Low-dose dexamethasone in the rat: a model to study insulin resistance

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Severino, C., P. Brizzi, A. Solinas, G. Secchi, M. Maioli, and G. Tonolo. Low-dose dexamethasone in the rat: a model to study insulin resistance. Am J Physiol Endocrinol Metab 283: E367–E373, 2002.—The main aim of this study was to set up a new animal model to study insulin resistance. Wistar rats (6 or 7 per group) received the following for 4 wk in experiment 1: 1) vehicle, 2) 2 µg/day subcutaneous dexamethasone, 3) metformin (400 mg·kg−1·day−1 os), and 4) dexamethasone plus metformin. In experiment 2 the rats received the following: 1) vehicle, 2) dexamethasone, 3) dexamethasone plus arginine (2%; as substrate of the nitric oxide synthase for nitric oxide production) in tap water, and 4) dexamethasone plus isosorbide dinitrate (70 mg/kg; as direct nitric oxide donor) in tap water. Insulin sensitivity was significantly reduced by dexamethasone already at week 1, before the increase in blood pressure (day 15) and without significant changes in body weight compared with vehicle. Dexamethasone-treated rats had significantly higher triglycerides, hematocrit, and insulin, whereas serum total nitrates/nitrites were lower compared with vehicle. The concomitant treatment with metformin minimized all the described effects of dexamethasone. In experiment 2, only isosorbide dinitrate was able to prevent the observed dexamethasone-induced metabolic, hemodynamic, and insulin sensitivity changes. Chronic low-dose subcutaneous dexamethasone (2 µg/day) is a useful model to study the relationships between insulin resistance and blood pressure in the rat, and dexamethasone might decrease insulin sensitivity and increase blood pressure through an endothelium-mediated mechanism.

glucocorticoids; metabolic syndrome; metformin; hypertension

THERE IS CONSIDERABLE evidence that abnormalities of glucose, insulin, and lipoprotein metabolism occur more frequently in untreated hypertensive patients than in normotensive subjects. It could be argued that the relationship between high blood pressure and metabolic disorder is incidental, but, on the other hand, there is evidence that changes in glucose, insulin, and lipoprotein metabolism play a role in the etiopathology and/or clinical course of hypertension. In human hypertension, subtle changes in adrenal steroid metabolism have been suggested (28, 29, 36), indicating that glucocorticoids, besides the well-known Cushing syndrome, might have an important role in the development of high blood pressure. Moreover, glucocorticoids have been shown to reduce cellular glucose uptake affecting the glucose transport system per se (7), with no direct effects on the insulin receptor (26). Thus glucocorticoids alter glucose metabolism, and in turn they have a role in the development of peripheral insulin resistance. Insulin resistance may be an impetus for the development of hypertension, impaired carbohydrate tolerance, and lipid alteration, but the underlying mechanisms are still unclear. Similar metabolic abnormalities occur in rodent models of hypertension. For example, endothelial dysfunction precedes hypertension in an experimental model of fructose-induced insulin resistance (9, 24), but fructose-induced insulin resistance is not easily comparable to humans. At the best of our knowledge, there is no established model of insulin resistance induced by dexamethasone without the dramatic catabolic side effects commonly seen with glucocorticoids. We previously showed that it is possible to increase blood pressure for long term in rats with subcutaneous doses of dexamethasone on the order of micrograms per day (31) without appreciable catabolic effects. This animal model is not associated with sodium retention but with sodium shift from the intracellular to the extracellular space (12), and the effects on blood pressure are opposite to those obtained by intracerebroventricular dexamethasone administration (32). The main aim of this study was to establish if our previously described “old model” of glucocorticoid-induced hypertension in the rat is indeed a useful “new” model to study insulin resistance in the metabolic syndrome. To do so we used our previously described animal model (31) to evaluate the effects of metformin, a well-established drug able to ameliorate insulin sensitivity in rats (25).

