C-peptide replacement improves weight gain and renal function in diabetic rats

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SUMMARY

Aim: Recent experimental and clinical data suggest that C-peptide replacement during type 1 diabetes exerts beneficial effects on diabetic nephropathy. The aim of this study was to determine if physiological C-peptide administration in replacement dose during 28 days had beneficial effects on metabolic status and renal functions in type-1 diabetic rats.

Methods: Four groups of rats were investigated: a non diabetic group treated with buffer (C group, n=6), three streptozotocin diabetic-induced groups treated with either buffer (D group, n=6), insulin (D-I group, n=6) or rat homologous C-peptide (D-C group, n=6). Weight gain was measured every week. All animals were housed in metabolic cages on day 28 for assessment of metabolic data. Blood and urine samples were collected to allow measurement of plasmatic osmolality, C-peptide concentration, sodium, and glucose losses and proteinuria. Glomerular filtration rate (GFR) was determined by creatinine clearance.

Results: All streptozotocin-treated animals were diabetic. Glycaemic control (mg/dl), was markedly improved in D-I (133±65) when compared with either D (547±49, P<0.05) or D-C (520±48, P<0.05) groups. Conversely, weight gain during the study, was improved in D-I and D-C as compared with D animals (135±13 and 41±18 vs 18±21 respectively), despite different glycaemic control. Diabetes-induced glomerular hyperfiltration (ml/min/kg), urinary protein leakage (g/kg/day), and Na urinary losses (mmol/100 g/day) respectively, were significantly (P<0.05) reduced in D-C (3.95±0.6 ; 0.8±0.66; 1.5±0.9) in comparison with D (4.95±0.8; 0.18±0.16; 3.7±2.1) and D-I (5±0.9; 0.19±0.11; 2.7±0.8) animals. Plasmatic osmolality was significantly increased in D group whereas there were no differences between C group and D-C group. Food and water intakes, urinary volume as well as urinary glucose losses were not significantly different between D-C and D groups.

Conclusions: C-peptide administration in replacement dose to streptozotocin diabetic rats induces weight gain regardless hyperglycaemia or glycosuria. Diabetic animals supplemented with C-peptide exhibit better renal function resulting in reduced urinary sodium waste and protein excretion together with reduction of the diabetes-induced glomerular hyperfiltration.

Key-words: C-peptide · Diabetic nephropathy · Weight gain · Glomerular hyperfiltration · Proteinuria · Sodium losses.

RéSUMÉ

La supplémentation en peptide-C améliore la prise de poids et les fonctions rénales du rat diabétique

Objectifs: Des données expérimentales et cliniques récentes ont montré que la supplémentation en peptide-C avait des effets bénéfiques sur l’atteinte rénale du diabète de type 1. L’objectif de ce travail était de déterminer les effets d’une supplémentation en peptide-C à des doses physiologiques, pendant 28 jours, sur le statut métabolique et les fonctions rénales du rat diabétique.

Méthodes: Quatre groupes de rats ont été étudiés : un groupe de rats non diabétiques supplémentés avec une solution saline (groupe C, n = 6) et trois groupes de rats diabétiques (streptozotocine, IV), supplémentés respectivement avec une solution saline (groupe D, n = 6), de l’insuline (groupe D-I, n = 6) et du peptide-C (groupe D-C, n = 6).

Les rats ont été pesés chaque semaine. À la fin de la période d’étude, les animaux ont été placés dans des cages métaboliqes. Différents paramètres biologiques ont été mesurés : l’osmolalité plasmatique, les concentrations plasmatiques en peptide-C, sodium et glucose, la glycosurie, la natriurèse et la protéinurie. Le débit de filtration glomérulaire (GFR) a été déterminé par la clairance de la créatinine.

