Original article

Nocturnal activity of 11β-hydroxy steroid dehydrogenase type 1 is increased in type 1 diabetic children

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Abstract

Aim. – The objective of this study was to investigate low-grade inflammation in children with type 1 diabetes (T1D) and its association with cortisol levels as well as its bioavailability through 11β-hydroxy steroid dehydrogenase type 1 (11β-HSD1) activity.

Methods. – Children with T1D (n = 45) and their non-diabetic siblings (n = 28) participated in the study. Interleukin-6 (IL-6) and high-sensitivity C-reactive protein (CRPs) were measured between 1400 and 1800 h. Glucocorticoid metabolites were measured in the first morning urine on clinic day and 11β-HSD1 activity was estimated by tetrahydrocortisol/tetrahydrocortisone (THF/THE) ratio.

Results. – Diabetic patients presented with an increased THF/THE ratio compared with controls (median: 0.68 [range: 0.45–1.18] vs 0.45 [0.27–0.98], respectively; P < 10⁻³). There was no difference between diabetic patients and controls for IL-6 (0.6 ng/mL [0.6–6.8] vs 0.6 [0.6–2.2], respectively; P = 0.43) and CRPs (0.4 mg/L [0–7.4] vs 0.3 [0–8.2]; P = 0.26, respectively). When adjusted for age, gender and BMI, the THF/THE ratio was significantly associated with CRPs (β = 0.32, P = 0.02) in diabetic patients, but not in controls.

Conclusion. – Low-grade inflammation assessed by plasma CRPs and IL-6 concentrations was not detectable in our cohort of T1D children. Nocturnal 11β-HSD1 activity was increased and associated with plasma CRPs concentration in diabetic patients. These results may be explained by either a direct or inflammation-mediated effect of the relative hepatic lack of insulin due to subcutaneous insulin therapy.

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Keywords: Type 1 diabetes mellitus; Children; Inflammation; Pituitary–adrenal axis; 11β-hydroxy steroid dehydrogenase type 1; High-sensitivity C-reactive protein; Interleukin-6

Résumé

Augmentation de l’activité 11β-hydroxysteroid déshydrogénase de type 1 chez les enfants diabétiques de type 1.

Objectif. – Mesurer l’inflammation à bas bruit chez des enfants présentant un diabète de type 1 (DT1) et chercher des associations potentielles entre cette inflammation et l’activité de la 11β-hydroxysteroid déshydrogénase de type 1 (11βHSD1) qui contribue à la biodisponibilité tissulaire du cortisol.

Métodes. – Quarante-cinq enfants DT1 et 28 frères et sœurs non diabétiques ont participé à cette étude. L’interleukine-6 (IL-6) et la protéine C-réactive de haute sensibilité (CRPs) ont été mesurées entre 14 h et 18 h. Les métabolites urinaires des glucocorticoïdes ont été dosés sur les premières urines du matin le jour de la consultation. L’activité 11βHSD1 a été estimée par le rapport tetrahydrocortisol/tetrahydrocortisone (THF/THE).

Résultats. – Les enfants diabétiques présentaient une augmentation du rapport THF/THE par rapport aux témoins (médiane 0,68, extrêmes [0,45–1,18] vs 0,45 [0,27–0,98], P < 10⁻³, respectivement). Il n’y avait pas de différence entre les deux groupes pour les dosages d’IL-6 (0,6 ng/ml [0,6–6,8] vs 0,6 [0,6–2,2], P = 0,43, respectivement) et de CRPs (0,4 mg/l [0–7,4] vs 0,3 [0–8,2], P = 0,26, respectivement). Après ajustement pour...
l’âge, le genre et l’IMC, le rapport THF/THE était associé à la CRPhs (β = 0,32, P = 0,02) chez les enfants diabétiques mais non chez les témoins.

Conclusions. – Nous ne mettons pas en évidence d’inflammation à bas bruit par les mesures de CRPhs et d’IL-6 dans notre cohorte d’enfants atteints de DT1. L’activité nocturne de la 11βHSD1 est augmentée et associée aux concentrations plasmatiques de CRPhs chez les enfants DT1. Ces résultats pourraient s’expliquer par la carence relative en insuline au niveau hépatique engendrée par l’insulinotherapie sous-cutanée.

