J. Endocrinol. Invest. 31: 238-242, 2008

Glucocorticoid effect on insulin sensitivity: A time frame

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ABSTRACT. It is well known that glucocorticoids induce insulin resistance, but the exact time scale in humans is not well known. The aim of the study was to determine the time scale of effects of pharmacologic doses of glucocorticoids on insulin sensitivity. Subjects were treated with repeated methylprednisolone infusions and oral prednisone for Graves' orbitopathy. Insulin sensitivity was determined using euglycemic hyperinsulinemic clamp (EHGC) before, during the first glucocorticoid infusion and after 2 months of treatment. EHGC started 2 h after the start of the glucocorticoid infusion, and lasted for 2 h. In another group of patients, insulin sensitivity was determined by short insulin tolerance test (SITT) before and during the first glucocorticoid infusion. SITT started 15 min after the start of the glucocorticoid infusion and lasted for 15 min. Ten subjects were included in each protocol. All were euthyroid during the study period. Four hours after the start of the glucocorticoid infusion significant reduction of insulin sensitivity was observed, which did not change for a further 2 months of glucocorticoid treatment [before 7.82 (95% confidence interval (CI) 5.35-10.29), first infusion, 4.93 (95% CI 2.99-6.87), after 2 months 5.36 (95%CI 3.91-6.81) mg/kg/min]. No significant change in insulin sensitivity occurred during the first 30 min of glucocorticoid infusion [before 139.7 (95%CI 94.1-185.3), during 146.7 (95%CI 106.3-187.1) μmol/l/min]. In humans, glucocorticoid-induced insulin resistance develops quickly, in about 4 h, and does not change during further glucocorticoid treatment.

(J. Endocrinol. Invest. 31: 238-242, 2008)

INTRODUCTION

Glucocorticoids are known to reduce insulin sensitivity. Their principal effects are to oppose the actions of insulin in the regulation of carbohydrate, lipid, and protein metabolism. In addition, glucocorticoids inhibit glucose-stimulated insulin secretion from pancreatic β-cells (1). Most of these effects take time to develop. However, some glucocorticoid effects progress rapidly, in minutes. Inhibition of insulin secretion was shown in the 8th min in the perfused rat pancreas, and in the 1st min in the perfused isolated islets (2). *In vivo* studies in rats showed that corticosterone caused an acute depression of plasma insulin and a decreased insulin response to glucose (3). In the isolated rat fat cells, dexamethasone at high concentration inhibited glucose uptake within 1 min of its addition to fat cells (4).

Although it is well known that glucocorticoids induce insulin resistance, the exact time scale in humans is not well known. If glucocorticoids rapidly induce insulin resistance, then even a short-term increase in their concentration could be detrimental. Therefore, a single dose or a short-term glucocorticoid use could induce significant insulin resistance. In rats, a single dexamethasone dose causes insulin resistance after 4 h (5). In humans, short-term glucocorticoid exposure decreases glucose tolerance, and prolonged glucocorticoid exposure induces insulin resistance (6-8). A single 1 mg dexamethasone dose did not significantly affect insulin-stimulated glucose disposal and oxidation. However, 2-day dexamethasone treatment produced a 40% decrease in insulin-mediated glucose disposal and oxidation in healthy human subjects (7, 8).

The objective of the present study was to determine the time scale of effects of pharmacologic doses of glucocorticoids on insulin sensitivity. We measured insulin sensitivity in subjects treated for

Key-words: Insulin sensitivity, glucocorticoids, glucose clamp, insulin tolerance test, glucocorticoid therapy.

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Accepted May 18, 2007.

Graves' ophthalmopathy with high glucocorticoid dose. To assess changes in insulin sensitivity after prolonged glucocorticoid exposure (more than 2 h) we performed euglycemic-hyperinsulinemic glucose clamp. To assess insulin sensitivity during the first 30 min of glucocorticoid infusion short insulin tolerance test (SITT) was used, as it takes only 15 min.

MATERIALS AND METHODS

Subjects and therapy protocol

Two protocols were used to determine insulin sensitivity. Protocol 1 consisted of euglycemic-hyperinsulinemic clamp, and in protocol 2 SITT was used.

Twenty subjects were included in the study, 10 in each of 2 protocols (subject's clinical and metabolic data are presented in Table 1). Subjects were glucocorticoid-treated for the severe Graves' orbitopathy. They had never been treated with glucocorticoids before. Pre-treatment OGTT was normal in all subjects. During the whole study period they were euthyroid, most of them on the block and replace treatment. Subjects were otherwise healthy, and they were not on any other medication (e.g. propranolol). The glucocorticoid treatment lasted for 6 months, and consisted of 6 identical therapy cycles. Each cycle was started with 500 mg of methylprednisolone dissolved in 500 ml normal saline as a 4-h iv infusion. The infusion was repeated after 48 h followed by po prednisone in a tapering fashion (40 mg/day for 7 days, then 30 mg/day for 7 days, then 20 mg/day for 7 days and then 10 mg/day for 7 days). After that a new therapy cycle would start with 500 mg methylprednisolone infusion.

