Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study\(^1\text{–}^3\)

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ABSTRACT

Background: The consumption of sucrose-sweetened soft drinks (SSSDs) has been associated with obesity, the metabolic syndrome, and cardiovascular disorders in observational and short-term intervention studies. Too few long-term intervention studies in humans have examined the effects of soft drinks.

Objective: We compared the effects of SSSDs with those of isocaloric milk and a noncaloric soft drink on changes in total fat mass and ectopic fat deposition (in liver and muscle tissue).

Design: Overweight subjects (n = 47) were randomly assigned to 4 different test drinks (1 L/d for 6 mo): SSSD (regular cola), isocaloric semiskim milk, aspartame-sweetened diet cola, and water. The amount of intrahepatic fat and intramyocellular fat was measured with \(^1\)H-magnetic resonance spectroscopy. Other endpoints were fat mass, fat distribution (dual-energy X-ray absorptiometry and magnetic resonance imaging), and metabolic risk factors.

Results: The relative changes between baseline and the end of 6-mo intervention were significantly higher in the regular cola group than in the 3 other groups for liver fat (132–143%, sex-adjusted mean; \(P < 0.01\)), skeletal muscle fat (117–221%; \(P < 0.05\)), visceral fat (24–31%; \(P < 0.05\)), blood triglycerides (32%; \(P < 0.01\)), and total cholesterol (11%; \(P < 0.01\)). Total fat mass was not significantly different between the 4 beverage groups. Milk and diet cola reduced systolic blood pressure by 10–15% compared with regular cola (\(P < 0.05\)). Otherwise, diet cola had effects similar to those of water.

Conclusion: Daily intake of SSSDs for 6 mo increases ectopic fat accumulation and lipids compared with milk, diet cola, and water. Thus, daily intake of SSSDs is likely to enhance the risk of cardiovascular and metabolic diseases. This trial is registered at clinicaltrials.gov as NCT00777647. Am J Clin Nutr doi: 10.3945/ajcn.111.022533.

INTRODUCTION

Sugar-sweetened beverages have been associated with obesity, cardiovascular disease, and the metabolic syndrome (1–5) and recently with increased ectopic fat accumulation, independently of other lifestyle factors (1, 6). Although semiskim milk is isocaloric to sucrose-sweetened regular cola, milk has a different macronutrient composition and has been associated with opposite health effects, namely weight loss and a decrease in blood pressure (7–10). The noncaloric aspartame-sweetened cola was introduced to fulfill the desire for sweet taste with no concomitant energy intake, and short-term (3–10 wk) intervention studies showed that diet cola promoted weight loss compared with regular cola (11, 12). On the other hand, artificially sweetened soft drinks have also been associated with obesity and the metabolic syndrome (5, 13). However, to our knowledge, no long-term interventions (beyond 10 wk) have examined the effect of beverages on ectopic fat accumulation in humans.

Our main aim was to test the hypothesis that sucrose-sweetened cola increases ectopic fat including VAT\(^4\), total body fat accumulation, and metabolic risk factors in a 6-mo controlled trial compared with 3 alternative beverages: isocaloric semiskim milk, aspartame-sweetened cola, and water.

SUBJECTS AND METHODS

Sixty healthy, nondiabetic subjects with a BMI (in kg/m\(^2\)) between 26 and 40, an age between 20 and 50 y, and a blood pressure <160/100 mm Hg were included in the study. The study was undertaken simultaneously at Aarhus University Hospital and Department of Nutrition, Copenhagen University, Denmark. All participants provided written informed consent. The study protocol was approved by the Ethics Committee of Middle Jutland, Denmark, and was conducted in accordance with the Declaration of Helsinki.

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\(^2\) Supported by grants from The Danish Council for Strategic Research, The Food Study Group/Danish Ministry of Food, Agriculture and Fisheries, Novo Nordic Foundation, and Clinical Institute at Aarhus University, Denmark. The semiskim milk was donated by the Danish Dairy Company, Arla Foods, but without any influence on the design, interpretation, or conclusions of the study.

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\(^4\) Abbreviations used: DXA, dual-energy X-ray absorptiometry; HFCS, high-fructose corn syrup; MRI, magnetic resonance imaging; SAAT, subcutaneous abdominal adipose tissue; SSSD, sucrose-sweetened soft drink; VAT, visceral adipose tissue.

