An association between a BclI restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women

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ABSTRACT

Obesity is likely to be a multifactorial disease with an important genetic component. Animal models of genetic and experimentally induced obesity suggest that glucocorticoid receptor (GR) activity plays a role in the aetiology and maintenance of the obese state. Glucocorticoid activity appears to be essential for the development of hyperinsulinaemia and subsequent fat deposition. In humans, glucocorticoid excess is associated with central fat distribution. We have therefore investigated the restriction fragment length polymorphisms of the human GR gene locus (GRL) and have sought associations of specific alleles with anthropometric measurements and indices of insulin secretion and resistance in obesity.

Fifty-six extremely obese, unrelated, non-diabetic premenopausal British Caucasian females and 43 age-matched, normal weight controls were studied. The obese subjects were characterized by fat distribution (waist to hip ratio), insulin secretion and insulin resistance (fasting insulin (FI)), an index of insulin resistance (HOMA), stimulated insulin secretion during an oral glucose tolerance test and insulin-mediated glucose disposal, steady-state plasma glucose). A BclI polymorphism (fragments of 4.5 and 2.3 kb) demonstrated significant association with indices of glucose metabolism in obesity; those subjects homozygous for the 4.5 kb fragment had elevated FI (Pc=0.012) and HOMA (Pc=0.012) values. The genotypic and allelic frequencies of the GRL BclI polymorphism were otherwise similar in obese and normal weight subjects. We postulate that the GRL BclI polymorphism may directly affect GR gene expression, or be in linkage disequilibrium with a possible mutation within one-three exons of the GR gene, and thereby modulate GR transcriptional activity on target genes involved in glucose and insulin homeostasis.

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INTRODUCTION

Obesity appears to be a multifactorial disorder determined by both environmental and genetic factors. Evidence suggesting a genetic aetiology comes from animal, twin, family and adoptee studies (Laskarzewski et al. 1983; Despres & Bouchard, 1984; Trayhurn, 1984; Poehlman et al. 1986; Stunkard et al. 1986; Zonta et al. 1987; Sorensen et al. 1989; Stunkard et al. 1990). Although we have identified an association of polymorphism in the 5' flanking region of the insulin gene with hyperinsulinaemia and central obesity (Weaver et al. 1992a), the search for other candidate genes for obesity has so far been unsuccessful (Weaver et al. 1990, 1992b).

Evidence from several animal models of obesity (Debons et al. 1978; White & Martin, 1990; Stubbs & York, 1991) indicates that glucocorticoid receptor activity, particularly within the central nervous system, may be involved in the aetiology of obesity. In fa/fa, genetically obese rats, the development or progression of obesity is reversed or attenuated by adrenalectomy (Saito & Bray, 1984). This effect is mediated by reduced food intake, altered feeding pattern (Freedman et al. 1985) and increased diet-induced brown adipose tissue thermogenesis (Holt et al. 1983). The fa/fa rats are characterized by basal hyperinsulinaemia (Zucker & Antaniadis, 1972), excessive secretion of insulin in response to various secretagogues (Rohner-Jeanrenaud & Jeanrenaud, 1985; Rohner-Jeanrenaud et al. 1986) or to electrical
stimulation of the vagus (Rohner-Jeanrenaud et al. 1983) and insulin resistance (Terretaz et al. 1986). Hypersecretion of insulin is evident from an early stage in development and is considered to lead to insulin resistance and obesity (Rohner-Jeanrenaud et al. 1983; Jeanrenaud, 1985; Rohner-Jeanrenaud & Jeanrenaud, 1985). Adrenalectomy prevents or abolishes hyperinsulinaemia by an additional direct effect on the pancreas, where glucocorticoid receptors in the β cells are down-regulated following glucocorticoid deprivation (Fischer et al. 1990).

Some physiological changes leading to obesity are thought to be determined by abnormalities of the autonomic system causing an imbalance between the sympathetic (reduced sympathetic drive to thermogenic tissues) and parasympathetic (increased stimulation of the pancreas) systems (Bray & York, 1979; Jeanrenaud, 1985). These abnormalities are reversed by treatment with a glucocorticoid receptor blocker (Langley & York, 1990). Obese adrenalectomized fa/fa rats display excessive sensitivity to glucocorticoids with respect to their dose-dependent increase in body weight, total body lipid content and plasma insulin levels when compared with lean adrenalectomized animals (Freedman et al. 1985, 1986). It appears that the gene expression of various enzymes is altered in genetically obese animals following adrenalectomy but not in slim littermates (Bray, 1990).