The study was approved by the Ethics Committee. All subjects gave informed consent.

Table 1 - *Subject's clinical and metabolic data. Values are presented as mean and 95% confidence interval for mean.*

	Protocol 1 Baseline	Protocol 1 Two months	Proticol 2 Baseline
Age (yr)	50.2 42.4-58.0		44.9 37.4-52.4
BMI (kg/m^2)	25.9 21.9-29.9	26.3 22.5-30.1	22.3 20-24.6
Glucose (mmol/l)	4.97 4.61-5.32	5.17 4.83-5.51	5.14 4.3-5.98
Insulin (mU/l)	11.40 5.38-17.43	12.90 10.06-15.74	15.4 11.88-18.92
Total cholesterol (mmol/l)	5.20 4.46-5.94	6.09 5.51-6.67	5.72 4.82-6.62
HDL cholesterol (mmol/l)	1.22 1.08-1.36	1.36 1.00-1.71	1.3 1.08-1.52
LDL cholesterol (mmol/l)	3.30 2.70-3.90	3.87 3.22-4.52	3.88 3.09-4.66
Triglycerides (mmol/l)	1.48 0.88-2.08	1.70 1.11-2.29	1.19 0.79-1.59
Systolic blood pressure (mmHg)	129.0 118.8-139.2	134.5 124.9-144.1	123.5 114.0-133.15
Diastolic blood pressure (mmHg)	79.0 72.1-85.9	85.0 81.2-88.8	76.0 71.9-80.1
FT_4 (ng/l)	11.5 10.1-12.8	10.6 9.3-11.9	11.63 10.2-13.06
TSH (mU/l)	2.5 1.9-3.1	2.1 1.2-3.0	2.02 1.25-2.79

BMI: body mass index; FT_4: free T_4.

Experimental protocols

Protocol 1 consisted of euglycemic-hyperinsulinemic clamp. Clamp was done 1 day before first glucocorticoid infusion, during the first glucocorticoid infusion and after 2 months of glucocorticoid therapy (during the first glucocorticoid infusion of the third therapy cycle). During the pre-treatment (first) clamp study saline was infused, in the same way as glucocorticoids during the second and third clamp. Clamp was started after 2 h of the glucocorticoid/saline infusion, and lasted for 2 h. Glucocorticoid/saline infusion and clamp terminated at the same time. Clamp started with a primed continuous infusion of crystalline insulin (Novo Nordisk, Bagsværd, Denmark) and was continued during 120 min at a rate of 0.1 U/kg/h. Blood glucose was maintained at euglycemic level by variable infusion of 20% glucose. The whole body glucose disposal rate (M, mg per kg per min) was calculated according to DeFronzo (9). For the further analysis insulin concentrations and glucose disposal rates obtained at 100, 110, and 120 min were averaged (steady state rate). Protocol diagram is given in Figure 1.

Protocol 2 consisted of SITT. SITT was used to assess insulin sensitivity because the test takes only 15 min. Using this test we were able to assess change in insulin sensitivity during the first thirty minutes of glucocorticoid application. The test was done on two consecutive days, before and during the first glucocorticoid infusion. SITT started 15 min after glucocorticoid/saline infusion was started and terminated 15 min later. SITT was complete 30 min after the glucocorticoid infusion started. SITT consisted of bolus iv injection of crystalline insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark) 0.05 U/kg. Blood samples for glucose determination were collected at 0, 3, 6, 9, 12, and 15 min after the beginning of the test. Insulin sensitivity (IS) was estimated as the slope of the regression line of the blood glucose versus time in the period from 3 to 15 min of the test. Insulin sensitivity obtained by SITT has an acceptable agreement with insulin sensitivity obtained by the euglycemic-hyperinsulinemic clamp (10). Protocol diagram is given in Figure 1.

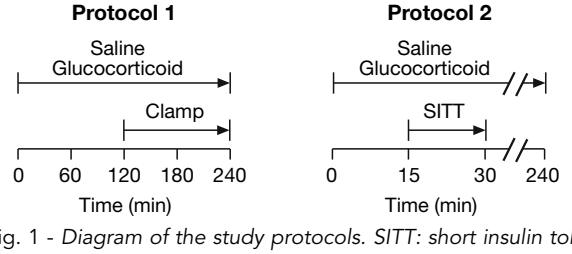

Fig. 1 - *Diagram of the study protocols. SITT: short insulin tolerance test.*

Assays

Glucose was determined using a Beckman Glucose Analyzer. Insulin was determined using a radioimmunoassay (INEP, Zemun). The intraassay coefficient of variation was 6.1%, and the interassay coefficient of variation was 16.8%.

