Effect of sucrose on inflammatory markers in overweight humans¹⁻³

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ABSTRACT
Background: Observational studies have found that dietary glycemic load is positively associated with C-reactive protein (CRP) concentrations in healthy humans, which suggests that the type of carbohydrate ingested influences inflammatory activity.
Objective: We investigated the effect of a diet with a high content of sucrose or artificial sweeteners on the inflammatory markers CRP, haptoglobin, and transferrin in overweight subjects.
Design: Overweight men and women consumed daily food and drink supplements containing either sucrose \( [n = 21; \text{body mass index (BMI, in kg/m}^2\)] : 28.0) or artificial sweeteners \( [n = 20; \text{BMI: } 27.6] \), predominantly from soft drinks \( (70\%\); average \( =1.3 \text{ L/d} \) for 10 wk.
Results: During the intervention, sucrose intake increased by 151% in the sucrose group and decreased by 42% in the sweetener group, resulting in a 1.6-kg weight gain in the sucrose group and a 1.2-kg weight loss in the sweetener group over 10 wk \( (P < 0.001) \). Concentrations of haptoglobin, transferrin, and CRP increased by 13%, 5%, and 6%, respectively, in the sucrose group and decreased by 16%, 2%, and 26%, respectively, in the sweetener group \( (\text{between-group differences: } P = 0.006, P = 0.01, \text{and } P = 0.1, \text{respectively}) \). Adjustment for changes in body weight and energy intake did not substantially influence this outcome.
Conclusions: The study shows that in the present group of overweight subjects a high consumption of sugar-sweetened foods and drinks increased haptoglobin and transferrin but had, at best, only a limited influence on CRP. Am J Clin Nutr 2005;82:421–7.

KEY WORDS C-reactive protein, CRP, haptoglobin, inflammatory markers, arteriosclerosis, cardiovascular disease, artificial sweeteners, sugar, sucrose, soft drinks, overweight, diabetes

INTRODUCTION
A biological marker of inflammation, C-reactive protein (CRP), has been shown in several prospective, nested, case-control studies to be associated with an increased risk of myocardial infarction, stroke, sudden death from cardiac causes, and peripheral arterial disease \( (1–3) \). Moreover, CRP and LDL-cholesterol concentrations measured at baseline in 27 939 apparently healthy women were found to be independent risk factors for first cardiovascular event at 8-y follow-up \( (4) \). The importance of CRP as a predictor of coronary heart disease is not clear, because different studies have provided varying results \( (4, 5) \). Elevated plasma concentrations of CRP have also been found to be a risk factor for type 2 diabetes independent of the classic risk factors, ie, obesity, smoking, exercise, and family history of diabetes \( (6) \). High concentrations of other inflammation-sensitive plasma proteins, including haptoglobin, have also been associated with type 2 diabetes, myocardial infarction, and stroke \( (7, 8) \). Recent evidence also links inflammation-sensitive plasma proteins to weight gain and obesity \( (9) \).

Although it is not known whether inflammation is a primary event that causes arteriosclerosis, type 2 diabetes, and perhaps obesity or whether it is a secondary event, it is important to identify environmental and lifestyle factors that may be influential. An observational study reported that a high glycemic load, ie, a high intake of rapidly digested and absorbed carbohydrates, was positively associated with CRP in healthy women \( (10) \). This finding was especially true in the overweight women, and the relation was independent of other risk factors \( (10) \). These results suggest that a proinflammatory process that may increase the risk of cardiovascular disease and type 2 diabetes may be exacerbated by a high intake of rapidly digested and absorbed carbohydrates. However, because of the observational nature of that study, it cannot be ruled out that the association was due to residual confounding caused by unmeasured and immeasurable factors.

In the present randomized, controlled trial we tested the hypothesis that overweight men and women who increase their intake of sugar-sweetened drinks and foods experience an increase in the inflammatory serum markers CRP and haptoglobin and a decrease in transferrin compared with a control group that consumed similar amounts of artificially sweetened beverages and foods.

