Abnormal release of incretins and cortisol after oral glucose in subjects with insulin-resistant myotonic dystrophy

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Abstract

Objective: Although the incretins, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), as well as glucagon and cortisol, are known to influence islet function, the role of these hormones in conditions of insulin resistance and development of type 2 diabetes is unknown. An interesting model for the study of hormonal perturbations accompanying marked insulin resistance without concomitant diabetes is myotonic dystrophy (DM1).

Design: The work was carried out in an out-patient setting.

Methods: An oral glucose tolerance test was performed in 18 males with DM1 and 18 controls to examine the release of incretins and counter-regulatory hormones. Genetic analyses were also performed in patients.

Results: We found that the increment in GLP-1 after oral glucose was significantly greater in patients, while there was no significant difference in GIP or glucagon responses between patients and controls, although long CTG repeat expansions were associated with a more pronounced GIP response. Interestingly, the GLP-1 response to oral glucose correlated with the insulin response in patients but not in controls whereas, in controls, the insulin response closely correlated with the GIP response. Furthermore, cortisol and ACTH levels increased paradoxically in patients after glucose; this was more pronounced in patients with long CTG repeat expansions.

Conclusions: This study showed that the GLP-1 and ACTH/cortisol responses to oral glucose are abnormal in insulin-resistant DM1 patients and that CTG triplet repeats are linked to GIP release. These abnormalities may contribute both to the severe insulin resistance and hyperinsulinemia in DM1 and to the preservation of adequate islet function, enabling glucose tolerance to be normal in spite of this marked insulin resistance in DM1.

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Introduction

Insulin secretion is adaptively increased in insulin resistance. Failure of this adaptation precedes the onset of impaired glucose tolerance and diabetes (1–4). The mechanism responsible for the increased insulin secretion in states of insulin resistance is still not established, however, although mediation by metabolic perturbations and/or autonomic activation have been proposed (5). Possible hormonal mediators include specific gut hormones functioning as incretins, i.e. hormones which are released by food intake and stimulate insulin secretion (6), most notably gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Alternatively, counter-regulatory hormones may be important, e.g. glucagon, since glucagon levels are known to be increased in insulin resistance (7). Furthermore, it has recently been suggested that altered cortisol responses to oral glucose in insulin-resistant states associated with abdominal obesity may also be of importance for the metabolic perturbations (8, 9).

Contradictory results have been published regarding differences in incretin responses between healthy subjects and patients with type 2 diabetes mellitus. One study has shown a reduced GLP-1 response in diabetes (10) whereas other studies have found normal responses (11). Furthermore, in impaired glucose tolerance (IGT), GLP-1 release has been shown to be normal whereas GIP release may be reduced (12). In order to examine the impact of insulin
resistance on changes in the release of incretin hormones, other conditions with insulin resistance not typically associated with IGT or diabetes are required. One such condition is myotonic dystrophy (DM1), the most common inherited form of muscle dystrophy among adults (13). The genetic defect causing DM1 is an expansion of a CTG triplet repeat in the DMPK gene on chromosome 19, encoding a serine-threonine protein kinase named myotonic dystrophy protein kinase (14). The number of CTG triplets in the gene varies in the normal population from 5 to 27, and >50 CTG triplets are associated with DM1 (14). There is some evidence of a direct genetic effect on the insulin receptor since the expression of insulin receptor mRNA and insulin protein are decreased in DM1 muscle (15). Whether a similar effect is present for other receptors has not yet been studied.

DM1 is characterized by pronounced insulin resistance in association with hyperinsulinemia (16, 17). However, in spite of the marked insulin resistance, glucose tolerance is often normal in patients with DM1 because of islet compensation and hyperinsulinemia (18, 19), and the prevalence of diabetes in DM1 populations ranges from 0 to 6.5% (13). Patients with DM1 might therefore be of interest for studies on mechanisms responsible for the adaptive and adequate hypersecretion of insulin in insulin resistance.

Previous studies on DM1 have shown that DM1 patients have an increased volume of GIP-producing cells in the duodenum (20). However, the responses of the incretin hormones during an oral glucose tolerance test in DM1 are not known. We and others have also reported abnormal regulation of the cortisol axis in DM1 including elevated trough levels (21, 22). Interestingly, increased adrenal cortex sensitivity to GIP has been demonstrated to cause food-dependent Cushing’s syndrome (23), providing a potential linking mechanism between the incretin and glucocorticoid pathways to insulin resistance. Whether cortisol release in response to glucose is excessive and/or related to GIP release in DM1 has, however, not yet been investigated.