Statistical analysis

Statistical analysis was performed using R version 2.3.0 (11). Data are presented as mean and 95% confidence interval (CI) for mean. Statistical analysis was done using t-test and linear mixed-effect models (R package nlme) (12). In the linear mixed-effect models analysis models were built using only time effect (as ordered factor) and both time (as ordered factor) and body mass index (BMI) (as continuous variable) effects. In common slopes model slope of the relation between BMI and the whole body glucose disposal rate was the same in all periods. In separate slope models, slopes of the relation between BMI and the whole body glucose disposal rate were different in each period.

RESULTS

Protocol 1 was used to assess insulin sensitivity during the last 2 h of the 4-h glucocorticoid infusion. Insulin concentration during the last 30 min of the clamp was not significantly different between the observed periods [161 (95%CI 111.91-209.73) *vs* 141 (95%CI 99.16-184.94) *vs* 156 (95%CI 127.43-185.13) mU/l, p=0.589]. However, there was a 40% reduction in the whole body glucose disposal rate 4 h after the infusion started [7.82 (95%CI 5.35-10.29) *vs* 4.93 (95%CI 2.99-6.87) mg/kg/min, p=0.001]. Two months later, the whole body glucose disposal rate was still significantly reduced (at an average of 21%) compared to the period before glucocorticoids [7.82 (95%CI 5.35-10.29) *vs* 5.36 (95%CI 3.91-6.81) mg/kg/min, p=0.012] (Fig. 2). BMI has no influence on changes in insulin sensitivity.

Protocol 2 was used to assess insulin sensitivity during the first thirty minutes of glucocorticoid infusion. Insulin sensitivity was measured using SITT, and no significant difference was found between the saline and glucocorticoid infusion [139.7 (95%CI 94.1-185.3) *vs* 146.7 (95%CI 106.3-187.1) mmol/l/min, p=0.788) (Fig. 3). BMI has no influence on changes in insulin sensitivity.

DISCUSSION

We observed a significant reduction in the whole body glucose disposal rate just 4 h after the start of glucocorticoid infusion. This reduction in insulin sensitivity was at a similar level during the following 2 months of glucocorticoid therapy. However, during the first 30 min of infusion no change in insulin sensitivity was detected. Thus, glucocorticoids, at least methylprednisolone, do not influence insulin sensitivity in less than 30 min. Nevertheless, in a few hours glucocorticoids exert full effect on insulin sensitivity. Previous studies showed that glucocorticoids reduce insulin sensitivity. However, most of the protocols using dexamethasone and glucocorticoid treatment were longer than 24 h (6, 8, 13, 14). Nevertheless, in a study by Schneiter and Tappy 1 mg dexamethasone was used and glucose metabolism study was carried out a few hours after the treatment. In this study neither the glucose infusion rate required to maintain euglycemia, nor carbohydrate and lipid oxidation were significantly altered by dexamethasone (7). There are 2 main differences between this study and ours. We used a much larger dose and a differ-

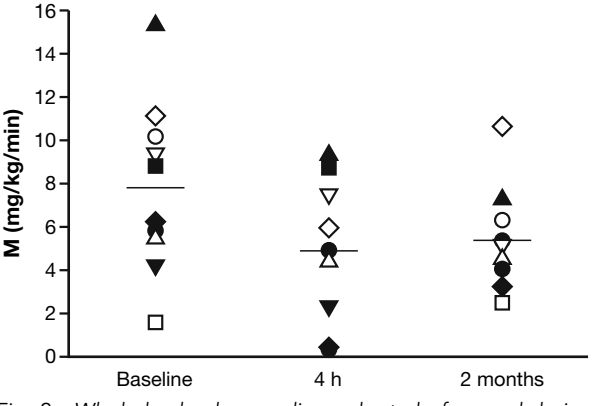

Fig. 2 - *Whole body glucose disposal rate before and during glucocorticoid treatment. Each patient is presented separately. Short straight line represents period average. Baseline period significantly differs from the other 2 periods. M: whole body glucose disposal rate (mg x kg x min).*