SUBJECTS AND METHODS
The main part of the study, with emphasis on the effect of energy intake, body weight, and body composition, has been described in detail elsewhere \( (11) \).

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Study design

The study had a parallel design with 2 intervention groups. For 10 wk one group received supplemental drinks and foods containing sucrose, and the other group received similar drinks and foods containing artificial sweeteners.

Subjects

Forty-two healthy, overweight subjects were included, and 41 (6 men and 35 women) completed the study. None of the subjects were dieting, and none of the female subjects were pregnant or lactating. Approval was obtained from the Ethical Committee of Copenhagen and Frederiksborg, and the study was performed in accordance with the Helsinki II Declaration. Each subject signed an informed consent document before the start of the study.

Diets

During the 10-wk intervention the subjects consumed a specific minimum amount of either sucrose-sweetened or artificially sweetened foods and drinks daily. The subjects were assigned to specific minimum amount of either sucrose-sweetened or artificially sweetened foods and drinks. The minimum intake of the experimental diet was regulated by the sucrose intake and corresponded to a sucrose intake of 125 g/d (level 1), 150 g/d (level 2), and 175 g/d (level 3). This corresponded to a total energy intake from sucrose supplements of 2738 kJ/d, 3285 kJ/d, and 3833 kJ/d, respectively. The sweetener group received an equivalent amount (by weight) of the sweetener group received an equivalent amount (by weight) of foods and drinks, resulting in an average energy intake of 694 kJ/d, 832 kJ/d, or 971 kJ/d at levels 1, 2, and 3, respectively. The artificial sweetener content of the intervention diet was 54% aspartame, 23% cyclamate, 22% acesulfame K, and 1% saccharin.

In the sucrose group ≈70% of the sucrose came from drinks (average: ≈1.3 L/d), and ≈30% came from solid foods. About 80% by weight of the supplements were beverages, and ≈20% by weight were solid foods. The beverages consisted of several soft drinks and fruit juices, and the solid foods consisted of yogurt, marmalade, ice cream, and stewed fruits. Some of the artificially sweetened products were low-fat, so the subjects in the sweetener group were given additional butter or corn oil to keep the fat intake in the 2 intervention diets as similar as possible.

The subjects were supplied with all the drink and food supplements at the Department of Human Nutrition. In addition to the food and drink supplements, the subjects were free to consume their habitual diet ad libitum.

Measurements

Several measurements were performed before, during, and at the end of the 10-wk intervention period. Body weight, fat mass (FM), and fat-free mass were measured at weeks 0, 2, 4, 6, 8, and 10. Subjects completed 7-d dietary records, 7-d diaries (for monitoring hunger, fullness, palatability of the food, and well being), week diaries (for recording illness, menstruation, altered diet, medication, or altered physical activity pattern), 24-h urine collections (to validate the dietary records), and diurnal appetite scores at weeks 0, 5, and 10. Waist and hip circumferences, sagittal height (height of abdomen when lying in a supine position), and blood pressure were measured, and blood samples were taken at weeks 0 and 10. In addition, subjects completed a 3-factor questionnaire about eating behavior (12) as well as a questionnaire about habitual physical activity before and after the intervention. The recordings of physical activity were used to match the 2 groups before the intervention and to monitor any changes after the intervention. After the intervention, subjects also completed a questionnaire about the experimental diet. Results concerning the 7-d diaries, the questionnaires, and the effects of the diets on fat-free mass and sagittal height have been reported elsewhere (11).

Anthropometry

Height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer at the screening visit. All other measurements were done in the morning after a 12-h fast. After voiding, body weight was measured to the nearest 0.1 kg on a digital scale (Seca model 708; Seca Mess und Wiegetechnik, Vogel & Halke GmbH & Co, Hamburg, Germany). Body composition was estimated by bioelectrical impedance by using an Animeter (HTS-Engineering Inc, Odense, Denmark). FM and fat-free mass were calculated as described previously (13). Waist and hip circumferences were measured with a tape measure. Blood pressure was measured in the supine position, after 10 min of rest, using an automatically inflating cuff (UA-743; A&D Company Ltd, Tokyo, Japan).

