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Evaluation of Insulin Sensitivity in Patients with Klinefelter's Syndrome

A Hyperinsulinemic Euglycemic Clamp Study

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Patients with Klinefelter's syndrome have a higher incidence of diabetes mellitus and the percentage of insulin resistance was reported to be high in these patients. However, little is known about the insulin sensitivity assessed by the hyperinsulinemic euglycemic clamp in these patients. In the present study, subjects included 13 newly diagnosed patients with Klinefelter's syndrome, and 9 age- and body mass index–matched healthy males. The hyperinsulinemic euglycemic clamp was performed in all patients and controls. Insulin resistance was present in five (38.5%) patients with Klinefelter's syndrome. Compared with control subjects, patients with Klinefelter's syndrome had elevated plasma concentrations of fasting insulin, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin, whereas they had reduced plasma free testosterone and total testosterone concentrations. The multivariate linear regression analysis showed that fasting glucose, fasting insulin, free testosterone, and total testosterone were independently associated with *M*-value. In conclusion, the present study by using hyperinsulinemic euglycemic clamp indicates the high prevalence of insulin resistance in Klinefelter's syndrome patients. However, these patients did not have reduced mean *M*-values compared with the controls, although their plasma insulin levels were significantly elevated. It is possible that hyperinsulinemia may be the primary metabolic abnormality rather than insulin resistance.

Key Words: Klinefelter's syndrome; insulin resistance; hyperinsulinemia; hyperinsulinemic euglycemic clamp; testosterone.

Introduction

There is a wealth of clinical and experimental data demonstrating that sex steroids are able to influence insulin sensitivity *(1)*. Pregnancy, where estrogen and progesterone concentrations are markedly raised, is also associated with reduced insulin sensitivity *(2)*. Artificially raised concentrations of the sex steroid hormones estrogen and progesterone found in women taking the combined oral contraceptives also affect glucose tolerance and produce a degree of insulin resistance *(3)*. In both sexes, insulin action is impaired and insulin secretion is increased during puberty *(4–8)*. Meanwhile, various clinical observations and experimental data from in vitro studies suggest that insulin and androgens interact and effects of androgens on insulin sensitivity appear to be sexually dimorphic. Castrated male rats showed increased insulin resistance, which was improved by low-dose testosterone replacement *(9)*. However, treatment with high-dose testosterone worsened insulin resistance in female rats *(10)*. Administration of supraphysiological amounts of dihydroepiandrostendione that also result in testosterone elevations has produced mild hyperinsulinemia in women, but had no effects on insulin sensitivity in men *(11,12)*. The modest hyperandrogenism characteristic of polycystic ovary syndrome has been proposed to be associated with insulin resistance *(13–17)*. Several prospective studies show that low levels of free testosterone are associated with increased risk for type 2 diabetes mellitus in middle-aged *(18,19)* and elderly men *(20,21)*.

Insulin has a wide range of acute metabolic and anabolic actions including stimulation of glucose uptake into peripheral insulin-sensitive tissues, suppression of hepatic glucose output, stimulation of glycogen synthesis, and an anti-lipolytic effect on adipose tissue. Impairment of each of these actions contributes to the consequences of insulin resistance for the organism *(22)*. The term "insulin resistance" in humans is frequently used synonymously with impaired insulin-stimulated glucose disposal as measured with the hyperinsulinemic euglycemic clamp. In the hyperinsulinemic euglycemic clamp, a desired dose of insulin is administered and euglycemia is maintained by a simultaneous

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Table 1
Mean Levels and Comparisons of Parameters
in Patient with Klinefelter's Syndrome (KS) and Control Group

Parameters	Patients with KS (n = 13)	Controls (n = 9)	p
Age (yr)	22.08 ± 1.38	22.56 ± 1.51	NS
BMI (kg/m^2)	23.65 ± 4.87	21.67 ± 2.70	NS
Fasting insulin (μU/mL)	12.10 ± 0.54	6.36 ± 0.62	<0.001
M-value (mg/kg/min)	5.89 ± 2.04	7.96 ± 1.79	NS
FSH (IU/L)	51.69 ± 15.29	4.00 ± 1.66	<0.001
LH (IU/L)	39.77 ± 6.89	3.89 ± 1.45	<0.001
FT (pg/mL)	7.38 ± 3.52	32.78 ± 14.42	<0.001
TT (ng/mL)	1.04 ± 0.17	6.44 ± 1.67	<0.001
E_2 (pg/mL)	69.92 ± 6.55	32.56 ± 4.95	<0.001
DHEAS (pg/mL)	338.62 ± 133.73	384.44 ± 120.82	NS
SHBG (nmol/L)	46.00 ± 5.83	31.89 ± 7.39	0.001

