Dexamethasone-induced insulin resistance shows no gender difference in healthy humans

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SUMMARY
Objective: Recent reports suggest that lipid-induced insulin resistance is more pronounced in men than in women. Whether such gender difference exists for other factors known to induce insulin resistance in healthy individuals remains unknown. We therefore assessed whether glucocorticoid-induced insulin resistance differs in men and women.

Methods: The insulin sensitivity and insulin secretion of 8 women and 7 men, all non-obese and healthy, were evaluated with or without administration of dexamethasone (2 mg/day during 2 days) by means of a two-step hyperglycemic clamp.

Results: Dexamethasone decreased insulin sensitivity to the same extent in men and women. The relative increases in insulin concentration observed after dexamethasone in the basal state, during the first phase of insulin release and at the two steps of hyperglycemia were similar in men and women. The hyperinsulinemia thus attained allowed to fully compensate for insulin resistance in both genders.

Conclusions: The effects of glucocorticoids on insulin sensitivity and insulin secretion show no gender difference in healthy humans.

Key-words: Glucocorticoid · Hyperglycemic clamp · Insulin sensitivity.

RéSUMÉ
Objectif : Il a récemment été suggéré que la résistance induite par les lipides était plus marquée chez l’homme que chez la femme. L’effet du genre sur d’autres agents capables d’induire une insulino-résistance n’est cependant pas connu. Dans cette étude, nous avons donc comparé les effets des glucocorticoides sur la sensibilité à l’insuline et la sécrétion d’insuline chez l’homme et chez la femme.

Méthodes : La sensibilité à l’insuline et la sécrétion d’insuline ont été évaluées à l’aide d’un clamp hyperglycémique chez 8 femmes et 7 hommes de poids normal. Cette mesure a été effectuée à 2 reprises, soit sous ou après l’administration de 2 mg/jour de dexaméthasone pendant 2 jours.

Résultats : La dexaméthasone a réduit de manière comparable la sensibilité à l’insuline chez les hommes et chez les femmes. La stimulation de la sécrétion d’insuline (phase rapide et phase lente) était aussi identique dans les 2 genres. L’hyperinsulinémie induite par la dexaméthasone a permis de rétablir un métabolisme glucidique quasi normal tant chez les hommes que chez les femmes.

Conclusions : Les effets de la dexaméthasone sur la sensibilité à l’insuline et la sécrétion d’insuline ne sont pas différents chez l’homme et chez la femme.

Mots-clés : Dexaméthasone · Résistance à l’insuline · Genre · Clamp hyperglycémique.
Insulin resistance is highly prevalent in affluent countries, and is tightly associated with several morbid conditions and risk factors for disease. The mechanisms underlying this condition remain unknown, but are most likely multiple, and may include genetic factors, sedentarity [1], and altered plasma or intracellular lipid metabolism [2]. Recently, it has also been recognized that increased tissue conversion of inactive to active glucocorticoids may also play a role [3].

Recently, it has been observed in both mice and humans that the insulin resistance induced by high plasma free fatty acid was gender specific: infusion of lipid emulsions reduced significantly insulin-mediated glucose disposal in males, but not in females [4-5]. This gender difference may be due to a reduction of insulin-receptor substrate 1 (IRS1) phosphorylation and of phosphatidylinositol kinase activation induced by fatty acids in males, but not in females [4]. Furthermore, observations done in mice [4] proposed a cellular mechanism responsible for this gender difference. Other studies have since reported contradictory findings [6-7]. This gender difference in lipid-induced insulin resistance remains therefore an opened question.

To further evaluate that effect of gender in the development of insulin resistance, we evaluated whether glucocorticoid-induced insulin resistance affects similarly males and females. The effect of a short-term treatment with the glucocorticoid analogue dexamethasone has been widely documented in humans and rodents. It involves both increased concentrations of plasma free fatty acids [8-10] and a direct inhibition of glucose transport in skeletal muscle [11]. Since impaired glucose tolerance and/or type 2 diabetes will result not only of insulin resistance, but also of an inadequate compensatory insulin secretion [12], we simultaneously assessed the effects of a 2-day administration of dexamethasone on insulin sensitivity and insulin secretion by means of a recently described two-step hyperglycemic clamp protocol [13].