Seven-day weighed dietary records

To monitor the subjects’ food consumption, three 7-d weighed dietary records were completed: in week 0 (before the intervention), and in weeks 5 and 10 (during the intervention). A 7-d dietary record was also completed 1–2 mo before the intervention to accustom the subjects to the method. Digital scales were used to weigh the food (Soehnle 8020 and 8009; Soehnle-Waagen GmbH & Co, Murrhardt, Germany), and the computer database of foods from the National Food Agency of Denmark (DANKOST 2.0) was used to calculate energy and nutrient intakes (14).

Urine samples

Subjects collected 24-h urine samples during the sixth day and every night of every dietary record period (weeks 0, 5, 10) to validate the dietary records. During these 24-h periods, subjects ingested a paraaminobenzenoic acid (PABA) pill with the 3 main meals (a total of 240 mg PABA/d) to serve as an indicator of complete urinary protein to ingested protein and calculation of dietary protein recovery has been reported elsewhere (11). Assays

Blood samples for glucose analysis were drawn into a test tube containing EDTA and fluoride, and blood samples for insulin, triacylglycerol, CRP, haptoglobin, and transferrin analyses were drawn into tubes with no additives. The tubes (except for insulin analyses) were kept on ice. Plasma glucose was analyzed by...
standard enzymatic methods (Boehringer Mannheim GmbH Diagnostica, Copenhagen, Denmark). Insulin concentrations in serum were measured by using radioimmunoassay (AutoDelfi Automatic Immunoassay System; Wallac Oy, Turku, Finland). Triacylglycerol concentrations in serum were analyzed by using the Test-Combination Triacylglycerols GPO-PAP method (Boehringer Mannheim GmbH Diagnostica). CRP concentration was measured by using the CRP (Latex) ultrasensitive assay (Roche Diagnostic Systems, Basel, Switzerland). Haptoglobin and transferrin were analyzed by using the immunoturbidimetric method (Roche Diagnostic Systems).

To assess insulin resistance the homeostasis model (HOMA IR) was used. The formula for the HOMA IR model is fasting insulin (in μU/mL) × fasting glucose (in mmol/L)/22.5 (16).

### Statistical analyses

Unpaired t test was used to test initial group differences. Repeated measures analysis of covariance (ANCOVA) was used to test interaction between diet groups (sucrose and sweetener) and time (5 and 10 wk). The mixed procedure in the STATISTICAL ANALYSIS SYSTEM (SAS) software package, version 8.2 was used (SAS Institute, Cary NC). Baseline values were included as covariate. When interaction of diet and time was significant, Tukey’s post hoc tests were applied.

The effect of diet on body weight, FM, CRP, haptoglobin, transferrin, triacylglycerol, HOMA IR, and blood pressure was tested by using ANCOVA with the mixed procedure in SAS; with week 10 values as response and week 0 values as covariate and subjects as random factor (Table 3). When adjusting for body weight changes or changes in energy intake, ANCOVA with week 10 values as response and week 0 values and body weight changes or changes in energy intake as covariate was used. Log transformation of data with skewed distributions was done when necessary.

### RESULTS

The subjects were randomly assigned to 2 intervention groups, which turned out to be well matched for sex, age, body size and composition, and habitual levels of physical activity (Table 1). Forty-one subjects completed the 10-wk study, but 4 subjects (2 from each group) had concentrations of CRP > 10 mg/L, either at baseline or at the end of the intervention, which could indicate infection. Therefore, statistical analyses were done both with and without these 4 subjects. Only the results for inflammatory markers varied with and without the 4 subjects; therefore, only these results are presented with the outcome from both analyses.

### Food intake

The records of ad libitum food intake (including food and drink supplements) showed that there were no significant differences between week 5 and week 10 values for any of the variables in the same diet group (Table 2). Although a significant interaction was observed between diet and time for several of the variables, the Tukey-Kramer adjusted post hoc tests showed that there were no withingroup changes from week 5 to week 10 (Table 2).

