CHAPTER 9

CONTRIBUTORY ROLES OF CIRCULATORY GLUCAGON AND GROWTH HORMONE TO INCREASED RENAL HAEMODYNAMICS IN IDDM PATIENTS

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The stimulatory effects of growth hormone (GH) and glucagon on renal function are well known, but it is uncertain whether these hormones are involved in the increase in renal function, characteristic of IDDM patients. Therefore, the circulatory levels of GH and glucagon were measured in 10 IDDM patients with an elevated glomerular filtration rate (GFR >130 ml/min per 1.73m²) and in 20 age and sex matched normofiltering patients (GFR ranging from 90 to 130 ml/min per 1.73m²). In the patients, fasting glomerular filtration rate and effective renal plasma flow (ERPF) were determined using ¹²⁵I-iothalamate and ¹³¹I-hippuran, respectively, during near-normoglycaemia. On a separate day, the levels of glucagon and GH were determined in the fasting basal state and after exercise. Multiple regression analysis disclosed that GFR was positively correlated with HbA₁c \( (r^2=0.18, \ p<0.01) \), glucagon \( (r^2=0.14, \ p<0.03) \) as well as exercise-stimulated GH \( (r^2=0.10, \ p<0.05) \). ERPF was independently associated with HbA₁c \( (r^2=0.24, \ p<0.005) \) and glucagon \( (r^2=0.18, \ p<0.01) \), whereas renal vascular resistance (RVR) was negatively correlated with stimulated GH \( (r^2=0.18, \ p<0.02) \). Kidney volume was positively correlated with HbA₁c \( (r^2=0.26, \ p<0.001) \) and inversely with RVR \( (r^2=0.16, \ p<0.01) \), but not with glucagon or stimulated GH. The present study suggests that circulatory GH and glucagon play a contributory role in the renal haemodynamic changes in IDDM.

Introduction

Elevations in glomerular filtration rate (GFR) are well recognized in insulin-dependent diabetes mellitus (IDDM) and can persist for many years after onset of the disease [1-4]. In experimental diabetes mellitus glomerular hyperfiltration appears to be involved in the pathogenesis of glomerulosclerosis and the progressive loss of kidney function [5]. Some clinical observations also support the suggestion that an elevated GFR might contribute to the subsequent development of diabetic nephropathy [6], but in other studies no such role could be assigned to glomerular hyperfiltration [7].

Moderate hyperglycaemia has been found to increase GFR [8,9], whereas improved metabolic control reduces renal haemodynamics as well as kidney volume [2,10-12]. Plasma levels of growth hormone (GH) and glucagon, which have a renal vasodilatory effect [13,14], are frequently elevated under circumstances of suboptimal metabolic control [15-18]. However, no differences in the diurnal profiles of GH and glucagon could

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be demonstrated between normo- and hyperfiltering IDDM patients [19], although recently an increased responsiveness to GH-releasing hormone has been observed in IDDM patients with glomerular hyperfiltration [20]. Thus, the contributory roles of these hormones in the maintenance of diabetic glomerular hyperfiltration are still controversial. Therefore, we employed an exercise test to stimulate GH physiologically and related GH as well as glucagon levels to renal function and kidney size in IDDM patients without clinical nephropathy.

**Patients and methods**

*Patients*

The study was approved by the local medical ethics committee and all patients consented to the procedure. Inclusion criteria to participate were an onset of disease before 30 years of age, glucagon-stimulated plasma C-peptide concentration <0.2 nmol/l, diabetes duration for at least 5 years, no arterial hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg), no orthostatic hypotension, serum creatinine ≤120 µmol/l, urinary albumin excretion rate (Ualb.V) ≤200 µg/min on three consecutive overnight urine samples, and no use of anti-inflammatory and antihypertensive drugs.