RESEARCH DESIGN AND METHODS

Experiment 1

Animals. Male Wistar rats, with a weight of 400 g, were used through the experiments in groups of 6 or 7 animals
each. All rats were housed in an automatically light-con-
trolled animal facility (12 h on, 12 h off) with constant
temperature (22 C°) and humidity, with free access to food
(Mil mice and rats GLP Diets, Mucedola Srl, Italy) and tap
water ad libitum. Two weeks before start of the experiment,
animals were accustomed to handling as well as blood pres-
sure and blood glucose measurements (by a vein puncture in
the tail). Four groups were thereafter treated for 4 wk:
1) vehicle: tap water and daily subcutaneous injection of 0.9%
NaCl (75 μl) at 8:00 AM and 8:00 PM; 2) dexamethasone
(Dex): tap water and daily subcutaneous injections of Dex (1
μg in 75 μl 0.9% NaCl) at 8:00 AM and 8:00 PM; 3) metformin
(Met): Met in tap water (3.5 mg/ml) and daily subcutaneous
injection of 0.9% NaCl (75 μl) at 8:00 AM and 8:00 PM; and
4) Dex + Met: Met dissolved in tap water (3.5 mg/ml) and
daily subcutaneous injection of Dex (1 μg in 75 μl 0.9% NaCl)
at 8:00 AM and 8:00 PM.

Methods. Three times a week systolic blood pressure was
measured in the morning (tail cuff method, Letica, Le 5001
pressure meter) in the conscious lightly restrained animal
after the animals were prewarmed at 38 C° for 10 min as
previously described (12, 31, 32); body weight and tap water
consumption were recorded at the same time. At days +8,
+14, and +26 after blood pressure measurement, in the
conscious rats blood from a tail vein was obtained for glucose
measurement with a reflectometer (LifeScan One Touch Pro-
file, Johnson-Johnson, Minneapolis, MN), immediately be-
fore and 30 min after rats received intraperitoneal fast-
acting insulin (1.6 U/kg Actrapid, Novo Nordisk). In this
“insulin-tolerance test,” the 1.6-U/kg dose and the 30-min
values were chosen to calculate the maximum decrease in
blood glucose. This method was, in our laboratory, repetitive
as shown in Fig. 1, where the effects of three different doses
of intraperitoneal insulin (0, 0.8, and 1.6 U/kg) on blood
glucose, over 80 min after insulin injection, are given. The
reflectometer was validated in our laboratory against an
automatic analyzer (Hitachi 912, automatic analyzer, Boeh-
Ringer Mannheim, using standard reagents) for values from
30 to 200 mg/dl (n = 68, r² = 0.921 P < 0.001).

At day +28 after the last blood pressure measurement,
while the rats were fasting from midnight, under pentobar-
bital sodium anesthesia a large blood sample from the
abdominal aorta was obtained. Serum cholesterol, triglycerides,
β-hydroxybutyrate and uric acid (Hitachi 912), Na⁺, and K⁺
(flame photometer, Beckman) were measured. One hundred
fifty microliters of blood were collected in a microhematocrit
capillary tube, spun at 5,000 rpm for 10 min, and used for the
determination of hematocrit. Insulin was measured in dupli-
cate by use of a rat insulin radioimmunoassay kit (Diasorin,
Stillwater, Minnesota, MN). Total nitrates/nitrates were
measured by a validated colorimetric assay kit (Cayman)
with high sensitivity (1 μM) for both NO₂⁻ and NO₃⁻ in serum
to achieve an estimate of in vivo nitric oxide (NO) production,
with nitrates (NO₂⁻) and nitrates (NO₃⁻) the end product of
NO (13). Intraerythrocyte Na⁺ was measured following a
published method (5) with the only modification being the
use of 0.5 ml full blood. Blood for free fatty acids was obtained
at the same time and collected in iced (+4°C) EDTA tubes; it
was immediately centrifuged at 4°C, and the plasma was
frozen at −80°C until assayed. Free fatty acids were mea-
sured by a colorimetric method using a Boehringer-Mann-
heim kit.

Heart, liver, and the left kidney were excised and weighed.
The measurement was corrected for body weight.

Histology. Liver and left kidney tissues after fixation in
10% buffered formalin were dehydrated with ethyl alcohol
and then included in paraffin. Sections of 5 μm were obtained
by a microtome (Top Rotary S-130, parish). Periodic acid-
Schiff coloration for polysaccharides has been used to evalu-
ate glycogen content in the hepatocytes. Two independent
observers not aware of the different treatments gave inde-
pendent comment on the histological material.

Steady-state glucose concentration. Steady-state glucose
concentration (24) was measured to confirm the findings of
the insulin-tolerance test in the period preceding the rise in
blood pressure observed in Dex-treated rats. Steady-state
glucose concentration was measured in an additional four
rats after 8 days of Dex treatment alone (before any signifi-
cant rise in systolic blood pressure) or with Met following
the described protocol of Reaven et al. (24). In brief, under anesthesia with pentothal sodium (Tiopental Sodico 0.5 g;
Farmaceutici Gellini), the right femoral vein was cannulated
for insulin and glucose infusion at the fixed doses of 2.5
mU·kg⁻¹·min⁻¹ and 8 mg·kg⁻¹·min⁻¹, respectively, for 3 h.
Tail blood samples for glycaemia were taken at 15-min inter-
vals during the last hour of infusion, and the four obtained
values were used to find the mean.

