, tissues were thawed in a homogenization buffer (pH 7.4; 0.25 M sucrose, 10 mM Tris, and 2 mM EDTA) to remove cell debris. Following centrifugation at 30,000 g for 10 min at 4°C, the supernatant was removed and the pellet resuspended in the homogenization buffer. The suspension was centrifuged again for 10 min at 30,000 g (4°C). The pellet was resuspended in a solution of 0.25 M sucrose, 10 mM Tris, and 2 mM EDTA and stored at −20°C for subsequent protein determination (bicinchoninic acid protein assay kit; Pierce, Rockford, IL) and Western blot analysis.

Preparation of mitochondrial lysates. To analyze the PKD4 and PDC-E1 protein levels from rat liver and muscle mitochondria, tissues were thawed in a homogenization buffer (pH 7.4; 0.25 M sucrose, 10 mM Tris, 2 mM EDTA, 2 mM dithiothreitol), disrupted by an Ultra-Turrax, and homogenized by a Potter-Elvehjem homogenizer (30 s, 1,400 rpm, 10–12 strokes). The homogenate was subjected to an initial low-speed centrifugation (400 g for 10 min, 4°C) to remove cell debris. Following centrifugation at 30,000 g for 10 min at 4°C, the supernatant was removed and the pellet resuspended in the homogenization buffer. The suspension was centrifuged again for 10 min at 30,000 g (4°C). The pellet was resuspended in a solution of 0.25 M sucrose, 10 mM Tris, and 2 mM EDTA and stored at −20°C for subsequent protein determination (bicinchoninic acid protein assay kit; Pierce, Rockford, IL) and Western blot analysis.

Western blot analysis. Mitochondrial proteins were separated on 4–12% Bis-Tris SDS-PAGE and transferred by semidry blotting onto nitrocellulose membrane (30 μg protein/lane). Membranes were blocked for 1 h at room temperature with Tris-buffered saline containing 0.05% Tween and 2% bovine serum albumin. Blots were incubated overnight at 4°C in blocking solution plus polyclonal antisemur against PKD4 (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) or PDC-E1α/β (1:10,000) (23). After being washed four times with blocking solution, membranes were incubated for 1 h at room temperature with secondary antibody (1:10,000, Santa Cruz Biotechnology). The membranes were then washed again four times (each 10 min) at room temperature. Bound antibodies were detected by enhanced chemiluminescence according to the manufacturer’s instructions. Immuno-reactive bands were visualized using a Lumi-Imager (Boehringer, Germany), quantified (software LumiAnalyst 3.0; Roche Diagnostics, Mannheim, Germany), and specified as arbitrary units referred per milligram of protein.

PDC activity. PDC activity was determined by the production of 14CO2 from [1-14C]pyruvate as described (31). Muscle and liver tissues were homogenized in 2 ml of a KCI-MOPS buffer (80 mM KCl, 50 mM MOPS, 40 mM NaF, 2 mM MgCl2, 0.5 mM EDTA; pH 7.4) containing protease inhibitor cocktail (Roche Diagnostics). The homogenate was subjected to differential centrifugation to obtain mitochondrial fraction as described above. The 14CO2 release assay was carried out in an air-tight reaction vessel containing a filter paper soaked with 100 μl of hyamine. The reaction mix contained 69 μl of a reaction mixture (72.5 mM phosphate buffer, pH 7.5, 21.7 mM potassium oxalate, 2.9 mM MgCl2, phosphotransacetylase (1.5 U/ml), 3.1 mM NAD+/H), 10 μl of 8 mM dithiothreitol, 1 mM thiamine.
pyrophosphate, 6.5 mM CoA, and 20 μl of the tissue suspension at 37°C in a shaking water bath. After preincubation for 2 min, the reaction was started by addition of 1 μl of [1-14C]pyruvate (100,000 counts/min, final pyruvate concentration 0.5 mM). After 20 min, the reaction was stopped by addition of 100 μl of 20% trichloroacetic acid with 30 mM pyruvate. 14CO2 was collected by a hyamine-soaked filter paper and was subsequently measured in a liquid scintillation counter. Total PDC activity was determined after preincubating disrupted mitochondrial preparation with lambda protein phosphatase for complete dephosphorylation of the complex (31).

