Carbonated soft drinks and carbonyl stress burden

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ABSTRACT — Carbonated soft drinks reportedly contain methylglyoxal (MG), which is strongly associated with human carbonyl stress. We sought to evaluate the effects of carbonated drink intake on human carbonyl stress. We measured MG levels in 4 commercial beverage brands, and evaluated the changes in plasma MG in healthy subjects following the intake of carbonated drinks. By 30 min after intake of samples containing high glucose and high MG, the levels of plasma MG, glucose, insulin and uric acid had increased significantly, and then returned to basal levels by 120 min. After intake of the low-calorie carbonated samples containing little MG, there were no increases in plasma MG. Our results suggest that glucose-containing carbonated soft drinks are associated with increases in not only glucose but also carbonyl burden.

Key words: Carbonated soft drink, MG, Carbonyl stress

INTRODUCTION

Carbonated soft drink consumption reportedly leads to cardiometabolic risk factors, such as hypertension, impaired glucose tolerance and chronic kidney disease (Dhingra et al., 2007; Winkelmayer et al., 2005; Saldana et al., 2007). Moreover, previous reports have shown that carbonated drinks contain methylglyoxal (MG), which is a highly reactive carbonyl compound and major precursor of advanced glycation end products (AGEs), and displays toxicity in cells and tissues (Tan et al., 2008; Fukunaga et al., 2004; Okado et al., 1996; Ramasamy et al., 2006). Food and beverages represent exogenous sources of MG (Nemet et al., 2006); however, few reports have evaluated the actual effects of drinking and eating such products on plasma MG levels.

Moreover, carbonyl stress caused by the accumulation of reactive carbonyl compounds is also associated with hypertension, diabetic complications and uremic states, and carbonyl stress plays a pathological role in these diseases (Wang et al., 2008; Beisswenger et al., 2003; Miyata et al., 2001; Nakayama et al., 2008). Therefore, whether the intake of carbonated soft drinks affects the carbonyl stress burden is of clinical importance.

In this study, we measured MG levels in 4 commercial beverage brands, and evaluated the changes in plasma MG levels and metabolic factors, such as glucose and uric acid (UA), after intake of 2 types of carbonated soft drink (regular and low-calorie).

MATERIALS AND METHODS

Beverage samples

We purchased 4 commercially available types of carbonated soft drink, including cola, lemon-lime soft drink, and 2 brands of diet cola (Table 1). Samples A (a cola) and C (a diet cola) were used for the loading tests.

Subjects

Subjects comprised 6 healthy volunteers (age range, 20 to 48 years) for loading tests with sample A, and 5 volunteers (age range, 22 to 48 years) for sample C. All subjects had normal renal function and no metabolic risk factors. After an 8-hr overnight fast, blood samples were obtained (pre) and subjects then consumed 300 ml of sample A or 500 ml of sample C. Blood samples were drawn after 30,
60 and 120 min. The Ethics Committee of Tohoku University approved this study protocol, and informed consent was obtained from all subjects.

Laboratory analyses
After centrifugation of blood, plasma was aspirated and stored at ~80°C until assayed. MG levels were assayed by derivatization with o-phenylenediamine (o-PD) and electrospray ionization liquid chromatography mass spectrometry (ESI/LC/MS) of the resulting quinoline derivatives, as reported previously (Nakayama et al., 2008). To obtain more precise data, we modified the analytical conditions of LC/MS. The gradient speed of the mobile phase was slowed (from 6 to 10 min) and the mass/charge ratio (m/z) was detected more precisely (from m/z 145 to m/z 145.07). The resulting plasma MG levels from this new method were lower than our previous data derived from the previous method, but high relativity between the new and old methods was observed for 30 plasma samples: 10 from healthy controls and 20 from patients with renal failure (regression equation: y = 0.89x - 92, R^2 = 0.97). Plasma insulin was measured by the chemiluminescent enzyme immunoassay method, and glucose and other laboratory data were measured using an automatic analyzer at our clinical laboratory. Chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Statistical analyses
SPSS version 11.0 software (SPSS Japan, Tokyo, Japan) was used to evaluate the changes in plasma before and after soft drink intake by analysis of variance with repeated measures and Dunnett’s test. Values of P < 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION
The MG concentrations in 4 types of carbonated soft drink are listed in Table 1. Samples A and B contained high concentrations of carbohydrates and significant levels of MG. Samples C and D were low-calorie drinks and contained very low MG levels.