The food records showed that there was a significantly higher intake of carbohydrate (both in gram and percent of energy) in the sucrose group than in the sweetener group during the intervention (Table 2). The increase in carbohydrate intake in the sucrose group was mainly due to an increased sucrose intake. Sucrose intake (both in gram and percent of energy) and the percentage of energy from fat and protein were higher in the sucrose group than in the sweetener group during the intervention (Table 2). The average difference in total energy intake between the 2 groups was 2.7 MJ/d during the intervention (Table 2). Changes in energy intake correlated positively with changes in sucrose intake ($r = 0.73, P < 0.0001$). The energy density of the diet was significantly lower in the sweetener group than in the sucrose group during the intervention. No differences were observed between the groups in intake of dietary fiber (in g/d); total fat (in g/d); saturated, monounsaturated, and polyunsaturated fat (in g/d); protein (in g/d); alcohol (in g/d; percent of energy); and total weight of food (in g/d).

### Validation of protein intake

Urinary protein excretion was estimated in 91 urine samples after excluding 32 samples (17 from the sucrose group and 15 from the sweetener group) that were incomplete, as indicated by a recovery < 85% of PABA. Data were available from 15 subjects in each group at each time point, with the exception of the sucrose group at week 10 ($n = 16$). The differences between urinary protein and self-reported dietary protein ranged from 1 to 13 g/d with no significant differences between groups and times. Dietary protein recovery ranged from 103% to 119%, also with no significant differences between groups and times. Urinary protein correlated significantly with dietary protein at all 3 time points, with the strongest correlations at weeks 5 and 10 (week 0: $r = 0.39, P < 0.05$; week 5: $r = 0.52, P < 0.01$; week 10: $r = 0.53, P < 0.01$).

### Serum concentrations of inflammatory markers

At baseline no differences were observed in clinical and biochemical characteristics between the sucrose group and the sweetener group (Table 3). After 10 wk, intervention mean serum CRP concentrations had decreased by 13% in the sucrose group and by 22% in the sweetener group (between-group difference: $P = 0.32$). After excluding 4 subjects with CRP concentrations > 10 mg/L, an increase in mean CRP concentrations by 6% in the sucrose group and a decrease by 26% in the sweetener group were found (between-group difference, $P = 0.1$) (Table 3). No correlation was observed between dietary changes and changes in CRP.

After 10 wk, serum concentrations of haptoglobin, using data from all subjects, had increased by 6% in the sucrose group and decreased by 15% in the sweetener group (between-group differences: $P = 0.02$). Serum transferrin concentrations increased

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Anthropometric characteristics of the subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose group ($n = 21$)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>33.4 ± 9.0</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>82.5 ± 7.7</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 2.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.1 ± 4.5</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.79 ± 0.02</td>
</tr>
</tbody>
</table>

1 All values are $\bar{x}$ ± SD. No significant differences were observed between the groups (unpaired t test).
TABLE 2
Average daily intake of energy and macronutrients in the sucrose and sweetener groups before (week 0) and during week 5 and week 10 of the food and drink supplementation after exclusion of 4 subjects.

<table>
<thead>
<tr>
<th>Carbohydrate (g/d)</th>
<th>Week 0 (baseline)</th>
<th>Week 5</th>
<th>Week 10</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>282 ± 24</td>
<td>382 ± 16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>385 ± 17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweetener</td>
<td>245 ± 16</td>
<td>222 ± 15</td>
<td>226 ± 13</td>
<td></td>
</tr>
<tr>
<td>(% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>49 ± 2</td>
<td>59 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweetener</td>
<td>46 ± 1</td>
<td>43 ± 2</td>
<td>45 ± 1</td>
<td></td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>71 ± 16</td>
<td>183 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>179 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweetener</td>
<td>40 ± 6</td>
<td>22 ± 3</td>
<td>22 ± 4</td>
<td></td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>34 ± 2</td>
<td>29 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweetener</td>
<td>34 ± 2</td>
<td>36 ± 1</td>
<td>34 ± 1</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>9659 ± 654</td>
<td>11 160 ± 513&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11 662 ± 582&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweetener</td>
<td>9187 ± 623</td>
<td>8764 ± 571</td>
<td>8593 ± 459</td>
<td></td>
</tr>
<tr>
<td>Energy density (kJ/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.4 ± 0.2</td>
<td>3.2 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.3 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweetener</td>
<td>3.0 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> All values are ± SEM. Four subjects were excluded because of C-reactive protein concentrations >10 mg/L, indicating possible infection. At weeks 0 and 10, n = 19 in the sucrose group and n = 18 in the sweetener group. At week 5, n = 18 in the sucrose group and n = 18 in the sweetener group. Only variables that were statistically different between the 2 groups are mentioned in the table.