Statistics. Results are presented as means ± SE; n indicates number of animals. Data were analyzed for statistical significance by one-way ANOVA followed by a post hoc analysis with Bonferroni correction. Parameters with P values <0.05 were considered to differ significantly.

RESULTS

PDC activity during fed and starved conditions. PDC activities in liver and muscle were analyzed in fed and starved male lean and obese ZDF rats. Active PDC activity was about twofold higher in the fed state compared with the fasting values in the same group of animals and was much higher in muscle compared with liver for both the groups (Fig. 1A). Values for the obese ZDF rats tended to be lower compared with those for lean rats, but the difference was not significantly different. The total PDC activities were comparable in liver and muscle tissues from the same group of rats under fed and starved states, respectively, but were significantly elevated in both muscle and liver from obese ZDF rats compared with lean ZDF rats (Fig. 1B). This suggests higher protein levels of PDC in obese ZDF animals than in lean rats. In agreement with this, Western blot analysis revealed that both E1α- and β-protein levels were increased approximately twofold in liver and muscle in obese ZDF rats compared with lean rats (Fig. 1D). The percent PDC activation (expressed as %active of total PDC) was significantly decreased during fasting in both tissues of both groups, and the percent active PDC in liver and muscle was significantly lower in obese ZDF rats than in their lean littermates (Fig. 1C). This suggests that PDC is maintained in the phosphorylated form at a higher level and thereby inhibited to a greater extent in obese ZDF rats compared with their lean littermates.

Amount of glucose used for the oGTT. The relevant metabolic tissues for glucose disposal after a glucose load are the liver and skeletal muscle (28). Because obese ZDF rats differed substantially in their body mass and fat mass compared with lean rats, we determined the lean body mass by 1H-MRS. Glucose dose was calculated in relation to their lean body mass to avoid any differences in the blood glucose profiles between lean and obese ZDF rats because of an inappropriate higher glucose load of the obese rats. Body mass, lean body mass, total body fat, and the dose of glucose administered during the oGTT are summarized in Table 1 for the intraindividual and interindividual oGTT.

Metabolic parameters and substrate oxidation. Glucose tolerance profiles were substantially different between lean and obese ZDF rats in both the intraindividual (Fig. 2A) and the interindividual setting of oGTT (Fig. 3A). Insulin-sensitive lean ZDF rats demonstrated a normal glucose tolerance,
whereas in obese ZDF rats, glucose tolerance was markedly impaired (Figs. 2A and 3A). In nicotinic acid-treated ZDF rats, glucose tolerance was significantly improved compared with untreated ZDF rats in both lean and obese ZDF groups although not normalized in obese rats (Fig. 2D). In addition, oxidative glucose metabolism during the oGTT was investigated by indirect calorimetry. Lean and obese ZDF rats were investigated with their respective control groups receiving saline instead of the glucose bolus. CHO in lean and obese ZDF rats receiving saline (control) remained unchanged at each time point studied, indicating that the oral administration per se did not interfere with the recording of V̇CO₂ and V̇O₂ (data not shown). In line with the impaired glucose tolerance profile in obese ZDF rats, CHO (Fig. 2B) and FO (Fig. 2C) differed substantially between the two groups of rats. After overnight starvation, the starting point of the respiratory quotient for both groups was close to 0.7, indicating predominant FO (data not shown). Thirty minutes after glucose administration, CHO peaked for both groups and remained elevated up to 90 min after the glucose load for the lean rats. However, CHO declined rapidly in obese rats, reaching baseline values ~1 h after the glucose load (Fig. 2B). Area under the curve calculated for CHO of lean animals was 45 ± 4 mg·3 h⁻¹·100 g lean body mass⁻¹, representing 22% of the administered glucose load, whereas the values for the obese ZDF rats were significantly lower (23 ± 2 mg·3 h⁻¹·100 g lean body mass⁻¹), representing 12% of the administered glucose load. Nicotinic acid caused increased CHO in both lean and obese ZDF rats paralleled by decreased FO (Fig. 2, E and F). Area under the curve calculated for CHO of nicotinic acid-treated rats was significantly elevated for both lean (137 ± 26 mg·3 h⁻¹·100 g lean body mass⁻¹) and obese (91 ± 14 mg·3 h⁻¹·100 g lean body mass⁻¹) ZDF rats. These increases demonstrate the suppressive role of FFA on CHO in both lean and obese rats and also indicate that the remaining difference in impaired CHO in obese rats did not exclusively depend on PFP levels.