We examined the changes in plasma MG levels after intake of sample A, which contained high glucose levels and the highest level of MG among the 4 drinks, and after intake of sample C, a diet-type drink. As shown in Fig. 1, plasma MG, glucose and insulin were increased in most subjects at 30 min after intake of sample A, while no remarkable change was observed in the test for sample C, except that the glucose level gradually increased. The increases in plasma MG at 30 min after intake of sample A were statistically significant, indicating that glucose-containing carbonated soft drinks may, at least partly, increase the carbonyl burden. In some subjects (4 and 5), the increases in both plasma MG and glucose were observed at 30 min (Figs. 1a and c), and these results may suggest that the increases in MG was due to secondary production from absorbed glucose. However, in other subjects (2 and 3), plasma MG was higher at 30 min without a concomitant increase in plasma glucose. Moreover, the amount of MG contained in sample A was 2.2 μmol (7.2 μmol/l, 300 ml), which was sufficient to raise the concentration from 110 to 170 mmol/l in 36 l water, which is similar to the body fluid volume in a person weighing 60 kg. Therefore, the increase in plasma MG was most likely due to direct absorption from the drinks. A previous report showed that significantly high levels of MG (from 3.3 to 19.3 μM) were present in 11 brands of carbonated soft drinks (Tan et al., 2008), and their findings are coincident with the results of the present study.

In addition, UA levels were slightly but significantly, higher at 30 and 60 min. The extent of these changes was very small, and thus, its effect may be of little clinical significance. However, a high level of UA is reportedly one of the independent risk factors for cardiovascular disease (Choi and Curhan, 2007), therefore it is of interest whether plasma UA is associated with habitual intake of carbonated soft drinks.

Curiously, in all subjects, although the changes were small, plasma glucose levels gradually increased after intake of sample C, which contained no carbohydrate, while insulin levels did not increase. The threshold level of insulin secretion is thought to be above 100 mg/dl, and thus, these increases in glucose were too small to stimulate beta cells to release insulin. These slow changes in glucose may be physiologic phenomena caused by circadian changes in hormones (e.g., insulin, glucagon and cortisol), but the exact mechanisms at work remains unclear.

In conclusion, glucose-containing carbonated soft

Table 1. Concentrations of methylglyoxal in 4 types of carbonated drink

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbohydrate (g/100 ml)</th>
<th>MG (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cola</td>
<td>11.3</td>
</tr>
<tr>
<td>B</td>
<td>Lemon-lime soft drink</td>
<td>10.0</td>
</tr>
<tr>
<td>C</td>
<td>Diet-type cola</td>
<td>0.0</td>
</tr>
<tr>
<td>D</td>
<td>Diet-type cola</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*MG, methylglyoxal
Changes in plasma MG, glucose, insulin and UA levels before and after intake of sample A (glucose containing carbonated drink) (a, c, e, g) or sample C (diet-type carbonated drink) (b, d, f, h). Subjects 1 and 2 participated in both tests. For sample A, the MG level (mean ± S.D.) at pre and 30 min was 113 ± 22 and 136 ± 34 nM, respectively (a). The glucose level at pre and 30 min was 94 ± 8 and 113 ± 18 mg/dl, respectively (c). The insulin level at pre and 30 min was 4.4 ± 2.3 and 14.1 ± 8.8 IU/ml, respectively (g). For sample C, the glucose level at pre, 30, 60 and 120 min was 85 ± 5, 89 ± 5, 93 ± 6 and 93 ± 6 mg/dl, respectively (d). P < 0.05 vs pre, P’ < 0.01 vs pre.

Fig. 1. Changes in plasma MG, glucose, insulin and UA levels before and after intake of sample A (glucose containing carbonated drink) (a, c, e, g) or sample C (diet-type carbonated drink) (b, d, f, h). Subjects 1 and 2 participated in both tests. For sample A, the MG level (mean ± S.D.) at pre and 30 min was 113 ± 22 and 136 ± 34 nM, respectively (a). The glucose level at pre and 30 min was 94 ± 8 and 113 ± 18 mg/dl, respectively (c). The insulin level at pre and 30 min was 4.4 ± 2.3 and 14.1 ± 6.2 IU/ml, respectively (e). The UA level at pre, 30 and 60 min was 5.0 ± 1.8, 5.3 ± 1.8 and 5.3 ± 1.8 mg/dl, respectively (g). For sample C, the glucose level at pre, 30, 60 and 120 min was 85 ± 5, 89 ± 5, 93 ± 6 and 93 ± 6 mg/dl, respectively (d). P < 0.05 vs pre, P’ < 0.01 vs pre.
drinks appear to lead to a transient increase in plasma MG levels. It is of great interest whether habitual intake of carbonated drinks enhances human carbonyl stress and UA levels, or is involved with enhanced cardiovascular events among these subjects. Further studies are required to address these issues.

REFERENCES