In view of the possibility of studying patients with DM1 as a model for pronounced insulin resistance in the presence of normal glucose tolerance, the primary aim of this study was to assess the release of incretins and counter-regulatory hormones, including glucagon and cortisol, during the oral administration of glucose in patients with DM1. The second aim was to study the effects of the genetic defect on these hormones by analysing the number of CTG triplet repeats in DM1 patients in relation to clinical and hormonal parameters.

Materials and methods

Subjects

Clinical data of the subjects are summarized in Tables 1 and 2. Eighteen men with adult onset DM1 were recruited from the Dystrophia Myotonica Center in Boden, northern Sweden, where the prevalence of the disease is exceptionally high (24). All patients included had clinically overt myotonia and muscular dystrophy. The diagnoses were based on genetic analyses. Two patients were smokers, two used snuff and two patients both smoked and used snuff. Eighteen age-matched male controls were recruited from healthy volunteers. None of the patients or controls was taking any relevant medication, had clinical or laboratory signs of endocrinological dysfunction (including diabetes mellitus and thyroid disease), cardiac failure, renal or hepatic insufficiency, infection or inflammation, and none was hospitalized at the time of the study. Furthermore, none of the patients or controls had a diagnosis or any symptoms of sleep apnoea.

Table 1 Clinical data of DM1 patients and controls. Levels are given as medians and 10th and 90th percentiles. There were 18 in each group.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th></th>
<th>Patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (full years)</td>
<td>38</td>
<td>23–62</td>
<td>39</td>
<td>22–60</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.6</td>
<td>19.8–30.7</td>
<td>24.7</td>
<td>18.9–30.3</td>
</tr>
<tr>
<td>Body fat (%) (n = 14)</td>
<td>21.4</td>
<td>15.0–31.0</td>
<td>34.4</td>
<td>16.2–50.7</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.92</td>
<td>0.80–1.0</td>
<td>1.0</td>
<td>0.81–1.1</td>
</tr>
<tr>
<td>HOMA insulin-resistance index</td>
<td>1.4</td>
<td>0.90–3.5</td>
<td>2.3†</td>
<td>1.4–5.5</td>
</tr>
<tr>
<td>Fasting plasma GIP (pmol/l)</td>
<td>16.0</td>
<td>10.4–20.3</td>
<td>16.5</td>
<td>11.3–25.9</td>
</tr>
<tr>
<td>Fasting plasma GLP-1 (pmol/l)</td>
<td>13.0</td>
<td>8.8–20.4</td>
<td>2.0‡</td>
<td>0.95–4.2</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>51.0</td>
<td>34.0–106.7</td>
<td>83.0†</td>
<td>55.6–210.4</td>
</tr>
<tr>
<td>Fasting plasma glucagon (ng/l)</td>
<td>63.5</td>
<td>49.7–84.3</td>
<td>72.5</td>
<td>46.4–142.6</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.4</td>
<td>3.8–5.1</td>
<td>4.6</td>
<td>4.0–5.3</td>
</tr>
<tr>
<td>Fasting serum cortisol (nmol/l)</td>
<td>548.50</td>
<td>365.7–659.1</td>
<td>487.5</td>
<td>183.7–617.7</td>
</tr>
<tr>
<td>Fasting plasma ACTH (ng/l)</td>
<td>38.0</td>
<td>21.6–74.0</td>
<td>51.6</td>
<td>17.0–83.4</td>
</tr>
</tbody>
</table>

GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1.

*P < 0.05, †P < 0.01, ‡P < 0.001 compared with controls.
This study was approved by the regional ethical committee, and all participants had given their informed consent to participate in this study.

**Sampling and measurements**

All participants were fasting and the baseline samples were collected at 0700 h. An indwelling cannula was inserted at least 15 min before the baseline sample. Peripheral venous blood samples were collected before and 30, 60, 120 and 180 min after ingestion of 75 g glucose.

HOMA insulin-resistance index (fasting glucose (mmol/l) \( \times \) fasting insulin (mU/l)/22.5) was calculated for all participants as an indicator of insulin resistance (25).

Body composition was measured by bioelectrical impedance analysis (Akern-RJL Systems, BIA 101; EL-DOT K/S, Fredriksværk, Denmark). The BIA failed to measure four patients due to too high resistance (>999 ohm). These patients were excluded from all analyses involving body fat mass.