**Table 1. Clinical characteristics.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group H (n=10)</th>
<th>Group N (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 ± 14</td>
<td>40 ± 12</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9 / 1</td>
<td>18 / 2</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>24 ± 13</td>
<td>21 ± 10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0 ± 2.4</td>
<td>23.8 ± 2.3</td>
</tr>
<tr>
<td>Retinopathy (O/B/P)</td>
<td>2 / 4 / 4</td>
<td>6 / 8 / 6</td>
</tr>
<tr>
<td>Ualb.V (µg/min)</td>
<td>25 (13 - 44)</td>
<td>15 (12 - 44)</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>93 ± 9</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>Hba1c (%)</td>
<td>8.0 ± 1.5</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>9.3 ± 2.3</td>
<td>7.7 ± 1.7</td>
</tr>
</tbody>
</table>

H: IDDM patients with glomerular filtration rate >130 ml/min per 1.73m²; N: IDDM patients with glomerular filtration rate ranging from 90 to 130 ml/min per 1.73m². Data are given as mean±SD, except for Ualb.V which is given in median and interquartile ranges. * Retinopathy: O: absent; B: background; P: proliferative; Ualb.V: urinary albumin excretion rate; Blood glucose: mean of three 8-sample-based 24-h blood glucose profiles.

The study groups were comprised of 10 patients with glomerular hyperfiltration, defined as a GFR>130 ml/min per 1.73m² [21], and 20 normofiltering patients (GFR between 90 and 130 ml/min per 1.73m²) (Table 1). The subjects from these groups, designated H for the hyperfiltering and N for the normofiltering patients, were individually matched with respect to sex, age and diabetes duration (within 5 years) to exclude any
Glucagon, growth hormone and kidney function

influence due to differences in clinical parameters. The proportion of patients with microalbuminuria, as well as retinopathy was not significantly different (Table 1). HbA1c values and mean blood glucose concentrations (obtained from the three 8-sample-based 24-h blood glucose profiles) tended to be higher in H than in N, but this difference did not reach statistical significance \( p=0.14 \) and \( p=0.08 \), respectively; Table 1). None of the patients consumed a protein or sodium-restricted diet. Besides insulin, no other medication was used.

Renal haemodynamics and kidney volume

The patients were studied after an overnight fast, both when measuring kidney function and during the exercise procedure to eliminate the confounding effects of recent nutrient intake. Supine GFR and effective renal plasma flow (ERPF) were determined simultaneously using \( ^{125}\text{I}-\text{iothalamate} \) and \( ^{131}\text{I}-\text{hippuran} \), respectively [22]. To minimize the possible effect of actual glycaemia on GFR and ERPF the patients were studied during near-normoglycaemia (blood glucose between 4.4 and 8.3 mmol/l [9]. Starting at 0800 h, a 5% glucose solution was infused intravenously at a rate 1 ml (0.28 mmol)/kg per h to which regular acting insulin (Velosulin H.M., Novo-Nordisk, Bagsvaerd, Denmark) was added in a dose of 1% of the total daily requirements per hour. After a 4 h period to normalize and stabilize blood glucose levels no further corrections of blood glucose were made and GFR and ERPF were measured over a 2 h clearance period from 1200 to 1400 h. The renal vascular resistance (RVR, in dynes.s/cm\(^2\) per 1.73m\(^2\)) was calculated by dividing the mean arterial blood pressure by the renal blood flow (RBF) multiplied by 80, where RBF was estimated from the ERPF and the haematocrit (HTC) using the formula: 

\[
\text{RBF}(l/min \text{ per } 1.73m^2) = \text{ERPF}(l/min \text{ per } 1.73m^2)/(1-\text{HTC})
\]

Kidney volume was determined by summation of the volumes of both kidneys, obtained by ultrasonography [23].

Exercise test

On a separate day following the renal haemodynamic studies, the patients performed a bicycle-exercise test with a fixed workload of 600 kpm for 20 min. The morning insulin dose was withheld. After 2 h rest, blood samples were taken at 1000, 1100, 1120 (i.e. directly at the end of exercise) and 1200 h from a cannula inserted into an antecubital vein.