<sup>2</sup> There were no significant differences in baseline values between the 2 groups (unpaired t test).

<sup>3</sup> P values were derived by repeated-measures analysis of covariance testing for interaction between diet groups and time (5 and 10 wk) with week 0 as a covariate. Tukey-Kramer adjusted post hoc tests showed that there were no within-group changes from week 5 to week 10.

<sup>4</sup>-<sup>6</sup> Significantly different from the sweetener group: <sup>a</sup> P < 0.001, <sup>b</sup> P < 0.01, <sup>c</sup> P < 0.05.

by 3% in the sucrose group and decreased by 1% in the sweetener group (between-group differences; P = 0.05). Exclusion of the 4 subjects with CRP concentrations >10 mg/L resulted in even more significant between-group differences for both haptoglobin and transferrin (P = 0.006 and P = 0.01, respectively) (Table 3). Changes in haptoglobin correlated positively with changes in sucrose intake (total group; n = 41; r = 0.33, P = 0.03; without excluded subjects; n = 37; r = 0.40, P = 0.01), whereas changes in transferrin and changes in sucrose intake only correlated after exclusion of the 4 subjects (n = 41: r = 0.27, P = 0.09; n = 37: r = 0.37, P = 0.03). A positive correlation between changes in CRP and changes in haptoglobin was found (n = 41: r = 0.38, P = 0.01; n = 37; r = 0.46, P = 0.004). Changes in haptoglobin correlated positively with changes in energy intake (n = 41: r = 0.40, P = 0.009; n = 37: r = 0.36, P = 0.03) as did changes in transferrin (n = 41: r = 0.40, P = 0.01; n = 37; r = 0.35, P = 0.03). Changes in haptoglobin correlated positively with changes in transferrin before exclusion (n = 41: r = 0.42, P = 0.006), whereas no significant correlation was observed after exclusion (n = 37; r = 0.31, P = 0.06). Adjusting for body weight changes and changes in energy intake did not substantially influence the results (Table 3).

Serum concentrations of triacylglycerol and HOMA IR and measurement of blood pressure

No differences were seen between the sucrose and the sweetener groups in triacylglycerol and HOMA IR concentrations during the intervention, both before and after exclusion of the 4 subjects (Table 3). No association was observed between changes in CRP and changes in triacylglycerol and HOMA IR. After 10 wk of intervention, systolic and diastolic blood pressure had increased in the sucrose group and decreased in the sweetener group, resulting in significant between-group differences (Table 3). No association was observed between changes in blood pressure and changes in CRP, haptoglobin, or transferrin.

Body weight

Body weight and FM increased in the sucrose group (1.6 ± 0.5 and 1.3 ± 0.5 kg, respectively) and decreased in the sweetener group (1.2 ± 0.5 and 0.3 ± 0.4 kg, respectively) during the 10-wk intervention. This finding resulted in significant between-group differences, amounting to 2.8 ± 0.7 kg body weight (P < 0.001) and 1.6 ± 0.7 kg body fat (P < 0.01) after 10 wk.
TABLE 3
Inflammatory markers, triacylglycerol, homeostasis model for insulin resistance (HOMA IR), and blood pressure before (week 0) and after 10 wk of intervention in the sucrose group and the sweetener group after the exclusion of 4 subjects