Metabolic blood parameters as well as PDC activity and PDK4 protein levels during an oGTT. The interindividual oGTT was performed at three time points (0, 60, and 120 min) after the glucose load to measure the levels of PDK4 and active PDC activity during this relatively short time period. Obese ZDF rats were obviously insulin resistant, as demonstrated by hyperinsulinemia under untreated (Fig. 3B) as well as under nicotinic acid-treated conditions (Fig. 3E), and glucose intolerant (Fig. 3, A and D). Despite their much higher serum insulin levels, lipolysis in untreated obese rats was only marginally suppressed compared with that of lean rats (Fig. 3C). Glucose intolerance was persistent in obese rats (Fig. 3D) even under conditions of suppressed lipolysis, where FFA were low in obese rats compared with untreated lean rats (Fig. 3F). PDK4 protein levels were significantly elevated in obese ZDF rats compared with lean rats, and, consequently, active PDC activities in muscle and liver of obese ZDF rats were significantly lower than for the lean group in untreated (Fig. 4, A–D) and nicotinic acid-treated rats (Fig. 4, E–H). Time course of the PDK4 protein levels during the oGTT in untreated rats revealed a rapid decline of PDK4 in the liver of lean and obese ZDF rats within 2 h as well as in muscle of obese ZDF rats only (Fig. 4, A and B). In nicotinic acid-treated rats, a similar rapid decline of PDK4 in the liver was detected, which was significant for obese rats only (Fig. 4F). The levels of active PDC activity in both groups peaked at 60 min after the glucose load under both untreated and treated conditions. The increases in active PDC activity in the liver were significantly higher in lean rats compared with obese ZDF rats (Fig. 4D) and were also higher in nicotinic acid-treated rats compared with untreated rats (Fig. 4, G and H), which was significant for all conditions except for livers of lean rats.

**DISCUSSION**

Both short- and long-term mechanisms are known for regulation of the PDC. Several groups have extensively investigated the regulation of PDC activity in starvation (16, 36, 37, 42, 43), insulin-deficient diabetes (chemically induced by streptozotocin) (15, 16, 21, 42, 43), insulin resistance, and diabetes in Otsuka Long-Evans Tokushima fatty rats (1, 2) and high sucrose- and high fat-fed rats (10). In starvation and chemical-induced diabetes, PDC activity is lowered, in part, by decreased PDP2 expression in rat heart and kidney (21). Increasing plasma FFA concentrations after intravenous lipid infusion (39) and dietary fatty acids (8) were shown to stimulate PDK4 gene expression in human skeletal muscle. The increase in PDK4 expression is suggested to be mediated by PPARs stimulation, since FFA are endogenous ligands for PPARs (42). However, little effort has been devoted so far to investigate rapid changes of active PDC activity and PDK4 expression during an oGTT. Here, we demonstrate rapid changes in active PDC activity, PDK4 levels, and in vivo glucose oxidation after an oral glucose load in lean rats and an impairment in these responses in obese ZDF rats. Furthermore, pharmacological reduction of serum FFA levels by pretreatment with the antilipolytic agent nicotinic acid confirmed the inhibitory role of elevated FFA on CHO and active PDC activity.

According to the nutritional state, active PDC activities were increased in both the liver and muscle from fed rats. They were significantly elevated in muscle compared with liver (Fig. 1A). The finding of elevated total PDC activity in obese ZDF rats (Fig. 1B) was consistent with the elevated expression of the E1 subunits (as a marker for other PDC proteins) (Fig. 1D) of the

Table 1. Body mass, lean body mass, fat mass (measured by 1H-MRS), and the absolute amount of the administered glucose for the oGTT in lean and obese ZDF rats