**Methods**

**Subjects**

Seven male and 8 female non-smoker subjects took part in this study. Their mean age was 30.1 ± 3.8 years (range 22-52) for male and 23.6 ± 0.6 (range 21-26) years for female. They all had normal body mass index: males: 21.6 ± 0.4 kg/m², (range: 19.8-22.9); females 21.9 ± 0.8 kg/m², (range 18.8-25.7). All subjects were apparently in good health and did not take any medication at the time the studies were performed. They all had moderate levels of physical activity and showed no history of diabetes or metabolic disorders among first degree relatives. The experimental protocol they accepted to follow had been accepted by the ethical committee of Lausanne University, School of Medicine, and every subject provided an informed written consent. Results regarding the female subjects have been reported in detail elsewhere [13].

**Experimental protocol**

Every subject was studied twice, with an interval of 2 to 6 weeks between the two experiments. Female subjects were studied during the follicular phase of their menstrual cycle (i.e. within 10 days of the beginning of their menses). On one occasion, he/she had been administered 0.5 mg 4 times a day, at 8 am, 12 am, 6 pm and 10 pm during the two days preceding the experiments, and 0.5 mg in the morning of the experiment. On the second occasion, he/she did not receive any medication. The order with which dexamethasone was administered was randomized. Both times, subjects were studied according to the same protocol [13]. They came to the metabolic investigation unit at 8 am after an overnight fast. Thereafter, they took place in a bed where they lay quietly in a semirecumbent position while watching video movies. One venous cannula was inserted in a vein of the left arm and was used for infusion of glucose and deuterated glucose. A second venous cannula was inserted into a vein of the right wrist and was used for periodic blood sampling. The hand of the subject was maintained in a thermostabilized box heated at 50°C to achieve partial arterialization of venous blood. Immediately after the cannulas were inserted, a primed 2 mg/kg continuous 20 mg/kg/min infusion of 6,6 ²H₂ glucose (CIL, Worcester, MA) was started. After one hour, a two-step hyperglycemic clamp procedure was applied. A primed-variable infusion of glucose 20% labeled with 1.25% 6,6 ²H₂ glucose was administered to increase plasma glucose to 2.5 mmol/l above basal values during the first hour, then to 5 mmol/l above basal values during the second hour. Blood samples were collected at times -60, 2, 4, 6, 8, 10, 15, 30, 60, 90 and 120 min for determination of plasma insulin concentrations, and at times -60, 30, 60, 90 and 120 min for determination of plasma 6,6 ²H₂ glucose enrichment.

**Analytical procedure**

Plasma glucose concentration was determined using a Beckman glucose analyzer II (Beckman Instruments, Palo Alto, CA). Plasma insulin concentrations was measured by radioimmunoassay (kit from Linco, St Charles, MO). Plasma free fatty acid (FFA) concentrations were measured colorimetrically using a kit from Wako (Freiburg, Germany). Plasma 6,6 ²H₂ glucose was measured by gas chromatography-mass spectrometry, as described earlier [14].

**Calculations**

Whole body glucose turnover was calculated by plasma 6,6 ²H₂ glucose dilution analysis (hot infusion model) [15]. Several parameters were calculated to assess insulin secretion. The first phase of insulin secretion was evaluated from the peak insulin concentration observed during the first 10 min of glucose infusion. The second phase of insulin secretion was evaluated at each step of hyperglycemia from the average values of plasma insulin obtained during the 2nd half hour of each plateau. Insulin sensitivity was evaluated by...
dividing whole body glucose turnover by plasma insulin concentrations. Endogenous glucose production was calculated during the last 30 min of each plateau of glycemia as the difference between whole body glucose turnover and the exogenous glucose infusion rate. To evaluate glucose-induced insulin secretion, the slope of the glucose-insulin curve was calculated as (insulin (120 min) — insulin (60 min))/glucose (120 min) — glucose (60 min)).