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Intervention

In a 6-mo randomized parallel intervention the participants were allocated to consume 1 of 4 commercially available test drinks: sucrose-sweetened regular cola (Coca Cola), aspartame-sweetened diet cola (Coca Cola), semiskim milk (Arla Foods), and still mineral water (Aqua d’or). The energy content and the composition of the beverages are shown in Table 1. Danish regular cola is sweetened with sucrose (50% glucose and 50% fructose). The subjects consumed 1 L of the test drink daily and were allowed to drink water, tea, coffee, and their usual amount of alcohol. Because of the nature of the intervention, the study was not blinded. The lifestyle changes of the subjects regarding diet and physical activity were monitored at baseline, after 3 mo, and at the end of the intervention with the use of a 7-d dietary record and a validated questionnaire concerning physical activity at work, during leisure time, and during sports (14). All beverages were free of charge and were handed out 2 or 3 times/mo from the research centers. Anthropometric measures and compliance checks were performed every 1.5 mo. All subjects were asked to bring the empty bottles or cartons to the research center for counting every 3–4 wk to monitor and strengthen compliance. Subjects who were randomly assigned to consume soft drinks were provided with toothpaste, instructed about dental hygiene, and examined by a dentist at baseline and every 1.5 mo throughout the intervention to check for caries or acid erosion of the enamel. None of the subjects developed these problems.

Clinical investigations

On the night before each test day, the subjects consumed a standardized evening meal (men: 4.2 MJ; women: 3.2 MJ) and refrained from any occasional medicine, alcohol, and vigorous exercise for 24 h. They fasted overnight, and on the following morning blood samples were collected (at 0800) and a 2-h oral-glucose-tolerance test was performed.

Blood samples for total cholesterol, HDL cholesterol, triglycerides, and glucose were analyzed by using routine laboratory methods at the hospital. Commercially available kits were used to assess leptin (Human Leptin ELISA kit; Mediagnost) and insulin (Human Insulin ELISA; DAKO). All analyses were collected at the hospital. Commercially available kits were used to measure the composition of the beverages are shown in Table 1. Danish regular cola is sweetened with sucrose (50% glucose and 50% fructose). The subjects consumed 1 L of the test drink daily and were allowed to drink water, tea, coffee, and their usual amount of alcohol. Because of the nature of the intervention, the study was not blinded. The lifestyle changes of the subjects regarding diet and physical activity were monitored at baseline, after 3 mo, and at the end of the intervention with the use of a 7-d dietary record and a validated questionnaire concerning physical activity at work, during leisure time, and during sports (14). All beverages were free of charge and were handed out 2 or 3 times/mo from the research centers. Anthropometric measures and compliance checks were performed every 1.5 mo. All subjects were asked to bring the empty bottles or cartons to the research center for counting every 3–4 wk to monitor and strengthen compliance. Subjects who were randomly assigned to consume soft drinks were provided with toothpaste, instructed about dental hygiene, and examined by a dentist at baseline and every 1.5 mo throughout the intervention to check for caries or acid erosion of the enamel. None of the subjects developed these problems.

Statistics

The effects of the beverages after the 6-mo intervention were assessed by 2 different analyses. First, we compared the groups with respect to the effects of the beverages with a univariate ANOVA with drink and sex as fixed factors. For each variable, the drink effect was expressed as the relative change between baseline and the 6-mo follow-up to take into account the possible differences at baseline between the drink groups. Sex was included in the model because of the unequal sex distribution of the 4 groups. It was determined to make pairwise comparisons between the 4 different beverages, and overall significance was determined by ANOVA ($P < 0.05$) by using a Bonferroni procedure for multiple comparisons. Second, within each beverage group, the absolute values of the variables at baseline were compared with the 6-mo follow-up values by using a paired $t$ test. This test was not stratified for sex because of the relatively few subjects in each group. A 2-tailed $P$ value $<0.05$ was

| TABLE 1 Composition and energy content of the 4 test drinks
drinks | Energy content (kJ/d) | Carbohydrate (g/100 mL) | Protein (g/100 mL) | Fat (g/100 mL) | Volume (mL) | Energy density (kJ/g) |
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose-sweetened regular cola</td>
<td>10.6</td>
<td>4.7</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>1.8</td>
</tr>
<tr>
<td>Milk</td>
<td>0</td>
<td>3.4</td>
<td>&lt;0.1</td>
<td>0</td>
<td>1000</td>
<td>1.9</td>
</tr>
<tr>
<td>Aspartame-sweetened diet cola</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>0.015</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>0</td>
</tr>
</tbody>
</table>

