

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

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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 ¹H-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 VAT⁴, 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/m²) 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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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 same laboratory and were analyzed in one batch. Blood pressure was recorded after a 10-min rest with a digital blood pressure apparatus (Colin Press-Mate). Insulin sensitivity was calculated from the HOMA index (15).

Intrahepatic fat, intramyocellular fat, VAT, and SAAT were measured with MR techniques by using a Signa Excite 1.5 tesla

twin-speed scanner (GE Medical Systems). MR spectroscopy of the skeletal muscle included a point-resolved spectroscopy (water suppression, echo time 27 ms, repetition time 3000 ms) on a 1.5 cm × 2 cm × 2 cm voxel positioned in the largest cross-sectional area of the tibialis anterior muscle. Full width at half maximum was 13.4 ± 2.2 Hz. The ¹H-MR liver spectroscopy technique was previously described (16). Full width at half maximum was 13.7 ± 3.3 Hz. The spectra were quantified by using the LCmodel software package (version 6.2; Stephen Provencher) by using either a dedicated muscle- or liver-spectroscopy fitting model. The data processing provided an estimate of the ratio of lipid to water in the tissue within the voxel (17). VAT and SAAT were quantified by MRI (body coil; fast-spin echo sequence; TE 8.5 ms; TR 600; slice thickness: 8 mm; field of view: 48 cm). Based on an axial slice at the top of the L3 vertebra, data processing was done by using the software package Hippofat (18). Recently, we showed that the amount of VAT quantified from one slice at the L3 level provides results similar to those obtained with a multislice protocol (19). A minority of the subjects were scanned at Copenhagen University (Herlev MR Center) following a slightly different protocol with the use of an Achieva 3.0 T MR imaging system (Philips Medical Systems) and a sense cardiac coil, but each individual was investigated by using the same scanner before and after the intervention. Total fat mass, lean body mass, and bone mass were determined by DXA (Hologic 2000/W QDR scanner; Hologic Inc). The DXA output was generated with the default settings of the Hologic software version 12.6.1.

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 drinks¹

	Sucrose-sweetened regular cola	Milk	Aspartame-sweetened diet cola	Water
Carbohydrate (g/100 mL)	10.6	4.7	0	0
Protein (g/100 mL)	0	3.4	<0.1	0
Fat (g/100 mL)	0	1.5	0	0
Energy (kJ/d)	1800	1900	15	0
Volume (mL)	1000	1000	1000	1000
Energy density (kJ/g)	1.8	1.9	0.015	0

¹ 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 \pm SDs. The results are presented as means \pm 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 ($P \leq 0.1$) between the groups. Accordingly, the results were analyzed as sex- and baseline-adjusted relative changes. Sixty individuals were randomly assigned to the intervention. Thirteen 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 221%; 95% CI: 44, 399; $P = 0.09$) and than in the diet cola group (by 227%; 95% CI: 50, 405; $P = 0.08$; **Figure 1C**). 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 subjects¹