<table>
<thead>
<tr>
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<th>oGTT (Without Nicotinic Acid)</th>
<th>oGTT After Pretreatment With Nicotinic Acid</th>
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<tr>
<td></td>
<td>Lean</td>
<td>Obese</td>
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<tr>
<td><strong>Intradividual</strong></td>
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<tr>
<td>Body mass, g</td>
<td>356±10</td>
<td>390±3</td>
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<tr>
<td>Lean body mass, g</td>
<td>333±9</td>
<td>299±3</td>
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<tr>
<td>Total fat mass, g</td>
<td>23±2</td>
<td>92±3</td>
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<tr>
<td>Glucose load, g</td>
<td>0.67±0.01</td>
<td>0.60±0.01</td>
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<td><strong>Interindiv</strong></td>
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<tr>
<td>Body mass, g</td>
<td>359±6</td>
<td>396±5</td>
</tr>
<tr>
<td>Lean body mass, g</td>
<td>332±6</td>
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</tr>
<tr>
<td>Total fat mass, g</td>
<td>26±1</td>
<td>90±3</td>
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<tr>
<td>Glucose load, g</td>
<td>0.67±0.01</td>
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Values are means ± SE; n = 8–16. Glucose load: 2 g glucose/kg lean body mass. MRS, magnetic resonance spectroscopy; oGTT, oral glucose tolerance test; ZDF rats, Zucker diabetic fatty rats.
complex and may be related to the hyperinsulinemic state of these rats (9). The correlation between a threefold increase in total PDC activity and a two- to threefold increase in E1 protein levels was demonstrated in male Sprague-Dawley rats after they were fed a high-sucrose, fat-free diet (10). The elevated E1 subunits in obese ZDF rats in liver and muscle could be interpreted as a protective mechanism for multiple phosphorylation of serine residues by increased protein levels of PDKs. As a consequence of increased total PDC activity and slightly reduced active PDC activity in obese rats compared with lean rats, the percent active PDC decreased in obese rats significantly (Fig. 1C). These results demonstrate the importance of both active and total PDC activity measurements when investigating the activity state of PDC (1, 2).

The male obese ZDF rat develops spontaneously a type 2 diabetes phenotype starting at the age of ~8–10 wk (12, 29). We used 16-wk-old rats for this study, and hence the obese ZDF rats suffered from overt diabetes resulting in a catabolic state with hyperglycemia and glucosuria. Despite this catabolic disease state, the obese ZDF rats still had a higher absolute body mass compared with their lean littersmates based on higher total body fat mass, but their lean body mass was already lower than that of the lean control group. Since we administered the amount of glucose for the oGTT based on the lean body mass, the obese ZDF rats received less glucose in absolute terms relative to the lean rats. Despite the lower amount of glucose for the oGTT, the obese ZDF rats demonstrated glucose intolerance, indicating their disease state of insulin resistance and type 2 diabetes. Whole body total CHO after the glucose load measured by indirect calorimetry was reduced, as was active PDC activity in the presence of elevated PDK4 protein levels in muscle and liver from obese ZDF rats compared with lean controls during both study conditions (without and with suppressed FFA). These results demonstrated impaired glucose oxidation during a glucose load in obese ZDF rats. Pharmacological reduction of serum FFA levels by nicotinic acid during the oGTT resulted in a significant increase in CHO in lean and obese ZDF rats paralleled by reduced FO. In addition, the increases in active PDC activity were higher compared with untreated lean and obese rats.

In both lean and obese ZDF rats, active PDC activity in liver and muscle increased rapidly within 1 h after oral glucose load (Fig. 4, C, D, G, and H). Earlier studies demonstrated that there was a period of hysteresis (3–4 h) before reactivation in
response to chow refeeding of fasted rats (17, 18). Parallel to active PDC activity, CHO reached its maximum level 1 h after glucose administration in untreated lean rats. From this it can be concluded that enhanced CHO is coupled to reactivation of PDC. For untreated obese rats it can be assumed that, at time\(*/H100560\) min, active PDC activity was already decreasing, as indicated by maximum CHO by time\(*/H1100530\) min (Fig. 2B). During the oGTT in lean rats, CHO appeared at much lower blood glucose levels relative to obese rats. Because of the insulin resistance of obese animals, mass action effects could enable glucose to be taken up independently of insulin action for subsequent oxidation or nonoxidative glucose metabolism. Therefore, CHO in obese rats probably results from this mainly insulin-independent substrate delivery. It might therefore be possible that PDK4 degradation had started already when substrate turnover was decreasing. The rapid changes of the active states of PDC in liver and muscle (Fig. 4, C, D, G, and H) occurred in the presence of high PDK4 protein levels in both tissues of obese ZDF rats. The high PDK4 protein levels linked to reduced PDC activity in obese relative to lean rats were in line with the role of long-term regulation of PDK4 by PPAR\(\alpha\) because of elevated levels of FFA in these rats.