|                     | Week 0 (baseline) | Week 10 | Week 10 adjusted for baseline values and body weight changes | Week 10 adjusted for baseline values and changes in energy intake | P
|---------------------|-------------------|---------|-----------------------------------------------------------|-------------------------------------------------------------|----
| CRP (mg/L)          |                   |         |                                                           |                                                             |     
| Sucrose             | 1.8 (0.9–3.0)     | 2.2 (1.2–3.0) | 2.1 (1.5, 2.8) | 0.10 | 2.1 (1.4, 3.0) | 0.17 | 2.0 (1.4, 2.8) | 0.22 |
| Sweetener           | 1.8 (0.8–4.5)     | 1.3 (0.8–2.8) | 1.4 (1.0, 1.9) | 0.006 | 1.4 (1.0, 2.0) | 0.004 | 1.5 (1.0, 2.1) | 0.04 |
| Haptoglobin (mg/dL) |                   |         |                                                           |                                                             |     
| Sucrose             | 138 ± 13          | 156 ± 15 | 149 (130, 171) | 0.001 | 155 (133, 181) | 0.004 | 145 (126, 167) | 0.041 |
| Sweetener           | 156 ± 15          | 132 ± 14 | 112 (98, 129) | 0.008 | 108 (92, 126) | 0.004 | 116 (100, 134) | 0.052 |
| Transferrin (mg/dL) |                   |         |                                                           |                                                             |     
| Sucrose             | 296(264–344)      | 312 (288–344) | 316 (305, 328) | 0.01 | 316 (303, 330) | 0.047 | 315 (302, 327) | 0.052 |
| Sweetener           | 288 (264–360)     | 282 (272–320) | 295 (284, 307) | 0.01 | 295 (282, 308) | 0.047 | 297 (285, 309) | 0.052 |
| Plasma triacylglycerol (mmol/L) |       |         |                                                           |                                                             |     
| Sucrose             | 1.1 (0.8–1.8)     | 1.2 (0.9–2.3) | 1.2 (1.0, 1.4) | 0.28 | 1.1 (0.9, 1.3) | 0.55 | 1.3 (1.1, 1.5) | 0.11 |
| Sweetener           | 1.1 (0.8–1.2)     | 0.9 (0.8–1.1) | 1.1 (0.9, 1.3) | 0.17 | 1.2 (1.0, 1.4) | 0.45 | 1.4 (1.2, 1.7) | 0.041 |
| HOMA IR\textsuperscript{10} |       |         |                                                           |                                                             |     
| Sucrose             | 1.0 (0.8–1.5)     | 1.3 (0.9–1.8) | 1.3 (1.1, 1.6) | 0.17 | 1.3 (1.0, 1.6) | 0.45 | 1.4 (1.2, 1.7) | 0.041 |
| Sweetener           | 1.1 (0.7–1.5)     | 1.2 (0.7–1.9) | 1.1 (0.9, 1.3) | 1.17 | 1.2 (1.0, 1.4) | 0.45 | 1.4 (1.2, 1.7) | 0.041 |
| Systolic BP (mm Hg) |                   |         |                                                           |                                                             |     
| Sucrose             | 117.2 ± 1.9       | 122.3 ± 2.4 | 121.8 (118.3, 125.3) | 0.002 | 120.8 (116.8, 124.7) | 0.059 | 121.3 (117.6, 125.0) | 0.013 |
| Sweetener           | 116.1 ± 2.1       | 113.1 ± 2.2 | 113.6 (110.0, 117.1) | 0.002 | 114.7 (110.6, 118.8) | 0.059 | 114.1 (110.3, 117.9) | 0.013 |
| Diastolic BP (mm Hg) |                   |         |                                                           |                                                             |     
| Sucrose             | 71.2 ± 1.6        | 75.6 ± 2.0 | 76.2 (73.3, 79.1) | 0.037 | 76.6 (73.3, 79.8) | 0.043 | 75.8 (72.7, 78.8) | 0.12 |
| Sweetener           | 73.3 ± 2.2        | 72.4 ± 1.5 | 71.7 (68.8, 74.7) | 0.037 | 71.3 (68.0, 74.7) | 0.043 | 72.2 (69.1, 75.3) | 0.12 |

\textsuperscript{1} Four subjects were excluded because their C-reactive protein concentrations were >10 mg/L, indicating possible infection. CRP, C-reactive protein; BP, blood pressure.