Résultats : Tous les rats traités avec la streptozotocine étaient diabétiques. La glycémie (mg/ml) a été rétablie dans le groupe D-I (133±65) comparé au groupe D (547±49, P<0.05) ou D-C (520±48, P<0.05). La prise de poids a été améliorée dans les groupes D-I et D-C comparés au groupe D (135±13 et 41±18 vs 18±21 respectivement), indifféremment de la glycémie. L’hyperfiltration glomérulaire (ml/min/kg) induite par le diabète ainsi que la protéinurie (g/kg/jour) et la perte de sodium (mmol/100 g/day) ont été significativement réduites (P<0.05) dans le groupe D-C (3.95±0.6 ; 0.8±0.66; 1.5±0.9) comparé aux groupes D (4.95±0.8; 0.18±0.16; 3.7±2.1) et D-I (5±0.9; 0.19±0.11; 2.7±0.8). Les quantités de nourriture et d’eau, le volume urinaire, ainsi que la glycosurie n’étaient pas significativement différents entre les groupes D-C et D.

Conclusion : La supplémentation en peptide-C pendant un mois de rats diabétiques améliore la prise de poids indépendamment de l’hyperglycémie ou de la glycosurie. Elle entraîne également une amélioration de la fonction rénale, par comparaison aux rats diabétiques non supplémentés, en réduisant la perte de sodium urinaire, la protéinurie et l’hyperfiltration glomérulaire.

Mots-clés : Peptide-C · Néphropathie diabétique · Prise de poids · Perte de sodium · Hyperfiltration glomérulaire · Protéinurie.

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Diabetes mellitus is a chronic metabolic disorder that induces degenerative complications in various organs, including nerves, heart, and kidneys [1]. C-peptide is considered to be a reliable marker of residual beta-cell function, and is secreted in the blood stream in equimolar amounts with insulin [2-4]. During the past decade, numerous studies in both humans and animals have demonstrated that C-peptide, although not influencing blood sugar control, might play a role in preventing and potentially reversing some of the chronic complications of type 1 diabetes, especially diabetic nephropathy [5-24]. Thus, C-peptide may be an active peptide with relevant physiologic effects different from and complementary to those of insulin [25].

Several studies in streptozotocin-induced diabetic rats treated with C-peptide showed improvement of renal function and reversal of some of the morphologic changes associated with diabetic nephropathy [20-22]. In humans, short- or long-term administration of C-peptide exerts a regulatory and physiologic influence on renal function in patients with type 1 diabetes [5,7,26-28]. Last but not least, successful islet transplantation has been associated with improvements in kidney graft survival rates and function among uremic patients with type 1 diabetes mellitus and kidney grafts [14,15]. The latter suggests that together with the positive effects of normalization of glycemetic control, successful islet transplantation exerts beneficial effects on kidney function, in part by restoring the C-peptide secretion, a situation closer to the endogenous pancreatic function. Furthermore, the recent discovery of the C-peptide receptor [29], strongly suggests that C-peptide exerts biological activity through a specific pathway. All these arguments raise the question of a therapeutic role of C-peptide as an active protective factor for the diabetic kidney.

The main objective of the present study was to study the effects of a 28 days infusion with physiological doses of either insulin or homologous C-peptide on metabolic effects, glomerular functions, renal sodium handling and urinary protein excretion in streptozotocin-induced diabetic rats.

**Material and methods**

**Animals**

Eight-week-old male Sprague-Dawley rats (Iffa-Credo, Charles Rivers France) with an initial weight of approximately 220 g were studied in four groups: control rats treated with buffer (C group, n=6), diabetic rats treated with buffer (D group, n=6), diabetic rats treated with insulin (D-I group, n=6), diabetic rats treated with rat C-peptide 2 (D-C group, n=6). All animals had free access to tap water and standardized chow throughout the study period.

Diabetes was induced by intraperitoneal injection of streptozotocin (65 mg/kg of body weight). Treatment with either insulin (3 UI/kg/day), C-peptide in equimolar amounts (50 pmol/kg/day) or buffer (which was also the vehicle for C-peptide) was initiated seven days after diabetes onset (blood glucose >15 mmol/l) and administered as a continuous intra-peritoneal infusion for 28 days by a mini osmotic pump, placed in the peritoneal cavity. The rate of insulin administration was calculated to permit the restoration of normal glycaemia and C-peptide infusion rate was calculated to be equimolar with the infusion of insulin. Body weight was measured every week. Blood samples for blood glucose measurements were taken from the tail. After 28 days, rats were sacrificed by an intra-muscular injection of 25 mg of ketamine chlorhydrate (Laboratoires Panpharma Z.I du Clairay 35133 Luire - Fougeres, France). The study protocol was reviewed and approved by the local animal ethics committee.