**Analytical methods**

Serum concentrations of cortisol and plasma concentrations of adrenocorticotropic hormone (ACTH) were determined in untreated samples by radioimmunoassay (RIA) using commercial kits obtained from Orion Diagnostica, Esbo, Finland (cortisol) and Nichols Institute Diagnostics, San Juan Capistrano, CA, USA (ACTH).

Plasma insulin concentrations were analysed with a double-antibody RIA technique. Guinea pig anti-human insulin antibodies, \(^{125}\)I-Tyr-human insulin as tracer and human insulin standard (Linco Research, St Charles, MO, USA) were used. Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco Research). Plasma glucose was determined using the glucose oxidase method.

Plasma glucagon concentrations were determined by double-antibody RIA using guinea pig anti-human glucagon antibodies specific for pancreatic glucagon, \(^{125}\)I-glucagon as tracer, and glucagon standard (Linco Research).

Plasma GIP levels were analysed with a double-antibody RIA technique using rabbit anti-human GIP antibodies, \(^{125}\)I-labeled human GIP and human GIP standard as previously described (26). The antibody used cross-reacts fully with human GIP, but not with the 8 kDa GIP, the nature and relationship of which to the synthesis or secretion of GIP is still unclear (27).

Plasma GLP-1 was determined with an RIA after extraction with ethanol as previously described (28). The antiserum is directed against the amidated C-terminus of GLP-1 and therefore measures mainly GLP-1 of intestinal origin.

Intra-assay coefficients of variation (CV) were as follows: for insulin <3%, glucagon <9%, GIP <6%, GLP-1 <6%, and for ACTH <3.2%. Interassay CV values were as follows: for insulin 6%, glucagon 12%, GIP 9%, GLP-1 10%, and for ACTH <7.8%. Total CV for cortisol was <12.9%. The detection limits, determined in undiluted samples, were as follows: for cortisol 7 nmol/l, ACTH 1 ng/l, insulin 12 pmol/l, glucagon 5 ng/l, GIP 1 pmol/l, and for GLP-1 1 pmol/l.

**Genetic analyses**

Genomic DNA was prepared from blood collected in EDTA-coated tubes according to standard procedures and digested with EcoRI or PstI according to the manufacturer’s instructions. Southern blotting and hybridizations were performed according to standard (29). The probe used was pM10M6 (14), a 1.4 kb fragment that flanks the expanded region of the *myotonic dystrophy protein kinase* gene. The allele sizes were calculated with the computer program DNAfrag version 3.03 (National Research Council of Canada).

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**Table 2** Clinical data of DM1 patients with short (<615) and long (>615) CTG repeat expansions. Levels are given as medians and 25th and 75th percentiles. There were 9 in each group.

<table>
<thead>
<tr>
<th></th>
<th>DM1 short CTG</th>
<th>DM1 long CTG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Percentiles</td>
</tr>
<tr>
<td>Age (full years)</td>
<td>37</td>
<td>26–55</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.8</td>
<td>19.9–27.3</td>
</tr>
<tr>
<td>Body fat (%) (n = 7)</td>
<td>32.1</td>
<td>21.5–46.6</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.90</td>
<td>0.84–1.02</td>
</tr>
<tr>
<td>HOMA insulin-resistance index</td>
<td>2.2</td>
<td>1.9–2.6</td>
</tr>
<tr>
<td>Fasting plasma GIP (pmol/l)</td>
<td>14.0</td>
<td>12.0–18.0</td>
</tr>
<tr>
<td>Fasting plasma GLP-1 (pmol/l)</td>
<td>3.0</td>
<td>1.0–4.0</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>82.0</td>
<td>62.5–94.0</td>
</tr>
<tr>
<td>Fasting plasma glucagon (ng/l)</td>
<td>72.0</td>
<td>55.0–101.0</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>4.7</td>
<td>4.1–4.7</td>
</tr>
<tr>
<td>Fasting serum cortisol (nmol/l)</td>
<td>534.0</td>
<td>355.5–592.5</td>
</tr>
<tr>
<td>Fasting plasma ACHT (ng/l)</td>
<td>51.9</td>
<td>46.4–68.2</td>
</tr>
</tbody>
</table>