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Mots clés : Diabète de type 1 ; Enfants ; 11β-hydroxysteroides deshydrogénase de type 1 ; Inflammation ; Axe corticotrope ; Interleukine-6 ; Protéine C-réactive

1. Introduction

The incidence of type 1 diabetes (T1D) is increasing worldwide in children and teenagers by an average of 3% per annum, but is up to 7% in children younger than 4 years [1–3]. This alarming increase calls for the development of new strategies for preventing chronic complications associated with the disease in this population. One strategy would be to identify new markers associated with the developing pathophysiology of these complications.

Markers of inflammation have been shown to be associated with microvascular complications and cardiovascular disease in T1D [4,5]. Furthermore, levels of interleukin-6 (IL-6) could be elevated in young adults with T1D, but no clinical evidence of microvascular or macrovascular complications, suggesting that a low-level chronic inflammatory state may play a key role in the early stages of atherogenesis and in the development of microvascular disorders [6].

Cortisol is the main hormone involved in the endogenous anti-inflammatory process. Local cortisol bioavailability is highly dependent on the activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme. Indeed, 11β-HSD1 is the essential agent in peripheral cortisol metabolism, converting inactive cortisol to the active hormone cortisol mainly in the liver, adipose tissue and brain. This enzyme, extensively studied in subjects with obesity and the metabolic syndrome, has been implicated in the regulation of their low-grade inflammatory states [7,8]. However, little is known of the peripheral activity of 11β-HSD1 in T1D.

Early recognition of changes in the balance between inflammatory and anti-inflammatory processes could be of interest in detecting the susceptibility for developing future complications of diabetes. Thus, the objective of the present study was to investigate low-grade inflammation in children with T1D and its association with cortisol levels as well as its bioavailability through 11β-HSD1 activity.

2. Patients and methods

2.1. Patients’ recruitment

Children with T1D of at least 1 year’s duration (n = 45) attending the diabetes clinic at the Children’s Hospital, CHU Bordeaux, France, were invited to participate, along with 28 non-diabetic siblings serving as controls. Informed consent was obtained from the participating children and their parents (clinical trial registration: NCT 01099956).

2.2. Data collection

Data regarding gender, age, duration of diabetes, body mass index (BMI), waist circumference (WC) and blood pressure were collected. The night before coming to the clinic, the patients were asked to collect their first morning urine. Patients with known urine emission or known hypoglycaemia during the night were not included. A blood sample taken by micropuncture was obtained for HbA1c (DCA Vantage HbA1c Reagent Kit, Siemens Healthcare Diagnostics, Suffolk, UK), capillary blood glucose (blood analyzer, Olympus Company, Center Valley, PA, USA), IL-6 (human IL-6 immunoassay, Bio-Rad Laboratories, Hercules, CA, USA); intra- and interassay coefficients of variation were 7.4% and 7.2%, respectively) and high-sensitivity C-reactive protein (CRPhs) (latex assay, Olympus; intra- and interassay coefficients of variation were 2.8% and 3.4%, respectively) measurement between 1400 and 1800 h.

Chronic hyperglycaemia was defined as a mean HbA1c value > 7.5%. Chronic exposure to hyperglycaemia was calculated by collecting all available HbA1c test results from the patients’ medical records. The time course surveyed by the HbA1c tests was calculated by multiplying the number of tests by 3 months – the periodicity of each test – and then dividing by the duration of the patient’s diabetes (in months). To minimize the effect of incomplete data, patients with an HbA1c survey of < 50% of the duration of their diabetes (n = 7) were excluded from the analysis of chronic exposure to hyperglycaemia.