Experiment 2

Animals and methods. To better characterize the possible
endothelial involvement in the development of insulin resis-
tance in this animal model, four additional groups of six
animals each were compared in a 28-day experiment. The
main reason for this second study was to evaluate if NO
production (measured, as said above, as total nitrates and
nitrates) was restored giving exogenously the endogenous
substrate for NO production, arginine. The arginine effects
were compared with those obtained by exogenous direct NO
gift through a NO donor, isosorbide dinitrate.

The experimental groups were as follows: 1) vehicle: tap
water and daily subcutaneous injection of 0.9% NaCl (75 μl)
at 8:00 AM and 8:00 PM; 2) Dex: tap water ad libitum and
daily subcutaneous injections of Dex (1 μg in 75 μl of 0.9%
Dex/H11001/day/Systolic blood pressure (SBP) increased significantly during these days, probably due to the rats becoming accustomed to the drug taste; thereafter, the daily water intake was similar to that of the other groups. Thus the average Met intake in the Dex + Met rats was 183–228 mg·kg⁻¹·day⁻¹ for days 1 and 2 and 280–333 mg/kg for the remaining days. Systolic blood pressure (SBP) increased significantly only in Dex rats from day +15, reaching the zenith at day +18 and remaining significantly elevated until day +28, with a net increase of >20 mmHg from basal (Fig. 2A). The remaining rats did not show significant changes in SBP through the study, although final SBP in Dex + Met rats was somewhat 5 mmHg more elevated, but not significantly, than in the other two remaining groups (vehicle and Met). Heart rate did not show significant difference during the study in the four groups of rats (Table 1). Body weight did not change significantly through the study in any group (Table 1).

The remaining rats did not show significant changes in SBP through the study, although final SBP in Dex + Met rats was somewhat 5 mmHg more elevated, but not significantly, than in the other two remaining groups (vehicle and Met). Heart rate did not show significant difference during the study in the four groups of rats (Table 1). Body weight did not change significantly through the study in any group (Table 1).

At the end of the 4 wk, serum insulin was significantly increased in Dex rats and decreased in Met rats compared with vehicle, whereas blood glucose was equal in the four groups (Table 1). Dex-treated rats showed also significantly lower (P = 0.032) levels of serum total nitrates/nitrites, whereas hematocrit, cholesterol, triglycerides, free fatty acids, and insulin were significantly higher compared with vehicle (Table 1). These effects were completely reversed by the concomitant use of Met (Dex + Met), but serum cholesterol remained higher than in vehicle. No significant changes for the other considered parameters were evident among the different groups of animals, including intraerythrocyte Na⁺ (12.1 ± 1.3, 10.4 ± 0.5, 9.8 ± 0.8, 12.5 ± 1.4, meq/10⁶ red blood cells for vehicle, Met, Dex, and Dex + Met rats, respectively).

Insulin sensitivity as estimated by the 30-min drop of blood glucose after intraperitoneal fast-acting insulin was significantly reduced in Dex-treated rats compared with the other groups at days +8, +12, and +26 (Fig. 2B). Similar results were obtained when insulin sensitivity was estimated by the steady-state plasma glucose during insulin/glucose infusion. Mean steady-state glycemia during the third hour of insulin/glucose infusion was significantly higher in Dex rats compared with Dex + Met rats: 128 ± 6 vs. 84 ± 5 mg/dl (P <
Table 1. Body weight, heart rate, biochemical serum parameters, and hematocrit at the end of the 4-wk experiment in the different groups of rats (experiment 1)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Met</th>
<th>Dex</th>
<th>Dex + Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>415 ± 18</td>
<td>400 ± 21</td>
<td>390 ± 17</td>
<td>395 ± 22</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>330 ± 25</td>
<td>340 ± 30</td>
<td>345 ± 28</td>
<td>365 ± 35</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40.2 ± 0.5</td>
<td>40.4 ± 0.2</td>
<td>42.0 ± 0.3†</td>
<td>41.4 ± 0.7</td>
</tr>
<tr>
<td>T-cholesterol, mg/dl</td>
<td>70 ± 6</td>
<td>72 ± 3</td>
<td>99.6 ± 9†</td>
<td>115 ± 10†</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>142 ± 18</td>
<td>121 ± 17</td>
<td>249 ± 20†</td>
<td>136 ± 18</td>
</tr>
<tr>
<td>Free fatty acids, mM</td>
<td>0.29 ± 0.14</td>
<td>0.25 ± 0.14</td>
<td>0.81 ± 0.18†</td>
<td>0.18 ± 0.08</td>
</tr>
<tr>
<td>β-hydroxybutyrate, mM</td>
<td>0.76 ± 0.3</td>
<td>0.72 ± 0.4</td>
<td>0.86 ± 0.4</td>
<td>0.79 ± 0.5</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>22 ± 2.1</td>
<td>14 ± 0.9‡</td>
<td>32 ± 2.3*</td>
<td>21 ± 1.9</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>77 ± 4.5</td>
<td>78 ± 2.4</td>
<td>75 ± 4.8</td>
<td>73 ± 3.2</td>
</tr>
<tr>
<td>Uric acid, mg/dl</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Nitrates/nitrites, µmol/ml</td>
<td>78 ± 9</td>
<td>62 ± 9</td>
<td>42 ± 10*</td>
<td>63 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE. Met, metformin; Dex, dexamethasone. *P < 0.05, †P < 0.01 vs. vehicle. ‡P < 0.05 vs. Dex.