Laboratory methods

Blood glucose was measured using a Yellow Springs glucose Analyzer (Model 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA). HbA\(_1\) was determined by col-ornitmetry [24].
Figure 1. Metabolic assessments during the exercise. Hyperfiltering O, normofiltering •
IDDM patients: see text and Table 1. Measurements at 1000, 1100, 1120 and 1200 h: 1 h before, at the start, directly after and 40 min after the exercise. Data are given in mean ± SEM. Differences in both IDDM groups: * denotes p<0.005 for growth hormone from preexercise, ** denotes p<0.01 for insulin from baseline.

Urinary albumin was measured by radioimmunoassay (Diagnostic Products Corporation, Apeldoorn, The Netherlands). Serum growth hormone (GH) concentrations were measured by radioimmunoassay (Farmos Diagnostica, Turku, Finland). Insulin-like growth factor-I (IGF-I) was measured by radio-immunoassay (Nichols Institute of Diagnostics, San Juan Capistrano, Calif., USA). Plasma glucagon was measured by radioimmunoassay, using antibody 30K (obtained from Professor RH Unger, Dallas, Tex., USA [25]). Plasma free insulin and plasma C-peptide concentrations were measured by radioimmunoassays.

Statistical analysis
Results are expressed as mean±SD or mean±SEM (Figures) for parametrically distributed data and as median (interquartile ranges) for non-parametrically distributed data. Data were compared with unpaired t-tests. Within group changes of parameters were evaluated using analysis of variance. Duncan's method [26] was used to adjustment for multiple comparisons. Multiple regression analysis was used to disclose the independent contribution of the various metabolic parameters to GFR, ERPF, RVR and kidney volume. P-values less than 0.05 were considered to be significant.
Results

Renal haemodynamic parameters and kidney volume

The renal haemodynamics and kidney volume measurements are given in Table 2. By selection, GFR was higher in H than in N, whereas ERPF was also clearly elevated in group H compared with N ($p<0.001$). The FF was similar in the groups H and N, but RVR was lower in group H than in group N ($p<0.001$). The difference in kidney volume was not significant. The mean blood glucose and insulin concentrations during the renal haemodynamic assessments were comparable between the groups (Table 2).

Table 2. Renal haemodynamics, corresponding plasma glucose and insulin levels, and kidney volume.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group H (n=10)</th>
<th>Group N (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular filtration rate (ml/min per 1.73m$^2$)</td>
<td>160 ± 22$^a$</td>
<td>109 ± 12</td>
</tr>
<tr>
<td>Effective renal plasma flow (ml/min1 per 1.73m$^2$)</td>
<td>670 ± 142$^a$</td>
<td>470 ± 74</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.24 ± 0.04</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Renal vascular resistance (dynes.s/cm$^2$ per 1.73m$^2$)</td>
<td>6654 ± 1422$^a$</td>
<td>9195 ± 1697</td>
</tr>
<tr>
<td>Kidney volume (ml/1.73m$^2$)</td>
<td>332 ± 99</td>
<td>291 ± 54</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>6.7 ± 1.4</td>
<td>6.1 ± 1.1</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>24.4 ± 11.5</td>
<td>23.9 ± 11.1</td>
</tr>
</tbody>
</table>

Group H: IDDM patients with glomerular filtration rate >130 ml/min per 1.73m$^2$; group N: IDDM patients with glomerular filtration rate ranging from 90 to 130 ml/min per 1.73m$^2$. Data are given as mean±SD. $^a$ denotes $P<0.001$ from group N.

Metabolic assessments during exercise

As expected, the circulatory level of GH increased in response to the exercise ($p<0.005$, Figure 1A). Plasma glucagon did not significantly change during the procedure (Figure 1B). Plasma insulin levels decreased ($p<0.01$, Figure 1C). Blood glucose ranged between 4.9 and 15.4 mmol/l and tended to increase at the end of the exercise (Figure 1D). No significant differences were found between the two groups in the absolute levels of growth hormone, glucagon, insulin and glucose neither at the separate measurements nor in the averaged values of the test ($p>0.10$ for all comparisons). Baseline plasma IGF-I levels were 0.35 ± 0.08 kU/l and 0.36 ± 0.07 kU/l in the groups H and N, respectively (NS).