2.3. Urine glucocorticoid metabolite measurement

Glucocorticoid metabolites were measured by liquid chromatography and mass spectrometry (ACQUITY UPLC System and TQD detector with electrospray ionization, Waters Ltd, Elstree, Hertfordshire, UK). Briefly, 6-alpha-methylprednisolone was used as the internal standard, and hydrolysis with β-glucuronidase was performed before dichloromethane extraction. Analyte concentration was related to creatinine concentration (analyte/cr). Total cortisol metabolite excretion was calculated as α and β tetrahydrocortisol (THF) + tetrahydrocortisone (THE) + cortols + cortolones. Whole-body equilibrium between cortisol and cortisone, as determined by the balance between tissue-specific activities of 11β-reductase and 11β-dehydrogenase activities, was inferred from the ratio of THF/THE. Renal 11β-dehydrogenase activity was inferred from the urinary cortisol/cortisone ratio.
Type 1 diabetic children and siblings characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients</th>
<th>Sibling control subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.0 [5.0–17.0]</td>
<td>10.0 [6.0–17.0]</td>
<td>0.9</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (Z score)</td>
<td>0.7 [–1.0–2.8]</td>
<td>0.4 [–1.2–2.8]</td>
<td>0.4</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>68.0 [48.5–91.0]</td>
<td>65.0 [52.0–103.0]</td>
<td>0.7</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>104 [85–136]</td>
<td>104 [86–128]</td>
<td>0.9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>58 [43–72]</td>
<td>56 [47–72]</td>
<td>0.2</td>
</tr>
<tr>
<td>Last HbA1c (%)</td>
<td>8.0 [6.5–10.5]</td>
<td>5.3 [4.4–5.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Last glycaemia (mmol/l)</td>
<td>8.2 [0.9–27.5]</td>
<td>4.8 [3.7–6.6]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>72 [13–136]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean HbA1c, since diabetes onset (%)</td>
<td>7.9 [6.5–11.0]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of HbA1c &gt; 7.5% since diabetes onset</td>
<td>9.5 [1–33]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of HbA1c &lt; 7.5% since diabetes onset</td>
<td>4 [0–27]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin doses (U/kg/d)</td>
<td>0.9 [0.3–1.8]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as medians [range]. BMI is expressed as Z score for sex and age. Comparisons were made using the Mann-Whitney test, with a P value < 0.05 indicating a statistically significant difference.

2.4. Statistical analysis

Continuous data are presented as medians [range]. BMI values are given as the Z score for gender and age, according to published data for the French population [9]. Comparisons were made using the Mann-Whitney test with a P value < 0.05 indicating a statistically significant difference. Factors potentially associated with IL-6, CRPhs and THF/THE were studied using a bivariate linear-regression model, with IL-6, CRPhs and THF/THE as dependent variables. The multivariate analysis included adjustments for age, gender and BMI (with standard deviations for gender and age). Because of their non-normal distribution, CRPhs and IL-6 results were log-transformed prior to the association studies.

3. Results

3.1. Patients’ characteristics

The study included 45 children with T1D and 23 siblings without the disease. Their clinical and biological characteristics are shown in Table 1. Children with T1D and their sibling controls were matched in terms of age, BMI, WC and blood pressure. Predictably, parameters associated with diabetes, such as the last HbA1c and last glycaemia values, were higher in patients with T1D than in the siblings.

3.2. Urine glucocorticoid metabolites and low-grade inflammation markers

Comparisons between diabetic patients and controls for urine glucocorticoid metabolites and low-grade inflammation markers are shown in Table 2. Diabetic patients presented with an increased THF/THE ratio (P < 10^{-3}) compared with the controls. There was also a trend towards higher THF/cr and lower THE/cr ratios in patients with diabetes, but no significant difference was evident between the groups for IL-6 and CRPhs concentrations.

3.3. Clinical factors associated with IL-6 and CRPhs

In diabetic patients, IL-6 concentrations (β = 0.34, 95% CI: 0.04–0.64; P = 0.03), but not CRPhs levels, were associated with age. When corrected for age, no association was found between either IL-6 or CRPhs and BMI, WC, blood pressure, last glycaemia and HbA1c values, doses of insulin, diabetes duration or chronic exposure to hyperglycaemia.

In the controls, IL-6 and CRPhs levels were only associated with BMI (β = 0.61, 95% CI: 0.14–1.05; P = 0.01) and β = 0.68, 95% CI: 0.30–1.06; P = 0.01, respectively) when corrected for age.

3.4. Factors associated with urine glucocorticoid metabolites

In diabetic patients, the THF/THE ratio was associated with age (Table 3), and this association remained even after parameters were adjusted for duration of diabetes. When adjusted for age, gender and BMI, the THF/THE ratio was significantly associated with CRPhs levels in diabetic patients, but not with IL-6 levels (not significant; Table 3). After adjusting for age, no association was shown for the THF/THE ratio with BMI, WC, blood pressure, last glycaemia and HbA1c values, doses of insulin, diabetes duration or chronic exposure to hyperglycaemia (data not shown). Neither THF nor THE was associated with BMI, WC, blood pressure, last glycaemia and HbA1c values, doses of insulin, diabetes duration, or chronic exposure to hyperglycaemia, IL-6 or CRPhs levels.