	Regular cola	Milk	Diet cola	Water	<i>P</i> value ²
Sex (<i>n</i>)					
Male	6	3	3	5	
Female	4	9	9	8	
Age (y)	39 \pm 6 ³	38 \pm 9	39 \pm 8	39 \pm 8	1.0
Body weight (kg)	97.8 \pm 12.5	94.7 \pm 15.3	92.2 \pm 10.9	101.7 \pm 22.4	0.5
BMI	31.3 \pm 2.9	31.9 \pm 2.8	32.8 \pm 3.8	32.2 \pm 4.6	0.8
Liver fat (AU) ⁴	0.037 \pm 0.04	0.127 \pm 0.1	0.21 \pm 0.2	0.093 \pm 0.09	0.10
Skeletal muscle fat (AU) ⁴	0.002 \pm 0.001	0.004 \pm 0.002	0.002 \pm 0.001	0.002 \pm 0.001	0.09
VAT (cm ³) ⁵	110.4 \pm 52.1	99.9 \pm 36.0	135.2 \pm 56.5	77.4 \pm 40.1	0.07
SAAT (cm ³) ⁵	281.5 \pm 70.8	345.5 \pm 91.7	298.8 \pm 106.3	278.9 \pm 112.2	0.4
Total fat mass (kg) ⁶	34.0 \pm 8.3	35.4 \pm 6.4	35.1 \pm 9.0	37.2 \pm 11.6	0.9
Lean body mass (kg) ⁶	61.5 \pm 11.5	56.8 \pm 14.1	54.9 \pm 11.1	57.5 \pm 12.0	0.7
Bone mass (kg) ⁶	2.8 \pm 0.4	2.6 \pm 0.7	2.6 \pm 0.4	2.7 \pm 0.6	0.6
Blood pressure (mm Hg)					
Systolic	125 \pm 7	127 \pm 16	134 \pm 15	125 \pm 12	0.3
Diastolic	73 \pm 10	76 \pm 9	81 \pm 8	74 \pm 9	0.2
Leptin (ng/mL)	25.7 \pm 22.1	38.0 \pm 20.8	43.4 \pm 28.7	36.31 \pm 26.5	0.4
Total cholesterol (mmol/L)	4.9 \pm 1.0	5.3 \pm 1.0	5.2 \pm 0.7	5.2 \pm 0.8	0.7
Triglycerides (mmol/L)	1.1 \pm 0.3	1.7 \pm 0.9	1.7 \pm 0.6	1.7 \pm 0.8	0.2
HDL cholesterol (mmol/L)	1.2 \pm 0.2	1.1 \pm 0.2	1.2 \pm 0.3	1.1 \pm 0.3	0.9
Fasting plasma glucose (mmol/L)	5.4 \pm 0.6	5.4 \pm 0.8	5.5 \pm 0.5	5.3 \pm 0.6	0.9
Fasting plasma insulin (pmol/L)	54.3 \pm 26.7	92.6 \pm 74.9	79.0 \pm 30.0	80.6 \pm 58.0	0.4
HOMA-IR	1.1 \pm 0.3	1.5 \pm 0.3	1.9 \pm 0.3	1.5 \pm 0.3	0.4

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 \pm SEM (all such values).

⁴ Determined by magnetic resonance spectroscopy.

⁵ Determined by magnetic resonance imaging.

⁶ Determined by dual-energy X-ray absorptiometry.

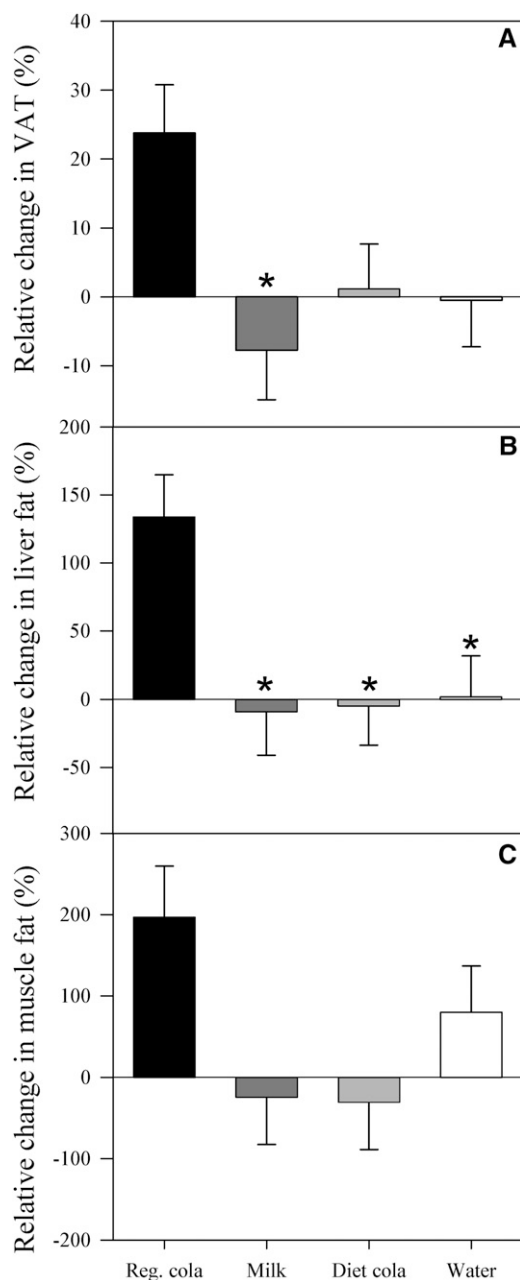


FIGURE 1. Mean (\pm SEM) relative changes in ectopic fat accumulation in VAT, liver, and skeletal muscle. Forty-seven volunteers drank 1 L of 1 of 4 test drinks daily for 6 mo: regular cola ($n = 10$), milk ($n = 12$), diet cola ($n = 12$), or water ($n = 13$). A: VAT was measured by magnetic resonance (MR) imaging. Liver fat (B) and skeletal muscle fat (C) were determined by ^1H -MR-spectroscopy. Data are sex-adjusted mean relative changes from baseline to 6 mo and were compared with a general linear model of univariate ANOVA; the overall P values adjusted for sex were $P = 0.03$ for VAT, $P = 0.01$ for liver, and $P = 0.048$ for muscle, with post hoc pairwise comparison between the 4 test drinks (corrected for multiple comparisons by Bonferroni procedure). The P values in the figure are expressed as pairwise comparisons with regular cola: * $P < 0.05$. Reg., regular; VAT, visceral adipose tissue.