There were no significant differences between these groups.
Figure 1 Response curves of (A) glucose, (B) insulin, (C) glucagon, (D) gastric inhibitory peptide (GIP), (E) glucagon-like peptide (GLP-1), (F) cortisol, and (G) ACTH after an oral glucose load of 75 g in 18 men with myotonic dystrophy (DM1) (●) and 18 healthy men (○). \( p < 0.01 \) (median and 25th and 75th percentile; Bonferroni correction for repeated analyses).
Statistical analyses

All statistics were performed using a commercial computer program, SPSS (SPSS, Inc., Chicago, IL, USA). We used Spearman’s rank correlation test ($r_s$) for correlation analyses and the Mann–Whitney U test exact $P$ value for comparisons between groups. Multiple regression analyses were also performed. Hormonal responses to oral glucose were analysed with repeated measurement analysis with Hotelling’s T as multivariate test. As post hoc test for individual time-points we used the Mann–Whitney U test with Bonferroni correction for relevant time-points.

In order to adjust for different baselines in the responses to oral glucose, we used the centered cumulative response ($\text{CCR} = (\text{area under the curve}) - (\text{baseline level} \times \text{total min of sampling})$).

For subgroup analyses, we divided the DM1 patients into two groups according to the number of CTG repeats (Table 2), using the median number of CTG triplet repeats as cut-off.

In cases where the hormone level was below the detection limit, the value was set to half the detection limit for statistical calculations. A $P$ value of $<0.05$ was considered significant.

Results

CTG repeats

The median number of CTG triplet repeats in DM1 patients was 614.5 (347–1088; 10th–90th percentiles), and was used as a cut-off for subgroup analyses.

Response to oral glucose

Fasting insulin was increased in DM1 patients whereas basal GLP-1 levels were reduced. In contrast, baseline GIP and glucagon levels did not differ between the groups (Table 1).

Following the administration of oral glucose, circulating glucose levels did not differ between DM1 patients and healthy controls during the first 2 h after glucose ingestion (Fig. 1A). In all but three patients, the 2-h glucose level was below the limit for IGT. In contrast, plasma insulin was markedly elevated in patients with DM1 and very high levels were present at 30 and 60 min (Fig. 1B). There was no significant difference in the glucagon or GIP responses (Fig. 1C and D). DM1 patients had a significantly greater increase in GLP-1 levels than controls ($P < 0.05$ for Δ30-min level, $P < 0.01$ for Δ60-min level and adjusted area under the curve respectively) (Fig. 1E).

The increase in GIP levels in response to oral glucose was considerably greater in patients with long CTG triplet repeat expansions compared with patients with short CTG triplet repeat expansions (adjusted area under the curve for short repeat patients: 3420 (1913–4121) and long repeat patients: 7073 (4163–11 663); $P < 0.05$) and compared with controls ($P < 0.05$) (Fig. 2A). There were no significant differences in the insulin (Fig. 2B), GLP-1, or glucagon (data not shown) levels or responses to oral glucose between patients with long and short CTG triplet repeat expansions. There was no difference in body fat mass between patient subgroups (Table 2).

Figure 2 Response curves of (A) GIP and (B) insulin after an oral glucose load of 75 g in DM1 patients with short (∆, $n = 9$) and long (○, $n = 9$) CTG triplet repeat expansions and in controls (∇, $n = 18$). $^bP < 0.01$ (short CTG expansions vs controls) and $^cP < 0.01$ (long CTG expansions vs controls) (median and 25th and 75th percentiles; Bonferroni correction for repeated analyses).
A˚ Johansson and others did not significantly change the results. Valid results from the body composition measurements were largely the same. Exclusion of the four patients without the GLP-1 response to oral glucose, the results were correlated to CCR and \( P \), significantly with higher GIP levels at all time-points following oral glucose in patients. In controls, GIP levels correlated positively to insulin, GLP-1, and cortisol for the group as a whole. Patients with long CTG repeat expansions and not in controls in conjunction with normal glucose tolerance, increased responses of insulin, GLP-1, and cortisol to oral glucose ingestion in patients and the control group, there was an interaction between time and GIP that independently predicted insulin levels \( P < 0.001 \). In DM1 patients, insulin levels were significantly predicted by an interaction between GLP-1 \( P < 0.01 \), GIP \( P < 0.05 \), and time per se \( P < 0.05 \).

