Plasma 15-F2t isoprostane concentrations are increased during acute fructose loading in type 2 diabetes

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Abstract

Objective. – Fructose consumption is increasing worldwide and is likely to play a role in metabolic disorders. Dietary fructose is often recommended for diabetic patients, as this form of carbohydrate leads to a lower postprandial rise in plasma glucose and insulin. However, fructose contributes to the generation of free radicals. The aim of this work was to investigate the acute effects of a fructose load in patients with type 2 diabetes mellitus (T2DM), compared with healthy controls, on several metabolic oxidative biomarkers, particularly plasma 15-F2t isoprostanes (15-F2t isoPs).

Research design and methods. – Six T2DM patients and six healthy subjects were recruited. All patients underwent a single fructose tolerance test (75 g of anhydrous fructose). Plasma 15-F2t isoPs concentrations, plasma total antioxidant capacity (TAS) and thiobarbituric acid reactive substances (TBARS) were measured at baseline, and at 60, 120, 180 and 240 min after fructose absorption.

Results. – Baseline plasma 15-F2t isoPs concentrations were significantly increased in T2DM patients compared with controls (310 ± 47 versus 237 ± 20 pg/mL, respectively; P < 0.01) and rose significantly (P < 0.01) to 414 ± 45 pg/mL in diabetic patients. No change in TAS or TBARS was observed in either group.

Conclusion. – Plasma 15-F2t isoPs are increased during acute fructose loading in T2DM. Knowing the potentially deleterious effect of plasma 15-F2t isoPs—in particular, vascular lesions—and in light of our results, it is necessary to reconsider fructose consumption in T2DM patients, as we can now show, for the first time, a possible association between acute fructose loading and deleterious effects in such patients.

Résumé

Les concentrations de 15-F2t isoprostanes plasmatiques augmentent durant une charge aiguë en fructose chez le diabétique de type 2.

Objectif. – La consommation de fructose augmente partout dans le monde et doit jouer un rôle dans les maladies métaboliques. Le fructose est souvent recommandé pour l’alimentation des patients diabétiques, car ce glucide provoque une faible augmentation de la glycémie postprandiale et de l’insuline. Le fructose contribue à la production de radicaux libres. L’objectif de ce travail était d’explorer les effets d’une charge de fructose chez des sujets diabétiques de type (T2DM) par rapport aux témoins sur plusieurs biomarqueurs du métabolisme oxydatif, en particulier les 15-F2t isoprostanes (15-F2t isoPs).

Patients et méthodes. – Six T2DM patients et six témoins ont été recrutés. Les concentrations plasmatiques de 15-F2t isoPs, la capacité totale antioxydante du sérum (TAS), et les TBARS ont été mesurés à l’inclusion et à 60, 120, 180 et 240 minutes après l’absorption de fructose.

Résultats. – Les concentrations basales de 15-F2t isoPs étaient nettement augmentées chez les patients diabétiques par rapport aux témoins (310 ± 47 contre 237 ± 20 pg/mL, P < 0.01) et ont augmenté de façon significative (P < 0.01) à 414 ± 45 pg/mL chez les patients diabétiques. Aucun changement pour les autres paramètres du stress oxydant n’a été observé dans les deux groupes.

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1. Introduction

Fructose is a monosaccharide, the sweetest form of naturally occurring carbohydrate (CHO), and relatively abundant in nature. However, throughout the 1970s and 1980s, the availability of fructose substantially increased because of the industrial production of high-fructose syrups, made mostly from cornstarch. Such high-fructose syrups are now used to sweeten soft drinks, fruit drinks, baked goods, jams, syrups and candies. The most recent data available suggest that fructose consumption is increasing worldwide [1]. Consequently, the potential role of fructose in the aetiology of obesity and metabolic diseases (such as insulin resistance and hyperlipidaemia) is currently much debated. It has also been proposed that an increase in fructose consumption may play a role in the increased prevalence of obesity over the past two to three decades [2]. On the other hand, fructose has been recommended to diabetic patients as a dietary replacement for glucose and sucrose. In fact, fructose produces less of a postprandial rise in plasma glucose and serum insulin than other common carbohydrates [3]. More important, and regarding energy homeostasis, fructose, unlike glucose, does not directly stimulate insulin secretion [4] because pancreatic β-cells have very low levels of the fructose transporter GLUT5 [5].