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 significant 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

TABLE 3

Changes from baseline to 6 mo in fatness, body composition, and metabolic risk factors¹

	Regular cola	Milk	Diet cola	Water	<i>P</i> value ²
	%	%	%	%	
Body weight	1.28 ± 1.1 ³	1.36 ± 1.1	0.114 ± 1.1	0.576 ± 1.0	0.8
Total fat mass	3.14 ± 2.7	1.42 ± 2.5	-0.52 ± 2.5	0.490 ± 2.6	0.8
SAAT	4.98 ± 2.8	3.10 ± 2.9	-2.79 ± 2.7	-4.30 ± 2.7	0.07
VAT/SAAT	18.1 ± 6.0	-12.5 ± 6.1	4.59 ± 5.5	3.90 ± 5.7	0.013 ^a
Lean mass	0.423 ± 0.8	1.43 ± 0.8	0.951 ± 0.8	-0.189 ± 0.8	0.5
Bone mass	-1.39 ± 0.6	0.571 ± 0.6	-0.926 ± 0.6	-0.909 ± 0.6	0.1
Leptin	25.2 ± 9.7	9.11 ± 8.6	-2.20 ± 8.6	3.72 ± 8.2	0.2
Total cholesterol	11.4 ± 3.2	0.634 ± 3.0	-5.89 ± 3.0	-0.159 ± 2.8	0.004 ^b
Triglycerides	32.7 ± 8.6	-0.301 ± 8.1	-14.1 ± 8.1	-14.2 ± 7.7	0.001 ^c
HDL cholesterol	6.24 ± 4.1	7.93 ± 3.9	1.03 ± 3.9	6.38 ± 3.7	0.6
Fasting plasma glucose	3.4 ± 3.4	5.7 ± 3.2	-1.0 ± 3.2	2.3 ± 3.2	0.5
Fasting plasma insulin	17.7 ± 25.4	33.1 ± 24.0	-5.0 ± 24.0	13.7 ± 23.5	0.7
HOMA-IR	21.6 ± 18.4	5.0 ± 16.2	-0.4 ± 15.7	16.8 ± 15.3	0.8

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): ^aSignificant difference between regular cola and milk (*P* < 0.01); ^bsignificant difference between regular cola and diet cola (*P* < 0.01), water (*P* = 0.05), and milk (*P* = 0.11); ^csignificant difference between regular cola and milk (*P* = 0.05), diet cola (*P* < 0.01), and water (*P* < 0.01).

³ Mean ± SEM values are relative changes adjusted for sex and baseline levels (all such values).

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

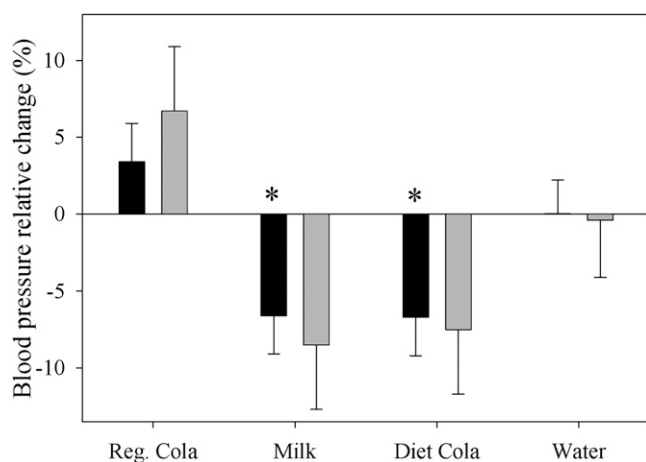


FIGURE 2. Mean (±SEM) relative changes in blood pressure. Forty-seven volunteers drank 1 L of 1 of 4 test drinks daily for 6 mo: regular cola (*n* = 10), milk (*n* = 12), diet cola (*n* = 12), or water (*n* = 13). Data are sex-adjusted mean relative changes from baseline to 6 mo. Black bars represent changes in systolic blood pressure, and gray bars represent changes in diastolic blood pressure. The data were compared with a general linear model of univariate ANOVA; the overall *P* values adjusted for sex were *P* = 0.01 for systolic blood pressure and *P* = 0.057 for diastolic blood pressure, with post hoc pairwise comparison between the 4 test drinks (Bonferroni corrected). The *P* values are expressed as pairwise comparisons with regular cola: **P* < 0.05. Reg., regular.