Values are mean ± SD. NS = not significant; BMI = body mass index; SHBG = sex hormone–binding globulin; DHEAS = dihydroepiandrostendione sulfate; FT = free testosterone; TT = total testosterone.

variable glucose infusion whose rate is adjusted based on frequent arterialized blood glucose determinations and a negative feedback principle. At steady state, the amount of glucose that is infused equals the amount of glucose taken up by the peripheral tissues and can be used as a measure of peripheral sensitivity to insulin, known as insulin-mediated glucose disposal or *M*. Insulin-mediated glucose disposal occurs only in muscle and in fat; muscle accounts for about 85% of this.

Klinefelter's syndrome (KS) is a developmental disorder of the testis resulting from the presence of an extra X (47 XXY) chromosome. It is characterized by low plasma testosterone, increased gonadotropin secretion, elevated estrogens, small testes, aspermatogenesis, gynecomastia, and a female fat pattern *(23,24)*. Patients with KS have a higher incidence of diabetes mellitus *(25,26)*. Furthermore, the percentage of insulin resistance was reported to be high in patients with KS *(27)*. Insulin resistance in this study was evaluated by two methods: the incremental area under the curve of serum insulin concentrations in response to a 75-g oral glucose load and the insulin suppression test *(27)*. However, the hyperinsulinemic euglycemic clamp is considered the gold standard for evaluation of insulin sensitivity *(28)*. Little is known about the insulin sensitivity assessed by the hyperinsulinemic euglycemic clamp in patients with KS. On the other hand, if sex hormones play a role in the development of insulin resistance in man, KS offers an interesting window into this relation because of not only androgen deficiency but also elevated gonadotropins and estrogens. Therefore, the aim of the present study was to evaluate insulin sensitivity using the hyperinsulinemic euglycemic clamp in patients with KS.

Results

The mean levels and comparisons of parameters in patients with KS and healthy controls are shown in Table 1. Compared with control subjects, patients with KS had elevated plasma concentrations of fasting insulin, FSH, LH, E_2, and SHBG, whereas they had reduced plasma FT and TT concentrations. The mean levels of age, body mass index, DHEAS, fasting glucose, and *M*-value did not significantly differ between the groups. The multivariate linear regression analysis showed that fasting glucose (Beta = 1.011, $t = 7.356$, $p < 0.001$), fasting insulin (Beta = 0.332, $t = 3.204$, $p = 0.005$), TT (Beta = 0.523, $t = 5.713$, $p < 0.001$), and FT (Beta = 0.222, $t = 2.431$, $p = 0.016$) were independently associated with *M*-value (adjusted $R2 = 0.963$; $F = 188.663$; $p < 0.001$). When the cut-off point was selected as 4.53 mg/kg/min for *M*-value, the sensitivity and specificity were 1.00 and 0.39, respectively. Thus, the five patients with KS (38.5%) with glucose disposal rates < 4.53 mg/kg/min were identified as having insulin resistance.

Discussion

The present study showed that insulin resistance was found in more than one third of men with KS by using hyperinsulinemic euglycemic clamp, irrespective of body mass index. Similar to our results, in a previous study, higher incidence of insulin resistance was reported in both patients with hypergonadotropic and hypogonadotrophic hypogonadism *(27)*. However, the subjects with KS did not have reduced mean *M*-values compared with the controls in the current study, although their plasma insulin levels were significantly elevated. We also found that testosterone is an

independent determinant of the *M*-value. Likewise, in several clinical studies, plasma testosterone concentrations are inversely related to insulin concentrations *(27,29,30)*. Haffner et al. also reported that lower testosterone correlated with whole-body glucose disposal in men *(31)*. On the other hand, we were unable to suggest any relationship between either gonadotropins or E_2 and insulin sensitivity in patients with KS. Liu et al. reported that insulin sensitivity or β-cell function was not significantly changed by recombinant human chorionic gonadotropin despite a significant increase in lean body mass and reduced fat mass in healthy older men *(32)*. Meanwhile, DHEAS and SHBG levels are not related to insulin sensitivity in our study. Similar to our results, in a more recent study, the inverse association between testosterone and insulin resistance, independent of SHBG, was reported in middle-aged nondiabetic men *(33)*. Additionally, in one study on healthy men, no association was found between serum DHEAS levels and insulin sensitivity *(34)*.