0.02), while serum insulin at the end of the 3-h infusion was comparable in the two groups.

At the end of the study, no significant difference in heart, liver, and kidney weight (corrected for body weight) was evident in the four groups of rats. Glycogen content, determined histologically in the hepatocytes, was reduced in Dex-treated rats compared with vehicle and Met-treated rats. Moreover, in Dex-treated rats, an increased deposit of lipids at the liver level was evident (Fig. 3). The addition of Met in Dex rats partially restored the content of glycogen at the liver level, dramatically reducing the liver lipids content. No significant differences in the different groups of rats were evident regarding kidney structure, although the glomerular apparatus in Dex rats appeared bigger than in the other groups (data not shown).

Experiment 2

All animals completed the 28 days of experiment, and water consumption was equal in the groups (30–37 ml·day⁻¹·rat⁻¹) without significant changes in rats given both Arg or Isn compared with vehicle or Dex. Rats treated with Dex + Isn did not show any significant change in SBP, heart rate, and body weight through the 28 days of experiments compared with vehicle (data for final measurement at day 28 are shown in Table 2). Isn treatment in addition to Dex was also able to completely reverse the effects of Dex treatment in terms of triglycerides, hematocrit, and delta glycemia changes after intraperitoneal insulin (Table 2). On the other hand, Dex + Arg rats did not significantly differ from Dex-only rats (Table 2). Thus only the direct NO donor Isn given in association with Dex was able to restore the conditions seen in the vehicle group.

DISCUSSION

In this paper we describe for the first time, to the best of our knowledge, a new animal model of glucocorticoid-induced insulin resistance possibly due to endo-
Table 2. Principal biochemical serum parameters, systolic blood pressure, and body weight in the four groups of animals at day 7 and at the end of the 28 days of experiment 2

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Dex</th>
<th>Dex + Arg</th>
<th>Dex + Ins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+7</td>
<td>+28</td>
<td>+7</td>
<td>+28</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>118 ± 2</td>
<td>119 ± 3</td>
<td>120 ± 5</td>
<td>151 ± 6†</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>405 ± 25</td>
<td>400 ± 21</td>
<td>386 ± 23</td>
<td>388 ± 30</td>
</tr>
<tr>
<td>Glycemia, mg/dl</td>
<td>75 ± 3</td>
<td>78 ± 4</td>
<td>72 ± 4</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>23 ± 20</td>
<td>21 ± 19</td>
<td>38 ± 2.3*</td>
<td>33 ± 2.5*</td>
</tr>
<tr>
<td>Δ% G</td>
<td>-44 ± 4</td>
<td>-41 ± 5</td>
<td>-27 ± 5*</td>
<td>-24 ± 4*</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>138 ± 19</td>
<td>232 ± 21†</td>
<td>215 ± 20†</td>
<td>135 ± 20</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.24 ± 0.13</td>
<td>0.79 ± 0.15†</td>
<td>0.68 ± 0.16</td>
<td>0.23 ± 0.10</td>
</tr>
<tr>
<td>Nitrates/nitrates, μmol/ml</td>
<td>64 ± 7</td>
<td>80 ± 8</td>
<td>35 ± 10*</td>
<td>41 ± 10*</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40.3 ± 0.7</td>
<td>40.0 ± 0.8</td>
<td>41.8 ± 0.5†</td>
<td>43.2 ± 0.4†</td>
</tr>
</tbody>
</table>

Values are means ± SE. +7 and +28 refer to treatment days. SBP, systolic blood pressure; TG, triglycerides; FFA, free fatty acids; Δ% G, blood glucose variation (% from basal) after 1.6 U insulin Actrapid ip. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. vehicle (same day).