In controls, no association was found between the THF/THE ratio or THF or THE concentrations and age, BMI, WC, blood pressure, IL-6 or CRPhs levels.
Table 2
Urine glucocorticoids metabolites and low-grade inflammation markers.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (n = 45)</th>
<th>Sibling control subjects (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatininuria (nmol/l)</td>
<td>9.5 [2.0–19.7]</td>
<td>12.0 [3.3–25.9]</td>
<td>0.10</td>
</tr>
<tr>
<td>F/cr (µg/nmol)</td>
<td>4.6 [1.0–30.9]</td>
<td>3.6 [2.6–13.3]</td>
<td>0.21</td>
</tr>
<tr>
<td>E/cr (µg/nmol)</td>
<td>6.5 [2.5–38.0]</td>
<td>5.6 [2.9–17.1]</td>
<td>0.15</td>
</tr>
<tr>
<td>THFs (5α + 5β THF)/cr (µg/nmol)</td>
<td>110 [39–396]</td>
<td>85 [45–188]</td>
<td>0.07</td>
</tr>
<tr>
<td>THE/cr (µg/nmol)</td>
<td>139 [55–441]</td>
<td>178 [67–508]</td>
<td>0.09</td>
</tr>
<tr>
<td>Total metab/cr (µg/nmol)</td>
<td>337 [164–1037]</td>
<td>344 [171–902]</td>
<td>0.97</td>
</tr>
<tr>
<td>F/E</td>
<td>0.68 [0.40–1.53]</td>
<td>0.72 [0.44–1.18]</td>
<td>0.68</td>
</tr>
<tr>
<td>5 α TFH/5β THF</td>
<td>0.12 [0.02–0.42]</td>
<td>0.12 [0.03–1.18]</td>
<td>0.07</td>
</tr>
<tr>
<td>THFs/THF</td>
<td>0.68 [0.45–1.18]</td>
<td>0.45 [0.27–0.98]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>0.60 [0.6–6.8]</td>
<td>0.60 [0.6–2.2]</td>
<td>0.43</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0.4 [0–7.4]</td>
<td>0.3 [0–8.2]</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Values are expressed as medians [range]. Comparisons were made using the Mann–Whitney test, with a P value < 0.05 indicating a statistically significant difference.

4. Discussion

The objective of our present study was to investigate whether low-grade inflammation and changes in cortisol or peripheral cortisol metabolism were present in children with T1D. It was found that the THF/THE ratio, suggesting nocturnal whole-body 11β-HSD1 activity, was increased in diabetic patients and that this increase was associated with CRPhs levels. de Lacerda et al. [10] attributed the diurnal variation in metabolic clearance rate of cortisol to variations of HSD activity. However, different studies have found that 11β-HSD1 activity undergoes minimal variations throughout the day [11,12]. On the other hand, the expression of 11β-HSD1 genes has been found to have a circadian rhythm in human adipose explants [13]. Our present study shows that diabetes has an impact on 11β-HSD1 activity.

IL-6 is a powerful inducer of the inflammatory hepatic acute-phase response and also stimulates hepatic production of CRPhs. IL-6 plasma concentration is doubled in young patients with a 12-year duration of diabetes, but no clinical evidence of microvascular or macrovascular complications compared with controls [6]. In our present population of children with shorter durations of diabetes (median of about 6 years), there was no difference in CRPhs or IL-6 levels. This suggests that the concentrations of these parameters may not be the earliest to be modified in T1D in children. However, strong associations were observed in both markers with BMI in controls that were lost in the diabetic children. This suggests that other associated factors could be precociously influencing CRPhs and IL-6 concentrations in diabetic children, and hyperglycaemia could be one of those factors [14,15]. However, neither recent nor chronic metabolic control criteria were associated with CRPhs and IL-6 levels in our study. In fact, the only factor associated with CRPhs (and marginally with IL-6) was the nocturnal THF/THE ratio.