However, lower levels of circulating insulin and leptin after fructose ingestion may inhibit the appetite less than consumption of other carbohydrates and, thus, increase calorie intake. Moreover, several lines of evidence suggest that a high-fructose diet [6] leads to several features observed in the metabolic syndrome, such as insulin resistance, hypertension and dyslipidaemia, usually presenting in some individuals as glucose intolerance up to overt type 2 diabetes mellitus (T2DM) [7]. Fructose also reacts to proteins, leading to protein glycation and the production of advanced glycation end-products (AGE) [8]. One possible mechanism is an increased generation of oxygen species somewhere along the fructose metabolic pathway [9], and acute oxidative damage to proteins. Plasma 15-F₂t isoprostanes (15-F₂t isopPs) appear to be a good marker of lipid peroxidation in vivo [10]. They are produced independently of enzyme activity by the action of free radicals on arachidonic acid. Plasma and urine concentrations of 15-F₂t isoPs are increased in diabetes [11]. A previous study demonstrated that an acute hyperglycaemic episode leads to an increase of F₂t isopPs generation in T2DM.

To determine the role of fructose in protein glycation and oxidative stress, we investigated the effects of acute fructose loading in people with T2DM on the generation of free radicals and, in particular, on plasma 15-F₂t isoPs.

2. Patients and methods

After approval by the ethics committee (Grenoble CPP, Comité de protection des personnes) and having obtained the participants’ written informed consent, we initially recruited, from December 2003 to July 2004, 16 subjects. Following an initial visit (complete medical history, physical examination, electrocardiogram, routine blood work and urinalysis), only 12 patients could completely follow the study protocol. These patients were from the diabetology department of our institutions in Grenoble, France, and were 18 to 65 years of age, nonsmokers and with no history of renal, hepatic or digestive failure. The T2DM patients had been treated with diet or oral hypoglycaemic agents, and had HbA1c levels lower than 8.5%. Patients were considered to have T2DM if they had been diagnosed between the ages of 40 and 70 years, treated with diet or oral hypoglycaemic agents, had no history of ketosis, and had shown stable glycaemic control and stable weight over the past six months. The ages of subjects in the control group were similar to those of the diabetic group as isoprostanes differ according to age. The study patients’ clinical characteristics are detailed in Table 1.

Patients with proliferative retinopathy or macular oedema, macroproteinaemia, kidney deficiency, autonomic neuropathy, severe dyslipidaemia, a recent cardiovascular event, or any inflammatory or malignant diseases were excluded from the study.

All patients underwent a single fructose–tolerance test (75 g of anhydrous fructose) at 0800 hour, with peripheral blood samples taken at 0, 60, 120, 180 and 240 min, using an indwelling venous cannula.

2.1. Measurement of metabolic parameters

Blood samples were obtained between 1000 and 1200 h. Samples were separated immediately after collection by centrifugation at 2000 g for 15 min and stored at –80°C for later analysis.

Table 1

<table>
<thead>
<tr>
<th>Patient clinical and biological characteristics</th>
<th>Diabetic patients</th>
<th>Control patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.5 ± 11</td>
<td>46.2 ± 12.6</td>
<td>NS</td>
</tr>
<tr>
<td>M/F</td>
<td>3/3</td>
<td>3/3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.1 ± 7.3</td>
<td>25 ± 3.5</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fasting glycaemia</td>
<td>7.5 ± 1.5</td>
<td>4.9 ± 0.88</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.5 ± 0.57</td>
<td>5.3 ± 0.37</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.1 ± 0.9</td>
<td>5.4 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.82 ± 0.49</td>
<td>2.78 ± 0.80</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.63 ± 0.51</td>
<td>1.48 ± 0.48</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.47 ± 0.60</td>
<td>1.01 ± 0.58</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Total cholesterol, triglycerides, lactataemia, HDL-C and LDL-C, and glucose were measured enzymatically using a specific automated analyzer (Modular, Roche Diagnostics, Meylan, France). Fructose was measured in plasma using a spectrophotometric method based on diphenylamine [12]. HbA1c was measured by HPLC using the Diamat system (Biorad, France).