*The subjects drank 1 L of 1 of 4 test drinks daily for 6 mo.*
considered significant. Last value carried forward was used for 2 subjects, who left the intervention after 5 mo. Otherwise missing values were not imputed. SPSS version 18.0 (SPSS Inc) was used for all statistical evaluations. Descriptive data are presented as means ± SDs. The results are presented as means ± SEMs (adjusted for sex) in the figures and as means and 95% CIs (adjusted for sex) in the text.

RESULTS

Baseline characteristics

The characteristics of the subjects at baseline are shown in Table 2. The 4 groups were well matched for age and BMI, but there was an unequal distribution of sex (ie, more men in the regular cola group) and a tendency for differences in VAT, skeletal muscle fat, and liver fat (P ≤ 0.1) between the groups. Accordingly, the results were analyzed as sex- and baseline-adjusted relative changes. Sixty individuals were randomly assigned to the intervention. Thirty subjects (all women) dropped out after randomization (4 randomly assigned to water, 5 randomly assigned to regular cola, and 4 randomly assigned to diet cola). Forty-seven subjects (30 women and 17 men) completed the study. At baseline, SSSD intake was not different between the 4 groups, and the mean intake in these individuals was 184 mL/d.

Effects on fat accumulation in VAT, liver, and skeletal muscle

Drinking regular cola resulted in a higher relative amount of VAT than did the other drinks (P = 0.03, ANOVA). In pairwise comparisons, the increment was 31% (95% CI: 10, 53; P < 0.05) compared with milk, 23% (95% CI: 3, 43; P = 0.14) compared with diet cola, and 24% (95% CI: 4, 44; P = 0.1) compared with water during the 6-mo intervention (Figure 1A). Moreover, the ratio of VAT to SAAT was significantly higher in the regular cola group than in the milk group (31%; 95% CI: 13, 48; P < 0.01; Table 3).

Accumulation of liver fat was higher after regular cola (overall P = 0.01, ANOVA) than after milk (143%; 95% CI: 50, 236; P < 0.05), diet cola (139%; 95% CI: 50, 227; P < 0.05), and water (132%; 95% CI: 43, 222; P < 0.05; Figure 1B). Furthermore, relative changes in muscle fat overall were greater in the regular cola group (P < 0.05, ANOVA) than in the 3 other groups. The post hoc pairwise analyses were nearly significant, with a higher amount of muscle fat in the regular cola group than in the milk group (by 227%; 95% CI: 50, 405; P < 0.05) compared with milk, 23% (95% CI: 3, 43; P < 0.05; Figure 1B). No significant differences in the accumulation of VAT, liver fat, and muscle fat were found between the milk, diet cola, and water groups during the 6-mo intervention (Figure 1, A–C).