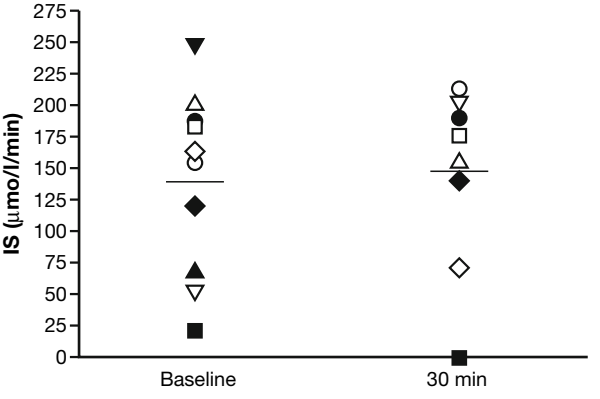

Fig. 3 - *Insulin sensitivity (IS) determined by short insulin tolerance test before and during the first 30 min of glucocorticoid infusion. Each patient is presented separately. Short straight line represents period average. There is no significant difference between the periods.*

ent glucocorticoid (methylprednisolone *vs* dexamethasone). In rats, however, a single dexamethasone dose induced whole body insulin resistance in about 4 h, a kinetics that is similar to one in our study (5). It is interesting to note that in the previous study by Besse et al. 2 days of low-dose dexamethasone administration induced insulin resistance in obese, but not in lean females (15). In our study, BMI, as a measure of obesity, did not influence glucocorticoid- induced insulin sensitivity change. Possible reasons for the difference between the previous study and ours could be different types of glucocorticoids used, different doses of glucocorticoids or differences in study group characteristics.

The classical, genomic model of steroid action requires several complex steps. This causes latency in genomic steroid effects (>30 min). In spite of this some glucocorticoid effects occur much faster, probably by the non-genomic mechanism (16). It is assumed that rapid non-genomic glucocorticoid effects are mediated by 3 different mechanisms: physicochemical interactions with cellular membranes (non-specific non-genomic effects), membrane-bound glucocorticoid receptor mediated non-genomic effects, and cytosolic glucocorticoid receptor mediated non-genomic effects (17). Some of the glucocorticoid effects on insulin secretion and fat cell metabolism occur by the non-genomic mechanism (18). Therefore, we wanted to assess if there is any change in whole body insulin sensitivity during the first 30 min of glucocorticoid application. However, glucocorticoids did not cause significant change in insulin sensitivity during the designated period. The most probable explanation is that glucocorticoid-induced insulin resistance is not caused by the non-genomic mechanisms, especially as we used very high doses of glucocorticoids. As previously noted low-dose dexamethasone administration induced insulin resistance in obese, but not in lean females (15). Our study group consisted of lean subjects with an average BMI of 22.3 kg/m^2. Therefore, subject characteristics could also explain the lack of early glucocorticoid effect. However, we used a statistical model to test BMI influence on insulin sensitivity change, and did not find significant effects. Therefore, we do not think that non-obesity is related to lack of early glucocorticoid effect.

Another possible explanation is that methodological problems precluded detection of the early insulin sensitivity change. We aimed to carry out insulin sensitivity measurement during the first 30 min of infusion, to be sure that only non-genomic effects were observed. However, it could be too early due to the delay in glucocorticoid distribution to tissues. In addition, early insulin sensitivity change might be too small to be detected by the SITT. On the other hand, a recent study by Darmon et al. used SITT and showed reduced insulin sensitivity after 4 h of glucocorticoid infusion (19).

Glucocorticoids are potent regulators of energy metabolism. They act on the liver, muscle and fat but also on central nervous system. Dexamethasone applied intracerebroventricularly causes insulin resistance (20). Glucocorticoids also influence quantity and quality of food intake and glucose allocation in the body (21, 22). Even relatively mild and short term increase in cortisol concentration causes changes of quality and quantity of food intake in humans (23). On the other hand, glucocorticoids quickly reduce insulin secretion (18). This causes glucose intolerance, even in subjects whose insulin sensitivity is unchanged (7). Moreover, we have shown that short-term (4 h) glucocorticoid exposure significantly reduces insulin sensitivity. Therefore, even a short-term increase in glucocorticoid concentration causes significant change in glucose homeostasis. Whether it is detrimental or advantageous would depend on the circumstances.

Another interesting finding in our study is that reduction in insulin sensitivity after 3 months of treatment was the same as after 4 h of treatment. This suggests that chronic treatment with glucocorticoids does not progressively worsen the degree of insulin resistance. The clinical implication of this finding is significant, as this means that most significant changes in glucose metabolism will occur very soon after institution of the glucocorticoid therapy.

In conclusion we have shown that glucocorticoid-induced insulin resistance develops quickly, in about 4 h. It does not seem that non-genomic mechanisms are involved, as there is no change in insulin sensitivity during the first 30 minutes of glucocorticoid exposure.

ACKNOWLEDGMENTS

This work was supported by a grant from the Ministry of Science and Environmental Protection of Republic of Serbia (145031).

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