\textsuperscript{2} Values are \(\bar{x} \pm \) SEM or median (interquartile range) for variables with skewed distributions. \(n = 19\) in the sucrose group and \(n = 18\) in the sweetener group.

\textsuperscript{3} There were no significant differences in baseline values between the 2 groups (unpaired t test).

\textsuperscript{4} Values are least-squared means of 10-wk values adjusted for baseline values; 95% CI in parentheses.

\textsuperscript{5} P values were derived by analysis of covariance with week 10 values as the response and week 0 values as the covariate.

\textsuperscript{6} Values are least-squared means of 10-wk values adjusted for baseline values and body weight changes; 95% CI in parentheses.

\textsuperscript{7} P values were derived by analysis of covariance with week 10 values as the response and week 0 values and changes in body weight as the covariates.

\textsuperscript{8} Values are least-squared means of 10-wk values adjusted for baseline values and changes in energy intake; 95% CI in parentheses.

\textsuperscript{9} P values were derived by analysis of covariance with week 10 values and changes in energy intake as the covariates.

\textsuperscript{10} HOMA IR = fasting insulin (\(\mu\)U/mL) \times fasting glucose (mmol/L)/22.5.

DISCUSSION

In the present study the sucrose diet produced a nonsignificant 6% increase and the sweetener diet produced a 26% decrease in CRP concentrations (\(P = 0.1\)). This trend toward an association between a diet high in rapidly digested and absorbed carbohydrates and CRP supports the findings of an earlier observational study (10). In the observational study the CRP concentrations were measured in 244 apparently healthy women, and the association between the subjects’ dietary glycemic load and CRP concentrations was examined (10). It was found that the median CRP concentration for the lowest quintile of dietary glycemic load was significantly lower than the CRP concentration for the highest quintile. This result remained after adjusting for several confounders such as age, BMI, history of hypertension, high cholesterol or diabetes mellitus, and lifestyle factors.

The lack of statistical significance in the present study may be due to a type II error as a result of the small number of subjects, which was only 19 and 18, respectively, in the 2 groups. However, the changes in CRP were relatively small, and, by taking into consideration that the reliability of CRP is poor (17), the results suggest that sucrose has little effect on CRP.

The sucrose diet increased concentrations of haptoglobin, and changes in haptoglobin concentrations were positively associated with changes in sucrose intake. This finding could indicate increased inflammation in the sucrose group and decreased inflammation in the artificial sweetener group. However, changes in haptoglobin concentrations were positively correlated with changes in energy intake, which suggests that haptoglobin may respond to the body’s energy flux rather than to the sucrose intake. However, it is not possible to reach a firm conclusion on this aspect because of the close covariation between energy and sucrose intakes.

Transferrin is a negative acute-phase protein, and a drop in transferrin concentrations therefore reflects increased inflammation. It was therefore contrary to our expectations that transferrin increased in the sucrose group and decreased in the sweetener group. Changes in transferrin concentrations were also positively associated with changes in energy intake, which could indicate...
that transferrin responds to the body’s energy flux. Adjusting for changes in energy intake did not eliminate the difference between the 2 groups, in either haptoglobin or transferrin, although the differences became less significant.

Earlier studies that investigated the effect of calorie-restricted diets (18), partial fasting (19), and total fasting (20), on various blood markers, have shown that concentrations of haptoglobin and transferrin decrease when subjects are in negative energy balance. In the study that investigated total fasting (18), transferrin concentrations rose again when the subjects received an 800-kcal diet (re-alimentation), although they were still in negative energy balance. Furthermore, haptoglobin started to increase already during the last period of fasting and increased further during re-alimentation.