The evidence that steroid hormones play a part in the pathogenesis of human obesity remains circumstantial. The polycystic ovary syndrome, Cushing's syndrome and obesity induced by pharmacological doses of steroids are characterized by a central type of fat distribution associated with hyperinsulinaemia, glucose intolerance and insulin resistance. This type of fat distribution has been shown to be genetically determined (Bouchard et al. 1988). Steroid hormones are also important in the growth and development of adipose tissue. Their effect was shown to be mediated by the differential distribution of glucocorticoid receptors (Rebuffe-Scrive et al. 1985, 1990). In view of the above evidence for the modulating role of glucocorticoids in the pathogenesis of obesity, hyperinsulinaemia and insulin resistance, we hypothesized that a mutation of the glucocorticoid receptor gene might be associated with obesity and/or hyperinsulinaemia in man.

MATERIALS AND METHODS

Patients

Fifty-six severely obese, unrelated, premenopausal Caucasian women were recruited from the Obesity Clinic at the Royal London Hospital for the study. They were non-diabetic, as documented by a normal 75 g oral glucose tolerance test (WHO criteria). Their mean body mass index (BMI; weight in kg/height in m²) was 42 (range 34–59) and their mean age 31 years (range 21–43). The majority of the women included in the study had a family history of obesity. The control group consisted of 43 normal weight, healthy female volunteers, of BMI range 20–25 and mean age 25 years (range 23–30), who were members of the hospital staff. Informed written consent was obtained from all the subjects.

Insulin resistance and insulin secretion

Different methods for the assessment of insulin sensitivity were used within a period of 10 days in the same obese patients. All subjects remained on a weight-maintaining diet for 6 weeks prior to the investigations. Not all obese patients were studied by each of the methods.

1. Fasting insulin (FI) was measured at 09.00 h after an overnight fast.

2. Relative insulin resistance was calculated using ambulatory fasting plasma insulin and glucose levels after an overnight fast by the homeostatic model of assessment (HOMA) method (Matthews et al. 1985).

3. Insulin-induced glucose disposal was measured after a 16-h fast by determining the steady-state plasma glucose (SSPG) during a simultaneous intravenous infusion of dextrose (420 mg/min) and insulin (0.77 U/kg body weight per min), using somatostatin (500 µg/h; Serono Laboratories (U.K.) Ltd, Welwyn Garden City, Herts, U.K.) to suppress endogenous insulin secretion. Measurements of plasma insulin and glucose were performed at the beginning of the infusion and then every 30 min for a further 150 min. Steady-state plasma insulin and glucose levels were achieved after 90 min of infusion. The arithmetic mean of the glucose measurements at 90, 120 and 150 min of the clamp period was taken to express a quantitative index of insulin resistance, SSPG. (Nagulesparan et al. 1979).

4. The insulin secretion in response to a 75 g oral glucose tolerance test was measured by calculating the area under the curve (AUC) of plasma insulin concentration over the period from 0 to 2 h, using the trapezoid method (Macintosh Excell).

Insulin assay

Serum immunoreactive insulin was determined by double-antibody radioimmunoassay, using Guildhay antisera (Guildhay Antisera Ltd, Guildford, Surrey, U.K.). The inter- and intra-assay co-
The efficiencies of variation were 10 and 7% respectively, with a minimal detectable limit of 3 mU/l.

Fat distribution

Fat distribution was assessed by a single observer using a flexible tape measure with the subject standing and breathing shallowly. The standardized measurements (in cm) were taken at a level half-way between the lower rib margin and the iliac crest for the waist, and over the widest hip circumference for the hip; they were expressed as a ratio, W/H (WHO, 1988).

Restriction fragment length polymorphism (RFLP) methods

DNA was extracted from 10 ml blood samples, digested with restriction enzymes and studied by the Southern blot hybridization method, using a $^{32}$P-labelled 1·1 kb human glucocorticoid receptor cDNA (Hitman et al. 1984; Hollenberg et al. 1985).

The following restriction enzymes (Northumbria Biological Ltd, Cramlington, Northumberland, U.K.) were used in search of RFLPs of the glucocorticoid receptor gene: BclI, BamHI, BglII, PstI, KpnI, PvuII,MspI, SacI, RsaI, TaqI, BglII, EcoRI, HindIII, HinfI, HaeIII, XbaI. However, two polymorphic alleles were detected, sized 2·3 and 4·5 kb, using BclI as previously described (Kidd et al. 1989).