### Table 2

Baseline characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Regular cola</th>
<th>Milk</th>
<th>Diet cola</th>
<th>Water</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (n)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
<td>39 ± 6²</td>
<td>38 ± 9</td>
<td>39 ± 8</td>
<td>39 ± 8</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>97.8 ± 12.5</td>
<td>94.7 ± 15.3</td>
<td>92.2 ± 10.9</td>
<td>101.7 ± 22.4</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>31.3 ± 2.9</td>
<td>31.9 ± 2.8</td>
<td>32.8 ± 3.8</td>
<td>32.2 ± 4.6</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Liver fat (AU)⁴</strong></td>
<td>0.037 ± 0.04</td>
<td>0.127 ± 0.1</td>
<td>0.21 ± 0.2</td>
<td>0.093 ± 0.09</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Skeletal muscle fat (AU)⁴</strong></td>
<td>0.002 ± 0.001</td>
<td>0.004 ± 0.002</td>
<td>0.002 ± 0.001</td>
<td>0.002 ± 0.001</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>VAT (cm²)⁵</strong></td>
<td>110.4 ± 52.1</td>
<td>99.9 ± 36.0</td>
<td>135.2 ± 56.5</td>
<td>77.4 ± 40.1</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>SAAT (cm³)⁵</strong></td>
<td>281.5 ± 70.8</td>
<td>345.5 ± 91.7</td>
<td>298.8 ± 106.3</td>
<td>278.9 ± 112.2</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Total fat mass (kg)⁶</strong></td>
<td>34.0 ± 8.3</td>
<td>35.4 ± 6.4</td>
<td>35.1 ± 9.0</td>
<td>37.2 ± 11.6</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Lean body mass (kg)⁶</strong></td>
<td>61.5 ± 11.5</td>
<td>56.8 ± 14.1</td>
<td>54.9 ± 11.1</td>
<td>57.5 ± 12.0</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Bone mass (kg)⁶</strong></td>
<td>2.8 ± 0.4</td>
<td>2.6 ± 0.7</td>
<td>2.6 ± 0.4</td>
<td>2.7 ± 0.6</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Blood pressure (mm Hg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>125 ± 7</td>
<td>127 ± 16</td>
<td>134 ± 15</td>
<td>125 ± 12</td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic</td>
<td>73 ± 10</td>
<td>76 ± 9</td>
<td>81 ± 8</td>
<td>74 ± 9</td>
<td>0.2</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>25.7 ± 22.1</td>
<td>38.0 ± 20.8</td>
<td>43.4 ± 28.7</td>
<td>36.31 ± 26.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.9 ± 1.0</td>
<td>5.3 ± 1.0</td>
<td>5.2 ± 0.7</td>
<td>5.2 ± 0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.1 ± 0.3</td>
<td>1.7 ± 0.9</td>
<td>1.7 ± 0.6</td>
<td>1.7 ± 0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.4 ± 0.6</td>
<td>5.4 ± 0.8</td>
<td>5.5 ± 0.5</td>
<td>5.3 ± 0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/L)</td>
<td>54.3 ± 26.7</td>
<td>92.6 ± 74.9</td>
<td>79.0 ± 30.0</td>
<td>80.6 ± 58.0</td>
<td>0.4</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

1 Forty-seven volunteers were randomly assigned to the 4 test groups, who drank 1 L of regular cola, semiskim milk, diet cola, or water daily for 6 mo. AU, arbitrary units; SAAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue.

2 Reflects an overall comparison of the 4 groups by ANOVA.

3 Unadjusted mean ± SEM (all such values).

⁴ Determined by magnetic resonance spectroscopy.

⁵ Determined by magnetic resonance imaging.

⁶ Determined by dual-energy X-ray absorptiometry.
Effects on body weight and body composition

No significant differences in the changes in body weight or total fat mass (determined by DXA) were found between the 4 groups during the intervention (Table 3). Total fat mass, however, showed a tendency to increase from baseline in the regular cola group at 6-mo follow-up (1.25 kg; 95% CI: -0.4, 2.7; P = 0.1). The relative amount of SAAT between the 4 groups tended to be different (P = 0.07, ANOVA), but no significant differences were identified in the post hoc pairwise analysis (after Bonferroni correction); however, both of the energy-containing beverages (regular cola and milk) resulted in an increase in the amount of SAAT during the intervention, whereas the noncaloric beverages (diet cola and water) resulted in a reduction in SAAT (Table 3). The overall effect on lean body mass was not significantly different between the 4 beverages, but tended to increase from baseline to the 6-mo follow-up within the milk group (798 g; 95% CI: -61, 657; P = 0.07). A nonsignificant tendency for a difference in the changes in bone mass between the 4 groups was found (P = 0.1, ANOVA), with a positive change in the milk group and negative changes in the 3 other groups after the intervention (Table 3).