have been taken up by these tissues. Thus, these effects may link the daily intake of regular cola to the development of the metabolic 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/amino 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 aspartame may reduce blood pressure in hypertensive rats by affecting 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 significant 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). Generally, 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 intervention, diet cola behaves much like water and does not increase 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 satiety 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 triglycerides 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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REFERENCES

1. Nseir W, Nassar F, Assy N. Soft drinks consumption and nonalcoholic fatty liver disease. *World J Gastroenterol* 2010;16:2579–88.

2. Malik VS, Popkin BM, Bray GA, Despres J, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* 2010;121:1356–64.
3. Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC, Hu FB. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *JAMA* 2004;292:927–34.
4. Palmer JR, Boggs DA, Krishnan S, Hu FB, Singer M, Rosenberg L. Sugar-sweetened beverages and incidence of type 2 diabetes mellitus in African American women. *Arch Intern Med* 2008;168:1487–92.
5. Dhingra R, Sullivan L, Jacques PF, et al. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. *Circulation* 2007;116:480–8.
6. Gaby AR. Adverse effects of dietary fructose. *Altern Med Rev* 2005;10:294–306.
7. Jauhiainen T, Korpela R. Milk peptides and blood pressure. *J Nutr* 2007;137:825S–9S.
8. Xu JY, Qin LQ, Wang PY, Li W, Chang C. Effect of milk tripeptides on blood pressure: a meta-analysis of randomized controlled trials. *Nutrition* 2008;24:933–40.
9. Pfeuffer M, Schrezenmeir J. Milk and the metabolic syndrome. *Obes Rev* 2007;8:109–18.
10. Barba G, Russo P. Dairy foods, dietary calcium and obesity: a short review of the evidence. *Nutr Metab Cardiovasc Dis* 2006;16:445–51.
11. Raben A, Vasilaras TH, Moller AC, Astrup A. Sucrose compared with artificial sweeteners: different effects on ad libitum food intake and body weight after 10 wk of supplementation in overweight subjects. *Am J Clin Nutr* 2002;76:721–9.
12. Tordoff MG, Alleva AM. Effect of drinking soda sweetened with aspartame or high-fructose corn syrup on food intake and body weight. *Am J Clin Nutr* 1990;51:963–9.
13. Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. Fueling the obesity epidemic[quest] artificially sweetened beverage use and long-term weight gain. *Obesity (Silver Spring)* 2008;16:1894–900.
14. Baecke JA, Burema J, Frijters J. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 1982;36:936–42.
15. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998;21:2191–2.
16. Moller L, Stodkilde-Jorgensen H, Jensen FT, Jorgensen JO. Fasting in healthy subjects is associated with intrahepatic accumulation of lipids as assessed by ¹H-magnetic resonance spectroscopy. *Clin Sci (Lond)* 2008;114:547–52.
17. Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* 1993;30:672–9.
18. Positano V, Gastaldelli A, Sironi AM, Santarelli MF, Lombardi M, Landini L. An accurate and robust method for unsupervised assessment of abdominal fat by MRI. *J Magn Reson Imaging* 2004;20:684–9.
19. Christiansen T, Paulsen SK, Bruun JM, Overgaard K, Ringgaard S, Pedersen SB, Positano V, Richelsen B. Comparable reduction of the visceral adipose tissue depot after a diet-induced weight loss with or without aerobic exercise in obese subjects: a 12-week randomized intervention study. *Eur J Endocrinol* 2009;160:759–67.
20. Assy N, Nasser G, Kamayse I, Nseir W, Beniashvili Z, Djibre A, Grosovski M. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. *Can J Gastroenterol* 2008;22:811–6.
21. Abid A, Taha O, Nseir W, Farah R, Grosovski M, Assy N. Soft drink consumption is associated with fatty liver disease independent of metabolic syndrome. *J Hepatol* 2009;51:918–24.