The present study was originally designed to test the effects of sucrose compared with artificial sweeteners on changes in intakes of ad libitum energy and macronutrients, body weight, and body composition (11). The decision to analyze frozen plasma was taken >5 y after the study was completed and after the results of Liu et al (10) had been published, which showed an association between glycemic load and CRP. It should be noted that CRP, transferrin, and haptoglobin are stable over long periods and have no diurnal variation (21). Using the original design to test the effect of a high glycemic load on inflammatory activity introduced a confounding difference in energy intake and change in body weight between the 2 diets. This difference could give rise to problems in interpreting the results, because overweight and obesity have been shown to be associated with elevated concentrations of inflammatory markers (19, 20, 22–24). The sucrose diet in our study caused an increase in total caloric intake and a weight gain of 1.6 kg in contrast to a weight loss of 1.2 kg in the group receiving the artificially sweetened drinks and foods. The minor changes in body weight did not have any significant effect on the differences in inflammatory markers between the 2 groups. In a recent study by Engström et al (9) inflammatory markers were linked to weight gain and obesity, and the researchers suggested that relations between inflammatory markers and weight gain reflect dietary factors that increase both weight and inflammatory markers. This suggestion is supported by our results, showing that changes in CRP and haptoglobin were unaffected when adjusted for body weight changes.

We conducted the present study in overweight subjects, because increased fatness is associated with increased inflammation (19, 20, 22–24). It may be possible that the subjects’ inflammatory response is dampened by previous exposure to sucrose, but the group receiving artificial sweetener acted as a control group. One could expect a decrease in CRP if sucrose intake has an influence on inflammatory activity.

Whereas the strength of the randomized design is a minimization of possible confounders, dietary trials such as the present one cannot be conducted in a double-blind fashion, which gives rise to other limitations. Although we aimed to conduct the trial in a blinded fashion (no information about the type of sweetener on the packaging of the drinks or foods), it was obvious that many participants could identify the artificial sweeteners by taste (11). Exercise is suggested to reduce inflammation (25), and, if the subjects who knew that they were getting the sucrose diet were more likely to exercise to compensate for the higher energy intake, then the markers of inflammation in the sucrose group would be artificially low. It does not seem to be a problem in this study because there was no significant between-group differences in the changes in the amount of physical activity or in the level of physical activity as recorded by the subjects after the 10-wk intervention (11).

In the present study no biological markers were available for objective information about dietary intake of fat and carbohydrate. The 24-h urinary nitrogen excretion was used as a marker to validate the dietary protein records, and the results show that significant agreement existed between the reported dietary protein intake and the urinary protein. A significant increase in body weight in the sucrose group was observed, which corresponds to the increased energy intake recorded during the intervention. In the sweeter group a significant decrease in body weight was observed, even though no significant decrease was observed in energy intake (594 kJ). However, 594 kJ/d over 70 d (41.6 MJ) would give rise to a weight loss of 1.2 kg (assuming 34 MJ/kg weight loss) (26), which corresponds to the observed weight loss of 1.2 kg. Thus, these results suggest that the compliance was good in both intervention groups.

The question is whether the intake of sucrose from soft drinks in the present study is realistic. In the sucrose group ≈70% of the sucrose came from drinks (average: ≈1.3 L/d) and ≈30% came from solid foods. This is actually similar to the mean volume and sugar content of the diet of American boys in 1996, slightly above that of American girls (27), and somewhat below the mean consumption of American adults (28). The results should therefore be applicable to a large proportion of the American population.

In conclusion, this study shows that, apart from causing weight gain and increasing blood pressure, a high consumption of sugar-sweetened drinks and foods may increase inflammatory activity in overweight subjects. This finding was independent of weight changes. The relative changes in inflammatory markers in the present study were small. Even though the between-group differences in haptoglobin and transferrin were statistically significant, it is doubtful that these differences are biologically important.

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AR and AA designed the core study, AR lead the conduct of the study, and both participated in the interpretation of the results. AA and SS generated the idea to analyze inflammatory markers in this study. LBS was responsible for the statistical analysis, and AA and LBS wrote the initial draft of the manuscript. All authors have read and corrected the manuscript. None of the authors had a personal or financial conflict of interest.

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