**Statistics**

All data in the text, tables and figures are expressed as mean ± 1 sem. The effects of dexamethasone on metabolic parameters in men and women were analyzed by paired t-tests. The comparison between men and women was assessed by an unpaired Student t-test. The correction of Bonferroni was used to account for multiple comparisons.

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**Figure 1**

Plasma glucose, insulin and FFA concentrations during the two-step hyperglycemic clamp in men (squares) and women (triangles) with (closed symbols) or without (open symbols) dexamethasone. *: p < 0.05 vs no without dexamethasone. All comparison men vs women were not significant.
Results

The glycemias obtained during the clamp studies with or without dexamethasone in male and female healthy subjects are shown in Figure 1. Fasting plasma glucose concentrations were similar in males and females and were not affected by dexamethasone. During clamp studies, the target glycemic values (2.5 mmol/l and 5 mmol/l above basal values during the first and second hour, respectively) were attained in all experiments. Dexamethasone administration increased significantly plasma insulin concentrations, both in fasting conditions and during the clamp (Fig 1). The relative increase in plasma insulin concentration elicited by dexamethasone was 164% vs 105% in males vs females in fasting conditions, 151% vs 122% during the first phase of insulin secretion, 108% vs 101% at the end of the first plateau of glycemia and 99% vs 127% at the end of the second plateau of glycemia (males vs females, ns in all cases) (Fig 1). Furthermore, the delta insulin/delta glucose ratio, which gives an estimate of the sensitivity of insulin secretion to changes in glycemia, were also increased to the same extent in males and females (from 99% to 198% in males and from 63% to 123% in females).

Plasma FFA are shown in Figure 1. Fasting FFA concentrations were significantly increased after dexamethasone in men but not in women. FFA were suppressed to comparable values during the clamps in men and women: –81% vs –89% in men and from –81% to –82% in women (Fig 2).

Table I shows the glucose infusion (M) and glucose rate of disappearance (GRd) during the clamp. Both were similar in males and females during the first plateau but were increased in men during the second plateau without dexamethasone. However, they were not significantly affected by dexamethasone neither in men nor in women and the difference between men and women with dexamethasone was not significant.

<table>
<thead>
<tr>
<th></th>
<th>M mmol/kg.min</th>
<th>M mmol/kg.min</th>
<th>GRd mmol/kg.min</th>
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<td>1st plateau</td>
<td>16.9 ± 1.1</td>
<td>13.6 ± 1.2</td>
<td>18.5 ± 0.9</td>
<td>15.8 ± 1.2</td>
<td>2.1 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>0.168 ± 0.019</td>
<td>0.140 ± 0.021</td>
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<td>2nd plateau</td>
<td>32.3 ± 2.5</td>
<td>27.2 ± 2.1</td>
<td>34.1 ± 2.2</td>
<td>25.8 ± 1.6</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>0.130 ± 0.013</td>
<td>0.123 ± 0.025</td>
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<td><strong>Dexamethasone</strong></td>
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<td>1st plateau</td>
<td>12.8 ± 1.6</td>
<td>12.7 ± 1.7</td>
<td>15.3 ± 0.9</td>
<td>17.3 ± 0.9</td>
<td>2.7 ± 0.5</td>
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<td>0.071 ± 0.009</td>
<td>0.083 ± 0.01b</td>
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<tr>
<td>2nd plateau</td>
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<td>27.7 ± 1.9</td>
<td>31.1 ± 4.4</td>
<td>27.0 ± 1.7</td>
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<td>0.063 ± 0.003</td>
<td>0.063 ± 0.010b</td>
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*: different from males; #: different from controls; M: glucose infusion rate; GRd: glucose rate of disappearance; GRd/I: glucose rate of disappearance/insulin concentration; EGP: endogenous glucose production.