Why do men with KS have high prevalence of insulin resistance or hyperinsulinemia? Insulin resistance, as determined by the hyperinsulinemic euglycemic clamp, reflects defective insulin action predominantly in skeletal muscle and liver. Because no patient in our study had chronic liver diseases that cause decreased hepatic extraction of insulin, it is possible that hyperinsulinemia is attributable to pancreatic β-cell hypersecretion compensating for insulin resistance. However, not only muscle glucose uptake but also adipose tissue lipolysis and suppression of glucose production are regulated by insulin. Therefore, potential androgen-mediated changes in body composition in an androgen deficiency state such as decreasing muscle size and increasing visceral fat mass may impair insulin sensitivity *(35–37)*. Administration of testosterone to female transsexuals or of ethinyl E_2 to male transsexuals caused a reduction in peripheral glucose uptake in the absence of any change in endogenous glucose production *(38)*. This indicates that the steroids have a peripheral site of action, and because skeletal muscle is responsible for the majority of peripheral glucose disposal *(28)*, it would appear that testosterone have a direct action on skeletal muscle to reduce insulin sensitivity. In this way, some studies have suggested that physiological testosterone replacement improves insulin sensitivity in middle-aged men with low testosterone levels *(35,39)*. In a more recent study, Chauhan et al. reported that administration of a gonadotropin-releasing hormone analogue was associated with a decrease in both testosterone levels and insulin action *(40)*. Furthermore, orchidectomized male rats when untreated or treated with high doses of testosterone show insulin resistance to both glucose uptake and incorporation of glucose into glycogen in skeletal muscle *(9)*. However, when treated with low doses of testosterone, all the metabolic abnormalities were corrected. Moreover, no effects of testosterone were observed on liver glycogen synthesis, suggesting that this steroid action is confined to peripheral tissues. Muscle glycogen stores have also been reported to be diminished in skeletal muscle from orchidectomized animals *(41)*. Administration of exogenous androgens has been reported to induce glucose intolerance and hyperinsulinemia in some studies *(9,42,43)*. Consequently, insulin sensitivity may be affected by decreased androgen levels directly in the target tissues, particularly muscles, therefore contributing to the development of the insulin resistance state in KS. This may also reflect indirectly that testosterone has a role in maintaining normal insulin sensitivity in men.

This study has some potential limitations. First, it has a small sample size. Second, the mechanisms underlying the association between plasma levels of testosterone and insulin-mediated glucose disposal are not entirely clarified by the present data. Another limitation of this study may be that no data after testosterone replacement are available, because hyperinsulinemic euglycemic clamp is uncomfortable for the patients and is difficult to perform and time consuming. Therefore, almost all of the patients with KS refused our offer to apply hyperinsulinemic euglycemic clamp after the testosterone replacement. The effects of testosterone administration on glucose metabolism and insulin sensitivity remain controversial *(9,35,39,42–47)*. It has recently been reported that treatment with anastrozole did not significantly affect insulin sensitivity in elderly men with low testosterone levels *(48)*. Various case reports describe induction of insulin resistance by treatment with synthetic androgens *(49)* or by abuse of anabolic steroids by young athletes *(50, 51)*. Further studies are needed to answer the question of whether testosterone treatment improves insulin sensitivity in KS.

In conclusion, patients with KS have a higher incidence of insulin resistance and hyperinsulinemia, and testosterone is an independent determinant of the whole-body glucose disposal rates on hyperinsulinemic euglycemic clamp. It is possible that hyperinsulinemia may be the primary metabolic abnormality rather than insulin resistance. Moreover, the observations presented here support the previous clinical and experimental data that androgens play an important role in the development of insulin resistance in man. Furthermore, the results of our study may be relevant in the understanding of the effect of chronic androgen deficiency state on insulin sensitivity in KS. However, the present study provides evidence of association rather than of causation. Therefore, determining whether testosterone replacement will decrease insulin resistance in KS will be of particular interest. Finally, chronic androgen deficiency–induced impairment in insulin sensitivity may have undesirable long-term health consequences.