Effects on blood pressure and other metabolic factors

Overall, a significant difference was found in mean relative changes in systolic blood pressure (P = 0.01, ANOVA) and a nearly significance difference in diastolic blood pressure (P = 0.06, ANOVA) between the 4 beverage groups (Figure 2). This finding appeared to be mainly due to the fact that milk (and diet cola) reduced blood pressure. Within the milk group, systolic and diastolic blood pressures decreased: 5.7 mm Hg (95% CI: -11, -0.9; P = 0.03) and 5.1 mm Hg (95% CI: -10, -0.3; P = 0.04), respectively, when compared with baseline. The reduction in blood pressure in the diet cola group was not significantly different from baseline (P = 0.1). Compared with the regular cola group, the mean relative change in systolic blood pressure was reduced by 10–15% in the milk and diet cola groups (P < 0.05; Figure 2). Similar changes were found concerning diastolic blood pressure, which, however, were not statistically significant in pairwise comparison with regular cola: P = 0.09 (milk) and P = 0.13 (diet cola) (Figure 2). The changes in blood pressure in the regular cola group were not significantly different from those in the water group.

The overall adjusted mean relative change from baseline in triglyceride concentrations was an increase of 32% and an increase of 11% in total cholesterol in the regular cola group, which was a significant increment as compared with the other groups (P < 0.01; Table 3). No statistically significant differences in lipids were found between the 3 other groups. The effect on HDL cholesterol was similar between the 4 groups (Table 3). No significant differences in the effects on glucose, insulin, and HOMA-IR were found between the 4 groups (Table 3).

From the questionnaires completed during the intervention, no significant differences between the 4 groups were found in relative changes in physical activity (P = 0.4) or total energy intake (P = 0.3), either at baseline or during the intervention (data not shown). Moreover, no changes in macronutrient composition were found between the 4 groups during the intervention, except due to the beverages under investigation (data not shown).

DISCUSSION

The current study showed that the relative changes in liver fat, skeletal muscle fat, and VAT were higher after daily intake of 1 L of an SSSD (eg, regular cola) for 6 mo than after daily intake of 1
L of isocaloric semiskim milk, diet cola (0 calories), or water. No differences in the relative change in ectopic fat accumulation were found between the milk, diet cola, and water groups. Furthermore, the relative changes in circulating concentrations of total cholesterol and triglyceride were higher in the regular cola group than in the 3 other groups. However, the relative change in body weight and total fat mass did not differ between the 4 test beverage groups. It is possible that the lack of statistical significance in body weight and fat mass was due to the relatively small number of subjects in the study, which limited the statistical power. Thus, on the basis of the data from this study, we cannot completely exclude the possibility that daily intake of regular cola plays a role in body weight gain or the development of obesity.

On the basis of the dietary questionnaire, the total energy intake was not different between the 4 groups during the study, which indicated that the consumption of energy-containing beverages (regular cola and milk) in the current study was generally compensated for by reducing the energy intake from other sources. For example, if there was no energy compensation for drinking 1 L of regular cola, the weight gain would have been 7–8 kg during the 6-mo period; however, it was 1.25 kg. Moreover, the specific effects observed after intake of the SSSDs (regular cola) in the current study may have been related more to the nutritional composition of the beverages than to the total energy intake (see below).

Because the large relative changes in VAT and liver fat after drinking regular cola will only result in smaller absolute changes in total fat mass, the increment in ectopic fat found in the current study could well be accounted for by the observed absolute changes in body weight and total fat mass.

Previous observational studies have also found that sucrose-sweetened beverages are associated with ectopic fat accumulation, particularly in the liver (20, 21), and elevated triglycerides and high blood pressure have been found to be associated with a high intake of SSSDs (5, 22, 23). Most negative health effects of sucrose-sweetened beverages have been linked to the content of fast-absorbable fructose (2, 5, 20, 21, 24). Fructose may directly enhance de novo lipogenesis, triglyceride production, and fat accumulation in the liver, and may subsequently increase circulating triglyceride and cholesterol concentrations (1, 25, 26). Moreover, regular cola intake resulted in enhanced fat accumulation in muscle and in VAT, which may have been related to the enhanced production of plasma triglycerides, which then may

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**TABLE 3**

Changes from baseline to 6 mo in fatness, body composition, and metabolic risk factors