The renal dysfunction. We previously showed that it is possible to increase blood pressure for long term in rats with subcutaneous doses of dexamethasone on the order of micrograms per day without appreciable catabolic effects and a complete inhibition of ACTH secretion (31) and without sodium retention (12). The main aim of this study was to establish if this old model of glucocorticoid-induced hypertension is a useful new model to study insulin resistance. Using our “pure glucocorticoid” model, characterized by the absence of catabolic side effects, we were able to show that dexamethasone-induced insulin resistance (increase in serum insulin concentration, decreased insulin tolerance, and higher state-state glucose concentration during exogenous insulin/glucose infusion) precedes hypertension and is accompanied by other features of the metabolic syndrome (increased serum free fatty acids, cholesterol, and triglycerides and decreased NO production as marker of endothelial dysfunction). Insulin resistance in our animal model clearly precedes the development of hypertension, and we were able to show that dexamethasone treatment reduces total nitrates/nitrates. Indeed, total nitrates/nitrates may be considered a good marker of NO synthase activity (13), suggesting an endothelial dysfunction as primus mover for the development of this dexamethasone-induced insulin resistance. NO undergoes a series of reactions with several molecules present in biological fluids as plasma and urine. The final in vivo products of NO are nitrite and nitrate. Since the relative proportion of nitrite and nitrate may vary, as a better index we measured the sum of the two as previously reported (13). The addition of metformin to dexamethasone treatment restores serum total nitrates/nitrates to the levels observed in the vehicle group, suggesting that metformin might have overcome the dexamethasone-induced endothelial dysfunction, the most probable pathogenetic mechanism of insulin resistance in this animal model. Pharmacological blockade of NO synthase activity has suggested an important role of its product, NO, in regulating insulin sensitivity and carbohydrate metabolism (1, 27). Data obtained in our second experiment confirm this hypothesis. Indeed, arginine did not modify dexamethasone-induced increase in SBP and serum triglycerides; it was not able to restore the circulating levels of nitrate/nitrates to the levels observed with vehicle and did not modify the dexamethasone-induced decrease in insulin sensitivity. When isosorbide dinitrate treatment is added to dexamethasone, serum nitrates/nitrates values higher than in the vehicle, although not significantly, were achieved, and only isosorbide was able to prevent the rise in SBP and in circulating triglycerides and the decrease in insulin sensitivity induced by dexamethasone alone. Mice with gene disruption of endothelial NO synthase show insulin resistance (21), stressing the importance of this system in the modulation of the peripheral insulin sensitivity at least in animals. Our data suggest that an endothelial disruption caused by dexamethasone treatment inactivates the NO synthase that has an important role in this newly described animal model of insulin resistance and metabolic syndrome.