Figure 2
Changes in plasma free fatty acid (expressed as fatty acid concentrations during the clamp — basal fatty acid concentration) during the two-step hyperglycemic clamp with or without dexamethasone. Same symbols as Figure 1: p < 0.05 or less from time 30 to time 120 men vs women. NS with vs without dexamethasone.
Endogenous glucose production (EGP, Tab I) was not different between men and women at plateau 1 or 2. In women, suppression of EGP was impaired at both plateaus of glycemia after dexamethasone. A similar trend was observed in men but the difference between the tests with and without dexamethasone did not reach statistical significance.

Insulin sensitivity, evaluated from the ratio of the glucose rate of disappearance to average plasma insulin concentrations (GRd/I) observed during the last 30 min of each glycemic plateau during clamp studies was similar in men and women (Tab I). Furthermore, it was reduced to the same extent in both genders (by 55°7% in males and 37°8% in females, (NS) for the first plateau and by 45°8% and 47°6% (NS) at the second plateau of glycemia).

Discussion

In healthy individuals, gender is known to affect insulin sensitivity. Several reports indicate that whole body insulin mediated glucose disposal is somewhat lower in females than in males, a difference which was attributed to a lower fat-free mass in females [16-18]. Besides body composition, gender may nonetheless exert additional effects on insulin’s actions since it has been reported that insulin stimulated-glucose utilization is higher in the skeletal muscle of healthy female than male subjects after ingestion of a glucose load [19]. Recent studies have suggested that there might be important gender differences in the way FFA affect whole body and skeletal muscle insulin sensitivity [4-5]. Elevated plasma FFA reduced whole body insulin-mediated glucose disposal in both male rats and men, but failed to do so in female rats and women. Furthermore, FFA reduced insulin-induced phosphorylation of insulin receptor substrate 1 in male but not in female rats [4]. Although these results have since been challenged by two studies [6-7], the issue of possible gender differences in the pathogenesis of insulin resistance remains an important and poorly investigated issue. Regarding fatty acid metabolism, it has recently been reported that, although male and female healthy subjects have similar fasting plasma free fatty acid concentrations and lipid oxidation rates, the rate of whole body lipolysis is substantially higher in females [20]. This observation implies that non oxidative lipid metabolism differs markedly in men and women and this may possibly affect the way lipids modulate insulin sensitivity.

In the present study, we evaluated whether dexamethasone-induced insulin resistance affects similarly both genders. The effects of dexamethasone on glucose homeostasis are highly complex and involve changes in whole body free fatty acid turnover, plasma insulin concentrations, alterations of muscle glucose transport (most likely at the membrane glucose transporter link), alterations of hepatic insulin sensitivity and glucose production [21]. Our present observations clearly indicate that the global effect of dexamethasone on whole body insulin sensitivity is not different in male and female humans. Whole body insulin sensitivity (suppression of fatty acid concentrations by hyperglycemia/hyperinsulinemia), and suppression of hepatic glucose production were similarly affected in both genders.

The experimental protocol we used also allowed us to evaluate insulin secretion, and to assess to which extent the increased insulin secretion observed after dexamethasone was able to compensate insulin resistance. The effect of a short-term administration of dexamethasone induced the same relative increase in plasma insulin concentrations in the fasting state, during the first phase of insulin secretion, and during both plateaus of glycemia in our graded hyperglycemic clamp studies. Furthermore, the observation that whole body glucose rate of disappearance and suppression of plasma free fatty acid concentrations remained normal during these hyperglycemic clamp studies indicates that the hyperinsulinemia induced by dexamethasone allowed to maintain normal whole body glucose disposal and lipolysis. This suggests that dexamethasone-induced muscle and adipose tissue insulin resistance could be fully compensated by increased insulin secretion in these healthy volunteers.

In summary, the results obtained in this study indicate that the effects of dexamethasone on whole body glucose metabolism, plasma free fatty acid concentrations, and endogenous glucose production are similar in male and female subjects. Furthermore, the compensatory increase in insulin secretion which occurs after dexamethasone is also comparable in both genders.

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References