In both lean and obese ZDF rats, there was a rapid down-regulation of PDK4 protein levels (Fig. 4, A, B, and F). A reduction in PDK4 activity and its protein after refeeding of starved rats was reported earlier (27, 36), but in contrast to the present study, PDK4 regulation was not reported to occur in such a short time period. It can be suggested that, in contrast to the previous studies, the fasting duration in our study was only 18 h, and furthermore, the oGTT represented an “acute glucose feeding” without any delay in intestinal carbohydrate digestion as required after chow refeeding. The short time period in which PDK4 was downregulated points toward an additional mechanism of short-term regulation of PDC. One triggering factor for the PDK4 protein downregulation is the change in serum insulin levels in the two groups of rats. It was reported that, after refeeding of 48 h-starved rats and after insulin administration to streptozotocin diabetic rats, PDK4 protein and mRNA levels were similar to those of fed animals within 48 h (42). The data from Lee et al. (27) indicated that 5-h

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**Fig. 3.** Blood glucose (A and D), serum insulin (B and E), and free fatty acids (FFA; C and F) during an oGTT (interindividual study) in lean (○) and obese (●) ZDF rats. oGTT was performed without nicotinic acid (A–C) as well as after pretreatment with nicotinic acid (D–F). Values are means ± SE; \(n = 5–8\). \(*P < 0.05\) vs. lean group. \(#P < 0.05\) vs. \(t = 0\). \(\$P < 0.05\) vs. oGTT without nicotinic acid.
Fig. 4. Pyruvate dehydrogenase kinase-4 (PDK4) protein levels measured by Western blot and expressed as arbitrary units/mg protein (A, B, E, and F) and active PDC activity (C, D, G, and H) during the oGTT (interindividual study: metabolic blood parameters shown in Fig. 3) in muscle (A, C, E, and G) and liver (B, D, F, and H) of lean (open columns, ○) and obese (black columns, ●) ZDF rats. oGTT was performed without nicotinic acid (A–D) as well as after pretreatment with nicotinic acid (E–H). Values are means ± SE; n = 5–6. *P < 0.05 vs. lean group. #P < 0.05 vs. t = 0. §P < 0.05 for delta increase t = 60 – t = 0 for lean vs. obese rats. $P < 0.05 for delta increase t = 60 – t = 0 vs. oGTT without nicotinic acid.
refeeding of overnight-fasted rats decreased muscle PDK4 mRNA levels by 77%, which was associated with a profound suppression of plasma FFA. However, a similar suppression of plasma FFA induced by nicotinic acid infusion failed to alter muscle PDK4 mRNA levels, indicating that the effect of insulin on PDK4 expression was independent of the reduction of FFA.

In the presence of hyperglycemia and obviously impaired glucose tolerance in obese ZDF rats (Figs. 2, A and D, and 3, A and D), CHO (Fig. 2, B and E) and active PDC activity (Fig. 4, C, D, G, and H) were significantly lower compared with normoglycemic and glucose-tolerant lean animals. Rapid increases (within 1 h) in both CHO and active PDC activity and a moderate decrease in PDK4 protein levels in lean rats indicate that allosteric effects and/or rapid effects of insulin on the phosphorylation state of PDC predominantly regulate PDC activity acutely during a glucose challenge. Experiments with pharmacologically reduced FFA levels confirmed the suppressive role of FFA on CHO and active PDC activity in both lean and obese ZDF rats. However, active PDC activity in obese ZDF rats after a glucose load was decreased even during suppressed FFA levels, which might be related to higher PDK4 protein levels. Thus impaired glucose oxidation in liver and muscle might at least partially contribute to hyperglycemia in obese ZDF rats.

REFERENCES