Endogenous glucocorticoid activity depends upon glucocorticoid output from the adrenal gland under the control of the hypothalamic–pituitary–adrenal (HPA) axis. However, intracellular glucocorticoid concentrations can differ greatly from blood levels due to the action of the 11β-HSD1 enzyme, which converts inactive cortisol to active cortisone [16]. In the liver, cortisol and cortisone are metabolized by irreversible inactivation by A-ring reductases, leading to urinary excretion of cortisone and cortisol metabolites (THE and THF) [17]. Thus, whole-body 11β-HSD1 enzyme activity may be assessed by the THF/THE ratio. However, in healthy people, whole-body regeneration of cortisol through 11β-HSD1 activity is mainly provided by visceral adipose tissue and the liver [16]. 11β-HSD1 has been found in other tissues, including the kidneys [18], but the influence of these forms on whole-body regeneration of cortisol is unknown. As our diabetic patients and controls presented with identical BMI and WC, it may be hypothesized that the difference between them in terms of THF/THE ratio were more likely the result of variations in hepatic 11β-HSD1 activity. Also, it may be assumed that variations in the THF/THE ratio were not due to changes in renal 11β-HSD type 2 activity, which converts cortisol to cortisone, as the E/F ratio was identical between groups. Previously, 11β-HSD1 enzyme activity was found to be normal in 24-h urine collections analyzed in adults [19] and children [20] with T1D. Interestingly, an increased ratio was found when glucocorticoid metabolites were analyzed in nocturnal urine samples. The circadian rhythm of cortisol secretion leads to low plasma cortisol concentrations and therefore low cortisol metabolite production at night. This...
basal condition allows the exclusion of stimuli such as food intake, exercise and stress, which can all greatly affect cortisol levels. For this reason, this nocturnal measure should help in detecting subtle changes in HPA-axis activity. Indeed, it was found that an increase in 11β-HSD1 activity was accompanied by a trend towards greater excretion of cortisol metabolites and less excretion of cortisone metabolites in diabetic patients. This result may be explained by nocturnal hypoglycaemia stimulating cortisol secretion. Although the absence of nocturnal hypoglycaemia was among our inclusion criteria, it was not possible to completely exclude the occurrence of unknown nocturnal hypoglycaemia.

Another explanation may stem from the stimulating effect of inflammation on 11β-HSD1 expression. It has been suggested that proinflammatory mediators such as IL-1β and tumour necrosis factor (TNF)-α increase 11β-HSD1 expression, thereby promoting local glucocorticoid availability and, thus, local anti-inflammatory action [7]. In our present study, the THF/THE ratio was associated with serum CRPhs concentrations in the diabetic children, but not in the controls, suggesting that a proinflammatory state could stimulate 11β-HSD1 expression in diabetes. However, the association was weak with IL-6 concentrations, and plasma CRPhs concentrations were not increased in the T1D children. This apparent discrepancy may result from the fact that CRPhs is mainly produced by hepatocytes whereas IL-6 is provided by T cells and macrophages. For this reason, it may be speculated that an hepatic proinflammatory state might stimulate hepatic 11β-HSD1 expression, thereby explaining the increase in THF/THE ratio. The latter would also not lead to elevated plasma CRPhs concentrations because of the increased intracellular production of cortisol by the liver.

As 11β-HSD1 gene transcription is increased by glucose concentration in hepatocytes [21], elevated 11β-HSD1 activity may be explained by an enhanced hepatic glucose output due to a global lack of insulin. However, as no association was found between THF/THE ratio and recent or chronic metabolic control criteria reflecting such a lack of insulin, this hypothesis is not considered likely. Alternatively, with a conventional insulin regimen, insulin is not delivered into the portal circulation, but through a non-physiological subcutaneous route, leading to a relative hepatic lack of insulin [22]. This could contribute to enhanced hepatic 11β-HSD1 activity either directly, as 11β-HSD1 gene transcription is decreased by insulin [23], or indirectly, by promoting an hepatic proinflammatory state [24]. Thus, the increase in THF/THE ratio in nocturnal urine could be explained by the relative hepatic lack of insulin at night due to subcutaneous insulin therapy.

In conclusion, low-grade inflammation, as assessed by plasma CRPhs and IL-6 concentrations, was not detectable in our cohort of children with T1D. However, nocturnal 11β-HSD1 activity was increased in diabetic patients and associated with plasma CRPhs concentrations. These results may be explained by either a direct or an inflammation-mediated effect of the relative insulin deficiency due to conventional insulin therapy. Thus, 11β-HSD1 activity may be an early marker of an inflammatory state associated with T1D.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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