Repeated measurement analyses
Repeated measurement analyses showed that there were significant time/group interactions for GLP-1 \( P < 0.05 \), glucose \( P < 0.001 \), insulin \( P < 0.05 \), glucagon \( P < 0.05 \), and cortisol \( P < 0.05 \). For GIP, there was no time/group interaction and no group effect. However, when dividing patients into two groups according to the number of CTG repeat expansions, there were no time/group interactions but significant group effects for GIP \( P < 0.05 \) and cortisol \( P < 0.05 \).

Correlations
In controls, GIP levels correlated positively to insulin levels at all time-points following oral glucose \( r_s = 0.48–0.60; P < 0.05–P < 0.01 \). In DM1 patients, there was a significant positive correlation between CCRs for GIP and insulin \( r_s = 0.54; P < 0.05 \). CCR and \( \Delta \) 30-min levels of GLP-1 correlated to CCR and \( \Delta \) 30-min levels of insulin in patients \( r_s = 0.48–0.58; P < 0.05 \) for all, but not in controls \( r_s = -0.34–0.06 \).

The morning levels of cortisol correlated negatively with morning GIP levels \( r_s = -0.55; P < 0.05 \) in patients. An increasing number of CTG repeats correlated significantly with higher GIP levels at all time-points following oral glucose intake \( r_s = 0.52–0.74; P = 0.05–P < 0.001 \) and to GIP CCR \( r_s = 0.65; P < 0.05 \).

Adjustments for body fat mass were performed in all the analyses above and, except for loss of significance of the GLP-1 response to oral glucose, the results were largely the same. Exclusion of the four patients without valid results from the body composition measurements did not significantly change the results.

Multiple regression analyses
In order to isolate the effects of the incretin hormones GIP and GLP-1 on insulin release after oral glucose tolerance test, linear multiple regression analyses were performed for each group separately after logarithmic transformation of data. Within the control group, there was an interaction between time and GIP that independently predicted insulin levels \( P < 0.001 \). In DM1 patients, insulin levels were significantly predicted by an interaction between GLP-1 \( P < 0.01 \), GIP \( P < 0.05 \), and time per se \( P < 0.05 \).

Discussion
We have confirmed in this study that subjects with DM1 exhibit a marked hyperinsulinemia, and we have shown that this is evident not only under basal conditions but also after oral glucose. This pattern is indicative of a severe insulin resistance, although this conclusion is based on indirect evidence and not on direct measurements. This supports previous findings of pronounced insulin resistance in DM1 patients \(16, 17 \). Since the DM1 patients did not have diabetes it is assumed that the hyperinsulinemia reflects adequate islet compensation for the reduced insulin sensitivity in order to maintain normal glucose homeostasis. DM1 is therefore a very interesting model for the studies of metabolic perturbations in severe insulin resistance with adequate islet compensation. In this study, we have focused on the possible contribution by incretin hormones, glucagon, and cortisol for the pronounced hyperinsulinemia after oral administration of glucose in DM1.

The patients had lower GLP-1 levels under baseline conditions than the control subjects. However, the value of this finding is unclear because the nature of the basal GLP-1 in the patients is not known. We used a C-terminally directed antibody to measure GLP-1, and this antibody detects both intact and metabolized forms of GLP-1 because the hormone is degraded from its N-terminal end \(28 \). Instead, a main finding of the present study was the augmented GLP-1 response to oral glucose in DM1 patients. This may contribute to the increased insulin response in these patients, corroborated by our finding that the GLP-1 response correlated with the insulin response in DM1 patients. This would, in turn, suggest that, in insulin resistance, increased GLP-1 secretion in response to oral glucose is required to ensure that exaggerated hyperinsulinemia maintains normal glucose tolerance. In line with this, subjects with impaired glucose tolerance have been shown to exhibit a GLP-1 response to oral glucose which is not different from that in healthy subjects, i.e. not exaggerated \(12 \). However, to establish this hypothesis, prospective studies are
required in populations at risk of developing IGT and diabetes.

Since a main stimulus for GLP-1 secretion is activation of L-cells from the luminal side of the gut (30), the augmented increase in GLP-1 after oral glucose in DM1 may be due to accelerated gastric emptying and/or intestinal motility (31). This would allow the glucose load to reach the GLP-1-producing cells in jejunum faster and in a greater concentration. Gastric emptying has been reported to be delayed in DM1 patients (32). However, retarded glucose delivery to the gut is unlikely since this would be reflected by a slower increase in circulating glucose, which was not observed. Other neuroendocrine factors might also have been involved in the exaggerated GLP-1 response to oral glucose in DM1, e.g. secretin or the neuropeptide gastrin-releasing polypeptide (6). In contrast, GIP does not seem to be involved in the regulation of GLP-1 since there were no persistent correlations between these incretins in our patients or controls, a finding which is in accordance with earlier studies (33). Finally, a negative feedback mechanism between insulin and GIP allowing an increased GLP-1 secretion, may be diminished in DM1 as has been reported in mice models of obesity-related diabetes syndromes (34).