Materials and Methods

Subjects included 13 newly diagnosed patients with KS, and 9 age- and body mass index–matched healthy males. The study was approved by the Ethical Committee of Gülhane Military Medical Academy and all subjects gave informed

signed consent prior to the study. Diagnosis of KS was based on clinical features, low levels of free testosterone, high levels of FSH and LH, and the demonstration of a chromosomal anomaly (47/XXY) on karyotype analysis. All controls had a normal gonadal development and their physical and biochemical findings were normal. Patients with a history of testosterone replacement, diabetes mellitus, or renal and hepatic diseases were excluded. None of the subjects was taking medications known to impact on insulin sensitivity.

Fasting blood samples were collected from patients and controls between 08:00 h and 08:30 h after overnight fasting. After sampling, blood was immediately chilled on ice and centrifuged, and serum as well as plasma aliquots were frozen at –80°C until assayed. All samples from individual subjects were analyzed in duplicate for each endocrine and biochemical parameter. Total plasma cholesterol, glucose, and triglyceride were measured by enzymatic calorimetric method with Olympus AU 600 autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). Serum insulin levels were measured by chemiluminescent immunoassay method (Immulite 1000, Diagnostic Product Corp., Los Angeles, CA).

Serum FSH, LH, and E_2 concentrations were measured by immunoradiometric assay with reagents from Radim Techland (Angleur, Belgium). The intra- and interassay CVs were 4.4% and 6.0% for FSH; 4.8% and 5.4% for LH; 4.4% and 6.0% for E_2. Serum free testosterone (FT) was determined by analog radioimmunoassay method (Diagnostic Systems Laboratories, Webster, TX). The intra- and interassay CVs for FT were 3.8% and 4.2%. Serum total testosterone (TT) and DHEAS levels were determined by radioimmunoassay with reagents from Diagnostics System Laboratories Inc. (Webster, TX). The intra- and interassay CVs were 9.3% and 11.0% for TT; 7.9% and 5.8% for DHEAS. Serum sex hormone-binding globulin (SHBG) levels were determined by chemiluminescence method using an automated hormone analyzer Immulite 1000 (Diagnostic Product Corp., Los Angeles, CA). The intra- and interassay CVs were 6.4% and 8.7% for SHBG. The normal ranges in our laboratory are <15 IU/L for FSH; <20 IU/L for LH; <60 pg/mL for E_2; 2.7–10.7 ng/mL for TT; 15–45 pg/mL for FT; 200–3350 pg/mL for DHEAS; and 13–71 nmol/L for SHBG.

The hyperinsulinemic euglycemic clamp was measured at 09.00 after an overnight fast, using a modification of the method as described previously *(52)*. In brief, two cannulas were inserted into an antebrachial vein or hand vein of each arm. One was used for intermittent arterialized venous sampling of blood for determination of glucose levels; the other was used for infusion of regular insulin and glucose. The right forearm was placed in a heating sleeve. Insulin infusion was started at a rate of 127.6 mIU/m^2 and decreased to 40 mIU/m^2 in the first 10 min. After reaching the steady-state velocity for the insulin infusion within 10 min in order to achieve steady-state insulin levels of approx 95 μU/mL (range 80–110) during the clamp, a variable infusion of 20% glucose was begun via a separate infusion pump and the rate was adjusted, on the basis of plasma glucose samples drawn every 5 min, to maintain plasma glucose between 95 and 105 mg/dL. The insulin infusion was continued at a rate of 40 mIU/m^2 for 110 min. The rate of total insulin-stimulated glucose disposal as the mean value for each 10 min interval during the last 30 min of a 2 h infusion [*M*-value (mg/kg/min)] was taken as the estimate of peripheral insulin sensitivity.

Statistical and Analytical Methods

Statistical analyses were performed by SPSS 10.0 (SPSS Inc., Chicago, IL, USA) statistical package. Descriptive statistics were shown as the arithmetic mean ± sd. Differences between control and study groups were investigated by Mann–Whitney U test. Parameters effects on *M*-value were calculated first by univariate linear regression and then by multivariate linear regression procedures as *M*-value were the dependent and the other parameters as the independent variables. *p* values less than or equal to 0.05 were evaluated as statistically significant *(53)*.

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