22. Havel PJ. Dietary fructose: Implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev* 2005;63:133–7.
23. Lê KA, Ith M, Kreis R, Faeh D, Bortolotti M, Tran C, Boesch C, Tappy L. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. *Am J Clin Nutr* 2009;89:1760–5.
24. Teff KL, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH, Keim NL, Cummings BP, Stanhope KL, Havel PJ. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metab* 2009;94:1562–9.
25. Collison KS, Saleh SM, Bakheet RH, Al-Rabiah RK, Inglis AL, Makhoul NJ, Maqbool ZM, Zaidi MZ, Al-Johi MA, Al-Mohanna FA. Diabetes of the liver: the link between nonalcoholic fatty liver disease and HFCS-55. *Obesity (Silver Spring)* 2009;17:2003–13.
26. Ferder L, Ferder MD, Inserra F. The role of high-fructose corn syrup in metabolic syndrome and hypertension. *Curr Hypertens Rep* 2010;12:105–12.
27. Malik VS, Popkin BM, Bray GA, Despres JP, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. *Diabetes Care* 2010;33:2477–83.
28. Brown CM, Dulloo AG, Montani JP. Sugary drinks in the pathogenesis of obesity and cardiovascular diseases. *Int J Obes (Lond)* 2008;32 (suppl 6):S28–34.
29. Cave M, Deaciuc I, Mendez C, Song Z, Joshi-Barve S, Barve S, McClain C. Nonalcoholic fatty liver disease: predisposing factors and the role of nutrition. *J Nutr Biochem* 2007;18:184–95.
30. Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterol Hepatol* 2010;7:251–64.
31. Louie JC, Flood VM, Hector DJ, Rangan AM, Gill TP. Dairy consumption and overweight and obesity: a systematic review of prospective cohort studies. *Obes Rev* 2011;12:e582–92.
32. Boelsma E, Kloek J. Lactotripeptides and antihypertensive effects: a critical review. *Br J Nutr* 2009;101:776–86.
33. Tucker KL, Morita K, Qiao N, Hannan MT, Cupples LA, Kiel DP. Colas, but not other carbonated beverages, are associated with low bone mineral density in older women: the Framingham Osteoporosis Study. *Am J Clin Nutr* 2006;84:936–42.
34. Kristensen M, Jensen M, Kudsk J, Henriksen M, Molgaard C. Short-term effects on bone turnover of replacing milk with cola beverages: a 10-day interventional study in young men. *Osteoporos Int* 2005;16:1803–8.
35. Maher TJ, Wurtman RJ. High doses of aspartame reduce blood pressure in spontaneously hypertensive rats. *N Engl J Med* 1983;309:1125.
36. Kiritsy PJ, Maher TJ. Acute effects of aspartame on systolic blood pressure in spontaneously hypertensive rats. *J Neural Transm* 1986;66:121–8.
37. de Koning L, Malik VS, Rimm EB, Willett WC, Hu FB. Sugar-sweetened and artificially sweetened beverage consumption and risk of type 2 diabetes in men. *Am J Clin Nutr* 2011;93:1321–7.
38. Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. *Diabetes Care* 2008;31:1311–7.
39. Odegaard AO, Koh WP, Arakawa K, Yu MC, Pereira MA. Soft drink and juice consumption and risk of physician-diagnosed incident type 2 diabetes: the Singapore Chinese Health Study. *Am J Epidemiol* 2010;171:701–8.
40. Nielsen SJ, Popkin BM. Changes in beverage intake between 1977 and 2001. *Am J Prev Med* 2004;27:205–10.
41. Bray GA. Soft drink consumption and obesity: it is all about fructose. *Curr Opin Lipidol* 2010;21:51–7.
42. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, Peppas M, Rayfield EJ. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci USA* 2002;99:15596–601.
43. Humphries P, Pretorius E, Naude H. Direct and indirect cellular effects of aspartame on the brain. *Eur J Clin Nutr* 2008;62:451–62.
44. Halldorsson TI, Strom M, Petersen SB, Olsen SF. Intake of artificially sweetened soft drinks and risk of preterm delivery: a prospective cohort study in 59,334 Danish pregnant women. *Am J Clin Nutr* 2010;92:626–33.
45. Maersk M, Belza A, Holst JJ, Fenger-Groen M, Pedersen SB, Astrup A, Richelsen B. Impaired satiety and satiety hormone response after sucrose-sweetened soft drink compared with isocaloric semi-skimmed milk: controlled trial. *Eur J Clin Nutr* (in press).