**Metabolic cages**

On days 21-28 after onset of treatment, all rats were put in metabolic cages (Tecniplast, Hohenpeissenberg, Germany) for 24 hours. Body weight, food and water intakes and urine volume were measured individually. Then urinary glucose, sodium, creatinine and protein excretions were analysed. Blood samples for measurement of osmolality, creatinine and C-peptide concentrations, were obtained through an intracardiac puncture.

**Biological products**

Streptozotocin (Sigma, St. Louis, MO) was freshly dissolved in sodium citrate buffer, 0.01 M, pH 5.5, and was used within 5 min of its preparation. The mini-osmotic pumps (model 2004, Alzet, Direct Corp, Cupertino, CA 95014) were filled either with sterile buffer for insulin pumps (HOE 21 P, Hoechst Marion Roussel, Deutschland GmbH, D-65926 Frankfurft (M)), Insulin (400 UI/mL, HOE 21 PH 400, Hoechst Marion Roussel, Deutschland GmbH, D-65926 Frankfurt (M)), or C-peptide (Synthetic rat C-peptide II, amino acid sequence: EVEDPQVA-QLELGGPGAGDLQTLALEVARQ, purchased from Epytop, Parc Scientifique Georges Besse, 190 rue Georges Besse, 30035 Nimes Cedex 1). Both this insulin and buffer, used in insulin pumps, are stable for a long period of time at body temperature.

**Analysis**

Plasma C-peptide residual levels were determined by radioimmunoassay (RIA; Linco Research Inc., USA). Blood glucose concentration was measured by means of One Touch Ultra (Lifescan; Johnson and Johnson Company, California, USA). Urinary sodium and glucose concentrations were determined by flame photometry (Elx6361, Eppendorf, Hamburg, Germany). Urine volumes were determined gravimetrically. Plasmatic osmolality was determined by the freezing point depression method. Creatinine urinary and
plasma concentrations as well as urinary protein concentrations were calculated using a colorimetric method (Jaffe).

Statistical methods and data presentation

All results are expressed as mean ± SEM. The significance of differences was calculated by analysis of variance (ANOVA) followed by the Tukey post hoc test. P-values were calculated with values from all rats in each group. A P value of less than 0.05 was considered statistically significant. All analyses were done by Statview software (Abacus Concepts, Berkeley, CA, USA) on Macintosh Imac (Apple Computer, Les Ulis, France).

Results

Weight gain

All animals except controls were diabetic following streptozotocin injection (data not shown). As shown in table I, diabetes induction resulted in failure to thrive that was supposed to be secondary to the lack of cellular glucose uptake, and dehydration secondary to urinary sodium and water losses.

Diabetic rats supplemented with buffer alone hardly increased their weight after 4 weeks of streptozotocin-induced diabetes (18±21 g). Conversely, diabetic rats supplemented with insulin, increased their weight as much as the control in the same time (181±52 g). Interestingly, C-peptide supplemented diabetic rats showed a slight increase of body weight (41±18 g, P<0.05 vs D group) while hyperglycaemia and glucosuria remained in the range of buffer-supplemented rats, suggesting that the reduction of cellular glucose availability was not the only determinant of failure to thrive during the initial state of diabetes.

Data from metabolic cages

Table II shows the results obtained with the metabolic cages in all groups after 28 days.

Before streptozotocin injection, there were no statistically significant differences between the study groups in body weight (table I), blood glucose, water and food intakes and urinary water loss (data not shown).

In diabetic rats, C-peptide infusion did not improve the blood glucose level (5.20±0.48 g/l), while insulin (1.33±0.65 g/l) improved glycaemia. The existence of a measurable C-peptide residual activity was considered to be secondary either to residual pancreatic activity or to a successful C-peptide supplementation by the osmotic pump. C-peptide residual activity was markedly decreased in diabetic animals supplemented either with insulin (0.7±0.2 nmol/l) or with buffer (0.2±0.4 nmol/l), as expected after streptozotocin treatment. Diabetic animals receiving C-peptide supplementation and control animals exhibited no difference in residual C-peptide activity, confirming thereby the efficiency of the osmotic pump.