The following genotypes were identified: 2·3 kb homozygous, heterozygous (2·3 kb/4·5 kb) and 4·5 kb homozygous. No differences in genotype or allelic frequencies of glucocorticoid receptor gene locus RFLPs were found between obese and slim subjects (Table 1).

In obese women, the mean FI values ± s.d. for 2·3 kb homozygous, heterozygous and 4·5 kb homozygous subjects were 19·8±1·9, 18·9±1·8 and 31·1±1·4 mU/l respectively. The mean HOMA values ± s.d. for 2·3 kb homozygous, heterozygous and 4·5 kb homozygous subjects were 5·02±2·1, 4·6±1·8, 8·1±1·5 respectively.

Initial one-way analysis of variance showed a significant difference between the three glucocorticoid receptor locus genotypes for FI ($F=3·2$, $P=0·05$; Cochran's C $P=0·2$) and HOMA ($F=3·3$, $P<0·05$; Cochran's C $P=0·09$). As the FI values for the heterozygous and 2·3 kb homozygous subjects were almost identical, for the purpose of further statistical analysis these groups were pooled together and comparison was performed for the effect of the presence or absence of the 2·3 kb allele (Table 2). A significant inhomogeneity was detected when subjects lacking the 2·3 kb allele (4·5/4·5 kb genotype) were compared with subjects possessing the 2·3 kb allele (4·5/2·3 kb and 2·3/2·3 kb genotype) for FI (Cochran's C $P=0·01$) and HOMA (Cochran's C $P=0·009$), therefore an independent t-test using
TABLE 1. The genotypic and allelic frequencies for BcII restriction fragment length polymorphism of the glucocorticoid receptor locus in the studied group of women

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genotypic frequency</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2·3/2·3 kb</td>
<td>2·3/4·5 kb</td>
</tr>
<tr>
<td>Obese</td>
<td>0·30</td>
<td>0·48</td>
</tr>
<tr>
<td>Control</td>
<td>0·26</td>
<td>0·58</td>
</tr>
</tbody>
</table>

Chi-squared test for genotypic (2 x 3 = NS) and allelic frequencies (2 x 2 = NS); NS = non-significant.

TABLE 2. Variation of anthropometric measurements and indices of insulin secretion and insulin resistance with glucocorticoid receptor genotypes of the studied obese women. The 2·3 kb negative group comprised 4·5 kb homozygous subjects; the 2·3 kb positive group contained both 2·3 kb homozygous and 2·3/4·5 kb heterozygous subjects. Mean values are given, with 95% confidence limits for the mean. The number of subjects (n) is also shown

<table>
<thead>
<tr>
<th>2·3 kb negative genotype</th>
<th>2·3 kb positive genotype</th>
<th>F ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 43)</td>
<td>1·9</td>
<td>NS</td>
</tr>
<tr>
<td>42·7 (39·4–46·0)</td>
<td>40·0 (38·2–41·8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0·85 (0·80–0·89)</td>
<td>0·85 (0·83–0·87)</td>
<td>0·1</td>
<td>NS</td>
</tr>
<tr>
<td>(n = 12)</td>
<td>(n = 44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/H</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FI (mU/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31·1 (24·6–39·4)</td>
<td>19·2 (15·8–23·3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMA</td>
<td></td>
<td>*</td>
<td>0·012</td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8·07 (6·3–10·4)</td>
<td>4·8 (4·9–7·8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 11)</td>
<td>(n = 39)</td>
<td>*</td>
<td>0·012</td>
</tr>
<tr>
<td>SSPG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 24)</td>
<td>2·0</td>
<td>NS</td>
</tr>
<tr>
<td>8·9 (6·4–12·3)</td>
<td>7·2 (6·3–8·2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 5)</td>
<td>(n = 24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (mU/l × min)</td>
<td></td>
<td>0·8</td>
<td>NS</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 572 (7643–17 521)</td>
<td>9256 (7388–11 595)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI = body mass index, W/H = waist to hip ratio, FI = fasting insulin, HOMA = index of insulin resistance using the homeostatic model of assessment method (see text), SSPG = steady-state plasma glucose achieved during simultaneous infusion of glucose, insulin and somatostatin (see text), AUC = area under the response curve of insulin secreted during a 75 g oral glucose tolerance test.