2.2. Oxidative balance parameters

The total antioxidant status (TAS) was determined by the capacity to inhibit the peroxidase-mediated formation of 2,2-azino-bisethylbensthiazoline-6-sulphonic acid (ATBS+) radicals in the presence of metmyoglobin. In this assay, the relative inhibition of ATBS+ in the presence of plasma is proportional to the antioxidant capacity of the sample. In brief, plasma (2.5 μL) was incubated for three minutes at 37 °C in a 96-well plate with a reaction mixture made up of 20 μL ATBS (20 mmol/L), 20 μL horseradish peroxidase (30 mU/mL) and 37.5 μL PBS (pH 7.4). The reaction was started with the addition of 20 μL hydrogen peroxide (final concentration, 0.1 mmol/L), and the increase in absorbance over six minutes was monitored at 405 nm. At the end of six minutes, the absorbance caused by the accumulation of ATBS+ in the test sample was read along with a control containing 2.5% PBS. The difference in absorbance was used to represent the percentage-inhibition of the reaction and given as trolox equivalents (TE).

Selenium-glutathione peroxidase activity (Se-GSH-Px; EC 1.11.1.19) was determined by the modified method of Gunzler et al. [13], using tert-butyl hydroperoxide as the substrate.

Lipid peroxidation intermediates were measured by the plasma thiobarbituric acid reactive substances (TBARS). These are products of oxidative degradation of polyunsaturated fatty acids, in particular, malondialdehyde (MDA). We used the modified method of Ohkawa et al. [14].

2.3. Plasma isoprostane

Peripheral blood for isoprostane measurement was collected in polyethylene tubes containing 3.8% (wt/vol.) trisodium citrate solution with indomethacin (as cyclooxygenase inhibitor) and butylated hydrotoluene (BHT); a free-radical scavenger). Plasma was obtained by centrifugation at 3000 g for 15 min at 4 °C. The plasma was then transferred to a polypropylene screw-cap tube and stored at −80 °C for later analysis.

After a purification step on affinity columns, the 15-F₂t isoPs was analyzed, using an enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer’s specifications. This assay is based on the competition between 15-F₂t isoPs and 15-F₂t isoPs-acetylcholinesterase conjugate for 15-F₂t isoPs-specific rabbit antiserum binding sites, as described previously [15].

2.4. Statistical analysis

Data are expressed as means ± S.D. Differences in individual variables during the fructose tolerance test were measured by repeated-measure one-way ANOVA, with a paired t-test and a significance level of P < 0.05. Otherwise, the Mann–Whitney test was used for paired measurements. Data were analyzed using Statview software.

3. Results

3.1. Clinical data

The main clinical characteristics of the patients are shown in Table 1. In the diabetic group, six patients were taking metformin, one patient, a glitazone, and another, a glinide. None
of the control subjects was taking medication. As for metabolic parameters, a significant difference was observed for body mass index (BMI), fasting plasma glucose and HbA1c between the two populations.

3.2. Metabolic parameters during fructose tolerance test

Baseline plasma fructose did not differ between the two groups (Fig. 1). A significant increase was observed in the two groups, and a significant difference was observed at 120 min between diabetic patients and controls (72 ± 0.03 versus 61 ± 0.14 μmol/L; \( P < 0.05 \)). Plasma glucose was significantly higher at baseline in diabetic patients and its elevation was significant, in contrast to the controls, in whom no significant rise was observed (Fig. 2). Baseline plasma lactate was higher in diabetic patients and increased significantly in both study populations, although the increase was significantly greater in the diabetics (Fig. 3).

![Fig. 2. Changes in plasma glucose during a fructose load in six diabetic patients and six healthy controls. Values are shown as means ± S.D. Those with superscript letters (a, b, c) are significant using the ANOVA test (\( P < 0.05 \)).

*\( P < 0.05 \) compared with controls at the same time during fructose loading (Mann–Whitney test); black squares, diabetic patients; white squares: controls.