Plasmatic osmolality was not significantly different between C (334±4.8 mosmol/l), D-I (324.4±2.1 mosmol/l) and D-C (344.6±4.3 mosmol/l) groups, while it was significantly increased in D group (361.4±4.4 mosmol/l, P<0.05 vs C, D-I, D-C groups).

Control and D-I groups did not present significant difference in food and water intake. In these groups, urinary volumes and glucosuria were not significantly different. D-C group differed significantly from the control group but not from D group for these parameters.

Glomerular dysfunction

During our study, GFR assessed by creatinine clearance (ml/min/Kg) (figure 2) was increased in both insulin (5.84±1.68, P<0.05) and buffer (5.38±2.28, P<0.05) supplemented rats, while C-peptide supplemented rats (4.27±0.74) had only a slight increase of GFR in comparison to control group (4±1).

Table I

C-peptide replacement induces weight gain. Weight gain was measured between the final weight at day 28 and the weight at the beginning of the study at day 0 when mini osmotic pumps were placed. ANOVA followed by the Tukey post hoc test was used for the statistical analyses. The superscript “a” indicates significant differences from group C at the same time point: P<0.05, “b” indicates significant differences from group D at the same point: P<0.05.

<table>
<thead>
<tr>
<th>Group/Mean ± SEM</th>
<th>Control</th>
<th>Buffer</th>
<th>Insulin</th>
<th>C Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight D0 (g)</td>
<td>261±9</td>
<td>205±35</td>
<td>290±42</td>
<td>215±20</td>
</tr>
<tr>
<td>Body weight D28 (g)</td>
<td>396±22</td>
<td>223±57</td>
<td>471±94</td>
<td>255±30</td>
</tr>
<tr>
<td>Weight gain (g)</td>
<td>135±13</td>
<td>18±21a</td>
<td>181±52</td>
<td>41±18b</td>
</tr>
</tbody>
</table>
We also observed that both buffer and insulin supplemented diabetic rats presented increased proteinuria (0.18±0.16 and 0.19±0.11 g/Kg/day respectively, P<0.05 vs C) after 4 weeks of diabetes, while C-peptide supplemented rats had significantly reduced protein excretion (0.08±0.06; P<0.05 vs D, D-I) (figure 3). Altogether, these results were consistent with a specific effect of C-peptide on glomerular function.

**Discussion**

The aim of our study was to clarify the effects of in vivo C-peptide replacement on metabolic status and renal function, including glomerular and tubular components, in diabetic rats. Several recent studies in both humans and rats suggested that C-peptide was a biologically active hormone and that the kidney was a potential target of its action.

The present findings indicate that a continuous four-week infusion, in physiological doses, of homologous C-peptide to rats with experimentally induced type 1 diabetes mellitus exerts beneficial effects on weight gain, urinary Na excretion and proteinuria, together with reduction of glomerular hyperfiltration when compared to untreated diabetics rats.

Weight loss during diabetes is mainly related to urinary glucose excretion because cells become unable to use glucose. Another factor could be also the osmotic diuresis resulting in hyperosmotic dehydration [30]. In insulin treated diabetic animals, weight gain is due to improvement of glycaemic control, and to decrease in urinary glucose excretion inducing a positive energy balance. In our study the glycaemic control was not improved by C-peptide infusion and urinary glucose excretion remained high (table II). As showed by metabolic data, food and water intakes were not increased by C-peptide treatment.
However, C-peptide influenced plasma osmolality, a parameter reflecting intracellular water composition. Indeed, plasma osmolality increased in diabetic rats meaning that these rats were dehydrated. The treatment with C-peptide allowed normal plasma osmolality therefore confirming the effect of C-peptide on the renal tubule.

Since C-peptide treated rats had a better renal Na handling we suggest that improvement of both water balance and renal Na handling contributes to improve weight gain during diabetes regardless of glycaemic control.

These results are of interest, since the effect of C-peptide on Na,K-ATPase had been described previously in experimental studies [10,11,13,18,19,31,32]. In humans, C-peptide increased the Na,K-ATPase activity in erythrocytes from patients with type 1 diabetes, and was closely correlated with the basal Na,K-ATPase activity of erythrocytes from patients with type 2 diabetes [10,31]. We have recently shown that in the medullary thick ascending limb (MTAL) C-peptide exerts a hormonal effect at a cellular level through PKC-α-dependent Na,K-ATPase phosphorylation [32]. From a physiological point of view, the C-peptide concentrations active on Na,K-ATPase were in the range of post-prandial plasma concentrations. This study was consistent with a contribution of C-peptide to renal Na handling during non-fasting periods. In the thick ascending limb of Henle’s loop, active Na,K-ATPase-dependent Na reabsorption and active Na reabsorption in the MTAL is mandatory for the generation of the osmotic gradient that ultimately allows water reabsorption and urine concentration [33].