Insulin resistance, before the development of type 2 diabetes and/or hypertension, may cause endothelial dysfunction with a key role in the pathogenesis of vascular complications (8, 33). Metformin is able to increase peripheral insulin sensitivity and insulin-mediated glucose uptake in the cells, increasing insulin-induced translation of GLUT4 from an intracellular pool to the plasma membrane and increasing the functional activity of the glucose carrier without altering the de novo synthesis of the glucose carrier both in vivo (15) and in vitro (15). In this way, metformin might reverse the concomitant endothelial dysfunction. Indeed, recently, the United Kingdom Prospective Diabetes Study (UKPDS) has shown that metformin treatment in overweight type 2 patients reduces significantly the occurrence of myocardial infarction (35), compared with sulfonylureas or insulin in overweight type 2 patients (34), suggesting additional effects of metformin (2) in addition to lowering glycemia. It is well known that glucocorticoids alter insulin sensitivity, and they have a role in altering glucose metabolism and blood pressure regulation. Most of the already described glucocorticoid animal models do not
take into account catabolism, with muscle atrophy (6, 17, 37) and increased blood pressure that can affect the measurement of insulin sensitivity by altering peripheral blood flow. Moreover, no data on the long-term glucocorticoid treatment are available as well regarding a possible endothelial involvement. We chose metformin as a well-established (25), both in vivo and in vitro, drug therapy to ameliorate peripheral insulin resistance in rats as well in humans. Its wide use in diabetes is mainly due to its effects on peripheral insulin action to increase glucose uptake and utilization (30), although recently a direct effect of metformin on restoring \(\beta\)-cell insulin secretion response in vitro has been described. The reduction in blood pressure induced by metformin in insulin-resistant rats is apparently through a direct mechanism with a NO-dependent relaxation (10). Indeed, metformin has been shown able to attenuate the development of hypertension in the spontaneously hypertensive rats (19) usually reported to be insulin resistant (4, 17). These effects of metformin on blood pressure are not present in other animal models of hypertension not characterized by insulin resistance (38), indicating that metformin is not able per se to decrease blood pressure. An additional alternative "nonesterified fatty acid hypothesis" may be added to explain glucocorticoid-induced insulin resistance (17). Our data show that dexamethasone treatment increases free fatty acids in plasma, and this might have contributed to insulin resistance, while metformin treatment restores free fatty acid metabolism (21), as we found in this experiment. Metformin increases the effects of infused \(l\)-arginine on lowering blood pressure, decreasing platelet aggregation and blood viscosity in non-insulin-dependent diabetes mellitus patients. We did not measure blood viscosity in our experiment, but metformin plus dexamethasone rats showed, compared with dexamethasone-only rats, lower hematocrit levels, lower triglycerides, and lower free fatty acids, all features that can explain a decrease in blood viscosity. Feeding rats with the NO synthase inhibitor \(N^\omega\)-nitro-\(l\)-arginine elevates serum triglycerides and cholesterol and lowers hepatic fatty acid oxidation, affecting the activity of hepatic carnitine palmitoyltransferase, the rate-limiting enzyme of fatty acid oxidation, increasing circulating free fatty acid due to a reduction in fatty acid oxidation (11). Indeed, raised free fatty acid concentrations have been associated with the development of hypertension (3), skeletal muscle insulin resistance (23), decreased insulin secretion (20), and fatty liver (14), this last being regarded as the hepatic consequence of the metabolic syndrome due to specific hepatic insulin resistance (14). In our experimental model, dexamethasone, together with the other classic features of metabolic syndrome such as insulin resistance, hypertriglyceridemia, was able also to increase the hepatic fatty acid accumulation, as observed in humans in whom nonalcoholic fatty liver disease is a feature of the metabolic syndrome with insulin resistance (14). Metformin dramatically reversed this fat accumulation together with the other features of the metabolic syndrome. Recently (39), metformin has been shown able to activate AMP-activated protein kinase in hepatocytes, leading to reduction in acetyl-CoA carboxylase activity, induction of fatty acid oxidation, and suppression of the expression of lipogenic enzymes. These new data may provide a unified explanation for the pleiotropic effects of metformin, in particular regarding modulation in circulating lipids and reduction in hepatic lipid synthesis and fatty liver, as we observed in our experiment. The effects on cholesterol, triglycerides, and free fatty acids were observed with dexamethasone treatment and reversed by the concomitant use of metformin. Thus we postulate that dexamethasone, by altering NO synthase expression, might alter lipid metabolism with an increase in free fatty acid and consequently insulin resistance. Because in human hypertension subtle changes in adrenal steroid metabolism have been suggested (28, 29, 36), indicating that glucocorticoids in addition to the well-known Cushing syndrome might have an important role in the development of high blood pressure, this animal model is useful to study this aspect of human hypertension.

In conclusion, the two most important findings of this study are as follows: 1) long-term low-dose subcutaneous dexamethasone induces insulin resistance that precedes hypertension, and both conditions are reversed by the concomitant treatment with metformin; and 2) dexamethasone-induced insulin resistance might be due to endothelial dysfunction. Our data indicate that low-dose dexamethasone-induced hypertension in rats, an old model (12, 31, 32) of hypertension, can be revisited as an endothelial dysfunction causing insulin resistance and consequently some of the commonly observed features of the metabolic syndrome (hypertension, lipid-metabolism alteration, fatty liver, etc.). In particular, the presence also of the hepatic consequences of the insulin resistance in humans (fatty liver) appears to complete the picture of the metabolic syndrome due to reduced insulin sensitivity. This model would be useful for studying insulin resistance-blood pressure relationships.

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C. Severino is at present working in the Servizio Diabetologia, Dipartimento Struttura Clinica Medica e Patologia Speciale Medica, University of Sassari as a postdoctoral fellow.

REFERENCES