![Fig. 3. Changes in plasma lactate during a fructose load in six diabetic patients and six healthy controls. Values are shown as means ± S.D. Those with superscript letters (a, b, c) are significant using the ANOVA test (\( P < 0.05 \)).

*\( P < 0.05 \) compared with controls at the same time during fructose loading (Mann–Whitney test); black squares, diabetic patients; white squares: controls.
Table 2
Changes in metabolic and oxidative balance during a fructose load in diabetic and control groups

<table>
<thead>
<tr>
<th>Group–parameters/time</th>
<th>T0</th>
<th>T60</th>
<th>T120</th>
<th>T180</th>
<th>T240</th>
</tr>
</thead>
<tbody>
<tr>
<td>D± TBARS (μmol/l)</td>
<td>2.21 ± 0.36</td>
<td>2.26 ± 0.45</td>
<td>2.31 ± 0.51</td>
<td>2.26 ± 0.48</td>
<td>1.97 ± 0.34</td>
</tr>
<tr>
<td>C TBARS (μmol/l)</td>
<td>2.40 ± 0.34</td>
<td>2.28 ± 0.38</td>
<td>2.25 ± 0.33</td>
<td>2.19 ± 0.27</td>
<td>2.33 ± 0.29</td>
</tr>
<tr>
<td>D± TAS (mmol/l)</td>
<td>1.21 ± 0.08</td>
<td>1.26 ± 0.09</td>
<td>1.22 ± 0.06</td>
<td>1.18 ± 0.08</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>C TAS (mmol/l)</td>
<td>1.28 ± 0.11</td>
<td>1.25 ± 0.13</td>
<td>1.22 ± 0.11</td>
<td>1.25 ± 0.13</td>
<td>1.25 ± 0.11</td>
</tr>
</tbody>
</table>

D±: Diabetic patients; C: Control patients.

Red-cell Se-GSH-Px was significantly higher in the diabetic patients compared with controls (426.7 ± 12.3 U/L versus 407.8 ± 13.1; P < 0.05). At baseline and during the fructose-loading test, no significant difference was observed between the groups for TAS and TBARS (Table 2). As for plasma F2α isoprostanes (Fig. 4), baseline levels were significantly higher in the diabetic patients. There was a significant rise in plasma 15-F2α isoPs concentrations between baseline and 240 min in both groups, but the increase was significantly greater in the diabetic patients versus nondiabetic subjects (104 ± 45 versus 47 ± 26 pg/mL; P < 0.01). No correlation was found between plasma 15-F2α isoPs and TAS or TBARS. The only variable positively related to 15-F2α isoPs was glucose (r = 0.761; P = 0.04).

4. Discussion

In this study, we investigated the effects of fructose in type 2 diabetic patients on several parameters of oxidative balance during the two hours following a 75-g fructose load. The main finding was an acute increase in plasma 15-F2α isoprostane. This indicates an increased free-radical generation of the compound from arachidonic acid in membrane and lipoprotein phospholipids [16]. An increase in oxidative stress during acute hyperglycaemia in type 2 diabetic subjects has been previously shown [17]. In the present study, we demonstrated for the first time a similar effect after an acute fructose load in type 2 diabetic patients. Interestingly, the effect on fructose was more
pronounced in the diabetic patients than in the control subjects at 120 minutes. Moreover, a significant glucose rise was observed in the diabetics and not in the controls.

Thus, most of the metabolic effects of fructose can be attributed to its rapid utilization in the liver and the bypassing of the phosphofructokinase regulatory step in the glycolytic pathway. Fructose can provide carbon atoms for both the glycerol and acyl portions of acylglycerol molecules [18]. Unlike glucose metabolism, in which the uptake of glucose is negatively regulated at the level of phosphofructokinase, high concentrations of fructose can serve as a relatively unregulated source of acetyl-CoA. Indeed, clinical studies have shown that fructose ingestion results in markedly increased rates of de novo lipogenesis [19,20]. This specific metabolism includes immediate hepatic increases in pyruvate and lactate production, and activation of pyruvate dehydrogenase, leading to esterification of nonesterified fatty acids, resulting in increased secretion of very-low-density lipoprotein (VLDL) when fructose is absorbed over a long period.