Correlation analyses

For GH the averaged baseline and exercise-stimulated levels were used separately in the regression analysis. For glucagon the averaged values of the whole procedure were taken since glucagon was not stimulated by exercise.

Simple regression analysis showed significant correlations between stimulated GH as well as glucagon and GFR (Figure 2A and B). The correlation between GFR and HbA1c did not reach statistical significance ($r=0.32$, $p=0.054$). ERPF was correlated with HbA1c and glucagon (Figure 2C and D), but not with GH ($r=0.28$, $p=0.13$).
Figure 2. Relationships between renal haemodynamics and metabolic parameters. Hyperfiltering ○, normofiltering ■ IDDM patients. A: exercise stimulated-serum growth hormone and GFR, $r=0.40$, $p<0.05$; B: mean plasma glucagon and GFR, $r=0.40$, $p<0.05$; C: HbA1c and ERPF, $r=0.42$, $p<0.02$; D: mean plasma glucagon and ERPF, $r=0.36$, $p<0.05$; E: exercise-stimulated serum growth hormone and RVR, $r=-0.43$, $p<0.01$; F: HbA1c and kidney volume, $r=0.61$, $p<0.001$. 
Table 3. Determinants of renal haemodynamics and kidney volume as assessed by stepwise multiple regression analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial correlation coefficient</th>
<th>Contribution to variance</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Determinants of glomerular filtration rate.</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HbA1c</td>
<td>0.42</td>
<td>18%</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.38</td>
<td>14%</td>
<td>p&lt;0.03</td>
</tr>
<tr>
<td>Stimulated GH</td>
<td>0.32</td>
<td>10%</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Multiple-(r)</td>
<td>0.67</td>
<td>44%</td>
<td>p&lt;0.002</td>
</tr>
<tr>
<td><strong>Determinants of effective renal plasma flow.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.48</td>
<td>24%</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.43</td>
<td>18%</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Multiple-(r)</td>
<td>0.62</td>
<td>39%</td>
<td>p&lt;0.002</td>
</tr>
<tr>
<td><strong>Determinants of renal vascular resistance.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stimulated GH</td>
<td>0.43</td>
<td>18%</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Determinants of kidney volume.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.51</td>
<td>26%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Renal vascular resistance</td>
<td>-0.42</td>
<td>18%</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Multiple-(r)</td>
<td>0.72</td>
<td>52%</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

RVR was negatively related to GH (Figure 2E). Basal GH levels were not correlated with renal haemodynamic parameters. Kidney volume was correlated with HbA1c (Figure 2F), but not with glucagon or GH.

Multiple regression analysis disclosed that HbA1c (\(r^2=0.18, p<0.01\)), glucagon (\(r^2=0.14, p<0.03\)) and stimulated GH (\(r^2=0.10, p<0.05\)) independently contributed to the GFR (multiple \(r=0.67, p<0.002\), Table 3). Also, HbA1c (\(r^2=0.24, p<0.005\)) and glucagon (\(r^2=0.18, p<0.01\)) showed independent relations with the ERPF (multiple \(r=0.62, p<0.002\), Table 3). RVR only showed a negative relation with stimulated GH (\(r^2=0.18, p<0.01\)) (Table 3). Kidney volume was positively related HbA1c (\(r^2=0.26, p<0.001\)) independent from the inverse relation with RVR (\(r^2=0.16, p<0.01\)) (multiple \(r=0.72, p<0.001\), Table 3). Blood glucose levels, IGF-I concentrations and clinical parameters such as age, diabetes duration, body mass index, retinopathy and Ua1b.V were not related to renal function or kidney size.