The contribution of C-peptide to the regulation of glomerular and tubular functions was suggested by several authors [5,18-22,32,34]. The most convincing experimental data concern the diabetes-induced glomerular hyperfiltration, consisting of a partial restoration of the renal functional reserve, and a significant decrease in urinary protein excretion in response to an intravenous infusion of C-peptide for 140 minutes [20]. Another study confirmed the effects on glomerular hyperfiltration rate and renal functional reserve, but not on urinary albumin leakage, after intravenous administration of homologous C-peptide to streptozotocin-induced diabetic rats for 14 days [22]. The glomerular response to C-peptide was dose-dependent and did not affect renal blood flow or total renal vascular resistance urinary albumin excretion [15].

Our data clearly show that physiological C-peptide replacement in diabetic rats prevents in part the failure to thrive induced by diabetes. This clinical effect is not related to an improvement of glycaemic control since glycaemias were not affected by C-peptide supplementation. Nevertheless, as shown by others previously in a two-week duration replacement model, C-peptide supplementation also prevents the development of both glomerular hyperfiltration and albuminuria despite lack of hyperglycaemia control.

The latter results were also observed in patients with microalbuminuria who were studied for 6 months in a double-blind, randomized, cross-over designed study [9]. In another study, diabetic patients receiving both renal and islet transplantation, with residual C-peptide secretion showed restoration of Na,K-ATPase activity in erythrocytes, reduction of natriuresis, and improvement of urinary albumin excretion [15].

In summary, our results indicate that C-peptide replacement in diabetic animals reduces glomerular hyperfiltration and proteinuria while it prevents in part diabetes-induced failure to thrive. C-peptide supplemented diabetic rats present with reduced Na losses together with an improvement of plasma osmolality, that may contribute to a better weight gain. From a physiological point of view, our results are consistent with a contribution of C-peptide

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### Table II

Data from the metabolic cages and C-peptide levels. Data are from metabolic cages on day 28 after implantation of the mini osmotic infusion pump in the four study group: control rats treated with buffer (C, n=6), diabetic rats treated with buffer (D, n=6), diabetic rats treated with insulin (D-I, n=6), diabetic rats treated with C-peptide (D-C, n=6). C-peptide was measured at the end of the study. ANOVA followed by the Tukey post hoc test was used for the statistical analyses. The superscript “a” indicates significant differences from group C at the same time point: P<0.05, “b” indicates significant differences from group D at the same point: P<0.05, “c” indicates significant differences from group D-C at the same point: P<0.05.

<table>
<thead>
<tr>
<th>Group/ Mean ±SEM</th>
<th>Control</th>
<th>Buffer</th>
<th>Insulin</th>
<th>C Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (g/l)</td>
<td>1.3±0.11</td>
<td>5.47±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33±0.65</td>
<td>5.20±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma C peptide (nmol/l)</td>
<td>1.3</td>
<td>0.26±0.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.76±0.24&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.3±0.11</td>
</tr>
<tr>
<td>P-Osmolality (mosmol/l)</td>
<td>334.6±4.8</td>
<td>361.4±4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>324.4±2.1</td>
<td>344.6±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Water intake (ml/24h)</td>
<td>50±10</td>
<td>194±71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47±7</td>
<td>214±41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Food intake (g/24h)</td>
<td>23±3</td>
<td>40±7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25±3</td>
<td>40±10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine volume (ml/24h)</td>
<td>15±11</td>
<td>129±64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17±4</td>
<td>173±41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary glucose losses (mmol/24h)</td>
<td>0.008±0.002</td>
<td>63.77±17.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42±0.25</td>
<td>50.49±9.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
supplementation to protection of renal function in diabetic animals. Further studies should emphasize on mechanisms of C-peptide induced improvement of renal troubles.

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References