Although GIP and GLP-1 have been shown together to fully account for the incretin effect (35), the relative contribution of each incretin to insulin secretion after oral glucose is not known. Our results suggest that, in healthy subjects, GIP is a more important insulin-releasing factor after oral glucose than GLP-1, since there was a closer correlation between the GIP and insulin responses than between the GLP-1 and insulin responses. In fact, the insulin response to oral glucose in the healthy subjects was independently predicted by the GIP but not the GLP-1 response. This would confirm a recent suggestion by Nauck (36) that GLP-1 is not a major incretin in healthy subjects, but rather functions as a so called ‘ileal break’. In contrast, among the insulin-resistant DM1 patients, GLP-1 appears to contribute to insulin release to a greater extent since, in the patients, the insulin response to glucose was independently predicted both by GLP-1 and by GIP and the influence of GLP-1 was greater than the influence of GIP. However, to allow a more definite conclusion on causal relationship between the incretins and insulin, these cross-sectional observations need to also be confirmed by intervention studies, e.g. by infusion of GLP-1 or GIP.

Interestingly, previous studies have shown that in type 2 diabetes, the incretin effect may be reduced or absent and that GLP-1 may be more important than GIP in stimulating insulin secretion (37, 38). Thus, the insulinotropic action of GLP-1 seems to be preserved in diabetes, whereas that of GIP is absent (38, 39). The reason for this is unclear, but a failure of the β-cell to respond to GIP due to a defective GIP receptor at the β-cell has been proposed (38, 40). Whether a GIP receptor defect exists in DM1 is not known but this is a possibility, considering the increased GIP levels among patients with long CTG triplet repeat expansions. Indeed, the genetic defect has been proposed to directly influence the expression of the insulin receptor, and at least one of the proteins encoded from the DM1 locus has been suggested to be involved in cell signaling (15, 41). Interestingly, we have shown here that the specific genetic defect in DM1, the increased number of CTG triplet repeats, correlates with an increased GIP response to oral glucose. These results corroborate recent findings from Rönnblom and co-workers (20) who showed that the volume of GIP-producing cells in duodenum was increased in DM1. The genetic defect has previously been associated with cognitive dysfunction and some clinical features, notably male hypogonadism and gonadal hormone levels (42, 43).

We found no significant alterations in glucagon regulation in our population of DM1 patients since glucagon levels decreased to a similar extent in both patients and controls in the present study, in accordance with earlier studies in healthy subjects (44). Therefore, insulin resistance in DM1 does not seem to have affected the glucagon cells. This is in contrast to the increased levels of glucagon in subjects with overt IGT and diabetes (45–47).

Our present study also suggests that food-related cortisol excess may exist in these patients since the cortisol release pattern was abnormal, with absence of the expected diurnal decrease at 30 min and actually an increase in cortisol levels at 60 min. This was associated with a concomitant increase in ACTH levels in patients with long CTG repeat expansions, suggesting that alterations in ACTH reactivity and flattened diurnal rhythmicity of cortisol, as demonstrated in earlier studies (21, 22), may be partly food dependent. Glucocorticoids have numerous effects on intermediary metabolism, including altered lipid metabolism, stimulation of gluconeogenesis, and inhibition of peripheral glucose utilization, and may thus contribute to insulin resistance. Aberrant adrenal sensitivity to GIP seems, however, not to be present among our DM1 patients, since there were no positive associations between GIP and cortisol levels. This is in line with findings in patients with adrenal incidentalomas, who displayed a similar pattern of cortisol release after oral glucose which seemed to be cortisol mediated, and not induced by GIP (48).

In conclusion, we have found alterations in GIP and GLP-1 responses to oral glucose in DM1 patients whereas glucagon levels were normal. Furthermore, the increased number of CTG triplet repeats in DM1 closely correlated with the release of GIP. We also found a paradoxical cortisol release after oral glucose in DM1 patients. These results indicate that altered incretin and cortisol, but not glucagon, secretion may...
contribute to the hyperinsulinemia and the severe insulin resistance in DM1.

Acknowledgements

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