*Calculation of the association of FI and HOMA values with glucocorticoid receptor locus genotypes showed significant inhomogeneity of variance, (Cochran's C: Pc = 0·01 and P = 0·009 respectively). Therefore, an independent t-test using separate rather than pooled variances was used, which was also significant (Pc = 0·012, P = 0·009). NS = non-significant.

The genotypic and allelic frequencies for BcII restriction fragment length polymorphism of the glucocorticoid receptor locus in the studied group of women.

Chi-squared test for genotypic (2 x 3 = NS) and allelic frequencies (2 x 2 = NS); NS = non-significant.

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DISCUSSION

This is the first study which has examined a glucocorticoid receptor gene locus as a candidate gene for human obesity and hyperinsulinaemia using a population-based approach. No association was found between the BcII RFLPs of the glucocorticoid receptor gene locus and obesity or fat distribution. However, in this preliminary study we have found a strong positive association between the BcII polymorphism of the glucocorticoid receptor gene locus (4·5 kb homozygous genotype) and the presence of hyperinsulinaemia (FI; Pc = 0·012) and relative insulin resistance (HOMA; PC = 0·012) in obese women. The fact that a positive genetic association was found with both these variables is not surprising, since they are not independent. No association was found between other measures of insulin resistance and the 4·5 kb homozygous genotype, but this could simply reflect the smaller number of subjects involved. The small number of subjects homozygous for the 4·5 kb allele raises the possibility of a spurious finding. Nevertheless, we consider the obese women as representative, as they comprised the majority of young obese females attending the obesity clinic at the time of these investigations. A multicentre study is being planned to investigate this association further.

What evidence is available to support the finding of an association between glucocorticoids and...
hyperinsulinaemia? Insulin secretion and biosynthesis are regulated by many factors, of which the most important are nutrients such as glucose, amino acids and hormones. Glucocorticoids have a pronounced effect on glucose homeostasis through both an increase in glucose production and a reduction in glucose utilization. Chronic glucocorticoid administration causes marked insulin resistance secondary to a post-receptor defect (Rizza et al. 1982). Elevated fasting and glucose-stimulated insulin levels are characteristically seen in adrenal hyperactivity or in chronic administration of glucocorticoids. More acutely, the administration of glucocorticoids reduces the insulin secretion response to various secretagogues (Kalhan & Adam, 1975; Barseghian et al. 1982; Pierluissi et al. 1986). The β cells are the only cells of Langerhan's islets to contain glucocorticoid receptors (Fischer et al. 1990), and this might imply a direct role of glucocorticoids on insulin biosynthesis and/or secretion. It has been shown that dexamethasone decreases steady-state insulin mRNA levels by more than 80%. This effect is not mediated through a change in gene transcription but rather through destabilization of insulin mRNA. The effect of dexamethasone is dose-dependent and mediated by the glucocorticoid receptor. The use of a competitive dexamethasone inhibitor for binding to the glucocorticoid receptor abolishes the dexamethasone effect (Philippe & Missotten, 1990). The glucocorticoid receptor is widely distributed and expressed in many tissues, and has been extensively investigated as a model for transcriptional regulation. The chronic effect of glucocorticoids is exerted by the regulation of gene expression, by means of transcriptional enhancement of the genes, which contain glucocorticoid response elements (GREs) near hormone-responsive promoters. GREs are active only on binding of the receptor–steroid complex. The glucocorticoid receptor consists of three structural domains, hormone-binding, DNA-binding and the modulatory region or N-terminal domain (Danielson, 1991). These regions are essential for the receptor to act as a transcription factor, effecting gene expression of the target genes. Mutations or polymorphism in these domains could therefore lead to the altered expression of genes involved in glucose and insulin homeostasis.

Possible explanations for the relationship of the BclI RFLPs of the glucocorticoid receptor gene locus and hyperinsulinaemia therefore include the following.

1. A direct effect of the BclI RFLPs on glucocorticoid receptor gene activity. However, no BclI sites are present in any of the sequences currently stored on Genebank. It would therefore seem likely that the BclI site is in the flanking region of the gene. So far there are no data on the effect of possible mutations within the flanking regions of the glucocorticoid receptor gene on receptor function.

2. Linkage disequilibrium of the BclI RFLP and a mutation within a coding region for the glucocorticoid receptor.

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