**Discussion**

In this study an exercise test was performed to stimulate GH levels physiologically and on both study days the patients were kept fasting to eliminate the effects of recent nutrient intake on hormone levels and renal function. Under these circumstances renal haemodynamic parameters were significantly related to circulatory levels of plasma glucagon, exercise-stimulated GH levels as well as HbA1c, whereas the absolute levels of these hormonal and metabolic factors were not significantly different between the hyper- and normofiltering IDDM patients. Possible between group differences in these parameters
could have been obscured because of the arbitrarily employed definition of glomerular hyperfiltration. A GFR above the upper normal limit of 130 ml/min per 1.73m² was designated as glomerular hyperfiltration, comparable to a cut-off value of 135 ml/min per 1.73m² used by others [10]. However, such a ‘control population-based’ definition has the limitation that subtle renal haemodynamic alterations in apparently normofiltering patients cannot be discriminated and is likely to represent a minimal estimation of the hyperfiltration phenomenon [27].

In the multiple regression analysis with the renal haemodynamic parameters as dependent variables, glucagon contributed to 14% and 18% of the variances in GFR and ERPF, respectively. The contributory effect of stimulated GH to GFR was 10% and amounted to 18% of the variance in RVR. In other studies plasma glucagon and GH concentrations were not different in normo- and hyperfiltering patients and were not correlated with renal haemodynamic parameters [1,4,19]. These negative results could possibly be explained by differences in study design, since stimulatory tests were not used [1,4,19] or single basal hormone levels were obtained [4]. Recently, an augmented GH response to GH-releasing hormone has been documented in hyperfiltering patients [20] supporting an association between an abnormal GH release and renal haemodynamic changes in IDDM. Long-term metabolic control, as determined by HbA1c levels, contributed to 18, 24 and 26% of the variances in GFR, ERPF and kidney volume, respectively. In accordance, other cross-sectional and intervention studies showed a relation of metabolic control with renal function and kidney size [4,10,11].

Several lines of evidence support that glucagon and GH are important humoral determinants of renal function in man. Infusion of glucagon to reach levels as encountered in poorly controlled IDDM acutely increases both GFR and ERPF in IDDM patients [14]. Conversely, the rapid decrease in renal function during the administration of the somatostatin analogue octreotide is closely related to reductions in plasma glucagon [28] and can be completely prevented by concomitant low-dose glucagon administration [29]. Furthermore, renal haemodynamics have been shown to be more responsive to increments in plasma glucagon in well-controlled IDDM patients than in normal subjects [14]. The role of circulatory GH in the maintenance of renal function is illustrated by the fact that acromegalic and GH-deficient patients show differences in GFR and ERPF of about 50% [30,31]. The abolished renal-stimulatory effects of amino acids in acromegaly as opposed to the enhanced response in GH-deficiency further supports that GH is involved in renal vasodilation [30,31]. GH increases GFR and ERPF indirectly by stimulating IGF-I synthesis [32,33]. In spite of the high concentrations of GH, plasma IGF-I levels are frequently found to be reduced in IDDM [34,35]. In agreement, plasma IGF-I levels were low in both groups of IDDM patients. In the interpretation of these data it should be noted that plasma IGF-binding proteins could have interfered with the presently used radioimmunoassay [36] and that free IGF-I concentrations were not measured. Disturbances in IGF-I binding proteins, modulating its biological activity [36,37], or alterations in renal IGF-I accumulation [35] might possibly explain why, in accordance with other observations [4,38], the low circulating IGF-I levels were not significantly correlated with renal haemodynamics in these IDDM patients. Moreover, an altered or variable renal haemodynamic responsiveness to circulatory IGF-I in IDDM cannot be excluded. Indeed, in vitro studies have shown increased expression of IGF-I receptors in
cultured mesangial cells from diabetic mice [39].

In conclusion, the present results suggests that circulatory glucagon as well as GH play a contributory role in the increase in renal haemodynamics in IDDM patients.

References

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