Increased generation of free radicals is a feature of hyperglycaemia in type 2 diabetes [21]. Moreover, fructose leads to free-radical generation through different mechanisms, including protein glycation, modifications in iron distribution, induction via the polyol pathway or a decrease in cell glutathione [1]. Fructose generates the active oxidant peroxynitrite (ONOO$^-$), and increases its production, which could cause cellular dysfunction through inactivation of enzymes or ion channels, and disturbance of mitochondrial respiration [22,23]. It has been shown that a high fructose or sucrose diet can increase blood pressure and induce insulin resistance [24]. In addition, defects in the antioxidant system have been observed in high-fructose-intake models. In a previous study, we showed that the activity of red-cell copper/zinc superoxide dismutase (CuZnSOD), Se-GSH-Px and blood GSH are significantly lower in rats fed a high-fructose diet, leading to insulin resistance [25].

Fructose significantly increases methylyglyoxal generation in vascular smooth muscle cells in a concentration-time-dependent manner [22]. This compound is highly reactive to proteins and leads to the formation of peroxy nitrite. Lipid peroxidation and TAS were not elevated in our diabetic patients, which could have been because of their excellent glucose control (HbA1c: 6.5 ± 0.57). These oxidative biomarkers did not change during fructose loading. As TAS is the result of several plasma compounds, the lack of change of this parameter suggests that acute free-radical generation has no influence on any component of TAS. It is possible that TAS and TBARS assays are less sensitive for detecting changes in the oxidative balance than 15-F_2t isopPs, as previously shown [17,21].

In our study, plasma 15-F_2t isopPs concentration rose by 33% in diabetic patients after fructose loading. This is similar to observations by Sampson et al. during acute hyperglycaemia in T2DM patients [17]. It is also comparable to observations with other models of oxidative stress [25]. Plasma 15-F_2t isopPs concentration is not only a biological marker of oxidative stress, but is also an independent factor associated with coronary artery disease. It also promotes endothelin production, increases platelet adhesion and reduces the vasodilator effects of nitric oxide [26,27]. These results suggest that quantification of 15-F_2t isopPs may offer a valuable tool for assessing the effects of a high-fructose diet on free-radical generation and, over the long term, on the consequences of cardiovascular disease [28].

Most recent data suggest that fructose consumption is increasing worldwide. Fructose currently accounts for approximately 10% of the average total energy intake in the United States [29]. Since insulin increases leptin release, lower circulating insulin and leptin after fructose ingestion may inhibit the appetite less than the consumption of other carbohydrates, thereby leading to increased calorie intakes [30]. As insulin and leptin serve as key afferent signals in the regulation of food intake, dietary fructose may again increase calorie intake and, thus, body weight, suggesting that dietary fructose may play a role in the growing obesity epidemic [1].

On the other hand, fructose has often been the ‘recommended sugar’ for diabetic patients because of its low glycaemic and insulinaemic scores. In fact, fructose results in lower postprandial glucose and insulin responses, in both healthy and diabetic people, than most other carbohydrate sources. However, there are concerns regarding the many deleterious consequences of fructose consumption in diabetics. More so than a high-glucose diet, a high-fructose diet can aggravate lipaemia (plasma VLDL triglycerides) in healthy men and in those who have the metabolic syndrome or T2DM [3,24]. Moreover, postprandial hyperglycaemia has recently been described as an independent predictor of cardiovascular mortality, via an increase in oxidative stress, in type 2 diabetics [21,31]. Similarly, our data suggest that postprandial hyperfructosaemia is likely to have the same consequences [21,28,31].

Thus, the present study raises new doubts concerning the safety of fructose in the diabetic diet. Our findings provide further evidence of the deleterious consequences of a fructose load in type 2 diabetic patients, even those whose diabetes is extremely well controlled, through free-radical generation. This could lead to an increase in vascular damage through proatherogenic processes by oxygen species. In this case—and in light of our findings—there is a need to reconsider fructose consumption, at least in large amounts, by type 2 diabetic patients.

Acknowledgements

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References