<table>
<thead>
<tr>
<th></th>
<th>Regular cola</th>
<th>Milk</th>
<th>Diet cola</th>
<th>Water</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent change (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>1.28 ± 1.1</td>
<td>1.36</td>
<td>0.114</td>
<td>0.576</td>
<td>0.8</td>
</tr>
<tr>
<td>Total fat mass</td>
<td>3.14 ± 2.7</td>
<td>1.42</td>
<td>-0.52</td>
<td>0.490</td>
<td>0.8</td>
</tr>
<tr>
<td>VAT/SAAT</td>
<td>18.1 ± 6.0</td>
<td>-12.5</td>
<td>4.59</td>
<td>3.90</td>
<td>0.013</td>
</tr>
<tr>
<td>Lean mass</td>
<td>0.423 ± 0.8</td>
<td>1.43</td>
<td>0.951</td>
<td>-0.189</td>
<td>0.5</td>
</tr>
<tr>
<td>Bone mass</td>
<td>-1.39 ± 0.6</td>
<td>0.571</td>
<td>-0.926</td>
<td>-0.909</td>
<td>0.1</td>
</tr>
<tr>
<td>Leptin</td>
<td>25.2 ± 9.7</td>
<td>9.11</td>
<td>-2.20</td>
<td>3.72</td>
<td>0.2</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>11.4 ± 3.2</td>
<td>0.634</td>
<td>-5.89</td>
<td>-0.159</td>
<td>0.004</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>32.7 ± 8.6</td>
<td>-0.301</td>
<td>-14.1</td>
<td>-14.2</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>6.24 ± 4.1</td>
<td>7.93</td>
<td>1.03</td>
<td>6.38</td>
<td>0.6</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>3.4 ± 3.4</td>
<td>5.7</td>
<td>-1.0</td>
<td>2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Fasting plasma insulin</td>
<td>17.7 ± 25.4</td>
<td>33.1</td>
<td>-5.0</td>
<td>13.7</td>
<td>0.7</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>21.6 ± 18.4</td>
<td>5.0</td>
<td>-0.4</td>
<td>16.8</td>
<td>0.8</td>
</tr>
</tbody>
</table>

1 Forty-seven volunteers drank 1 L/d of 1 of the 4 test drinks for 6 mo: regular cola (n = 10), milk (n = 12), diet cola (n = 12), and water (n = 13). SAAT, subcutaneous abdominal adipose tissue; VAT, visceral adipose tissue.
2 Reflects an overall comparison of the 4 groups by ANOVA. A post hoc pairwise comparison of all groups was also performed (with Bonferroni correction).
3 Significant difference between regular cola and milk (P < 0.01); 4 significant difference between regular cola and diet cola (P < 0.01), water (P = 0.05), and milk (P = 0.11); 5 significant difference between regular cola and milk (P = 0.05), diet cola (P < 0.01), and water (P < 0.01).
6 Mean ± SEM values are relative changes adjusted for sex and baseline levels (all such values).
have been taken up by these tissues. Thus, these effects may link
the daily intake of regular cola to the development of the me-
tabolic syndrome, diabetes, and cardiovascular diseases (26–28)
and may play a role in the increased prevalence of nonalcoholic
fatty liver diseases (29, 30).

Semiskim milk was found to have neutral effects, as compared
with water, on ectopic fat accumulations and on circulating
concentrations of lipids. The lack of effect of milk, as compared
with isocaloric regular cola, on ectopic fat accumulation may
have been mainly due to the fact that milk contains no fructose
in its carbohydrate fraction. Moreover, the content of proteins/
amingo acids and lipids in milk may have specific effects on
appetite and metabolism, which may also have influenced the
current results (9). On the other hand, the discussion that milk
(and dairy products) may prevent weight gain or induce weight
loss in obese individuals (31) was not supported by the current
intervention, in which no differences in body weight or total fat
mass were found between the 4 beverage groups. Furthermore,
milk significantly decreased both diastolic and systolic blood
pressure by 5–6 mm Hg compared with baseline and in a relative
decrease in blood pressure of 10% to 15% compared with the
regular cola group, and in a 7% decrease in systolic blood
pressure compared with the water group. These findings agree
with those of previous studies, in which milk intake was shown
to be associated with lower blood pressure, which may have been
due to the content of various proteins (eg, lactotripeptides) in
milk (8, 32). These effects of the daily intake of milk are likely to
help prevent cardiovascular diseases.

In the current study, we found no effects of regular cola on
bone mass; however, negative effects of regular cola on bone
mass have repeatedly been discussed, and some observational
(33) and short-term intervention studies (34) have found cola
intake to be negatively associated with bone mass.

The suggestions from observational studies that intake of diet
cola can result in obesity, type 2 diabetes, and the metabolic
syndrome (5, 13) are not supported by the findings of our current
intervention, ie, that the effect of diet cola on fatness, ectopic fat,
and metabolic factors is mainly neutral and very similar to that of
water. Also, diet cola was found to have effects nearly similar to
those of milk in reducing blood pressure. This finding agrees with
those of a few earlier publications, which have shown that as-
partame may reduce blood pressure in hypertensive rats by af-
flecting the brain content of tyrosine (35, 36). This possible effect
of aspartame should indeed be investigated further. Thus, the
current findings concerning the effects of artificially sweetened
beverages agree with those of a very recent observational study, in
which the positive association between artificially sweetened soft
drinks and the development of type 2 diabetes disappeared after
adjustment for various anthropometric and lifestyle factors (37),
which indicated reverse causation between artificially sweetened
beverages and obesity and type 2 diabetes. Thus, individuals with
weight problems may drink more diet products.

The main limitation of this study was the small number of
participants, which limited the power of our statistical analyses.
Moreover, the study was unblinded, which may have affected the
subjects’ behavior to counteract some of the expected effects of
the beverages, such as weight gain in the energy-containing
beverage groups. Consequently, the possibility of finding sig-
ificant differences between the 4 beverage groups was reduced,
but our current conclusions were not affected. Our study also
raised other questions, particularly in relation to the effect of
other fructose-containing foods and beverages on ectopic fat
accumulation; in this context, the effect of fruit juice is of interest.
Some observational studies indicate that the intake of fruit juice
is associated with weight gain and type 2 diabetes (38, 39). Gen-
ernally, besides fructose, fruit juice also contains micronutrients,
such as antioxidants and polyphenols, which may counteract
some of the negative health effects of sucrose and fructose on
inflammation and metabolism. Thus, it may not be possible to
directly translate the current findings obtained with SSSDs to
fruit juice; therefore, the effects of various fruit juices have to be
tested directly.

Consumption of 1 L of test drink daily should be compared
with the average daily intake of soft drink in the United States,
which is 1.8 L among young American men (ages 12–29 y) and
0.5 L among all Americans. In fact SSSDs contribute 150 kcal to
the average daily American diet (40, 41). In the United States,
SSSDs often contain HFCS. HFCS is made by an industrial
enzymatic isomerization of glucose to fructose and contains
~55% fructose (26). HFCS is likely to result in similar or even
further negative health effects as compared with sucrose-
sweetened beverages, which were tested in the current study.

There are several alternatives to regular cola, in which water is
the primary natural choice. Moreover, milk is a much healthier
alternative, as was also shown in the current study. In our in-
tervention, diet cola behaves much like water and does not in-
crease fat mass or ectopic fat accumulation. There may, however,
still be other health concerns regarding aspartame-sweetened
cola that were not evaluated in the current study (42–44).

In a recent study, we investigated the acute effect (over 4 h) of
the same 4 beverages used in the current study on appetite and
appetite-regulating hormones and found that milk induces more
satiation than does regular cola (45), which indicates that drinking
regular cola would increase the risk of positive energy balance as
compared with milk. However, the current long-term intervention
does not support the conclusion from the acute investigation,
which indicates that extrapolating observations from acute
studies on appetite regulation to long-term effects on body weight
may be very misleading.

In conclusion we found that daily intake of regular cola
increases ectopic fat accumulation and concentrations of tri-
glycerides and total cholesterol compared with other drinks. To
improve the health of the population, the intake of SSSDs should
be reduced considerably.

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interpreting the data, and writing the manuscript; AB: helped plan the study
design, conducted a portion of the clinical intervention, and edited the man-
uscript; HS-J, SR, EC, and HT: conducted the MRI and MR spectroscopy;
SBP and AA: took part in the funding, design, and editing of the final man-
uscript; BR: designed the study and took part in funding the study, interpreting
the data, and writing the manuscript; BR: had primary responsibility for the
final content. All authors read and approved the final manuscript. None of the
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