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Full Length Research Paper

Does an acid load promote liver desaturases and increase serum lipids?

Arne Torbjørn Høstmark[1]*, Marianne Sylvana Haug Lunde[1] and Einar Eilertsen[2]

[1]Section of Preventive Medicine and Epidemiology, University of Oslo, Norway, Box 1130 Blindern, 0318 Oslo, Norway. [2]National Institute of Occupational Health, Department of Administration, Lab. Animal Unit Box 8149 Dep, N-0033 Oslo, Norway.

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Sugar sweetened and acid containing soft drinks may influence the serum lipids. We raised the question whether intake of acid beverages in general might influence serum and liver triglycerides, and hepatic desaturases, which govern triglyceride synthesis. Thirty male rats were divided into 6 groups and given the same food, but various beverages: sucrose-cola, cola light, phosphoric acid, acetic acid or water. Serum triglycerides and HDL, liver triglycerides and the fatty acid distribution in liver lipids were determined. Liver desaturase indexes were calculated; Delta9-desaturase by the palmitoleic to palmitic acid (and oleic to stearic acid) ratios, and Delta5- desaturase by the arachidonic to linoleic acid ratio. Correlation, ANOVA and Mann Whitney tests were used to study associations and differences. After 6 weeks the sugar-cola group had lower food intake, higher fluid intake and urinary acid excretion, higher hepatic desaturase indexes and serum triglycerides, and lower HDL than the other groups. The hepatic desaturase indexes correlated positively with each other ($p < 0.01$), with liver TG (p 0.01), and with 18 h urinary acid excretion ($p < 0.01$). Rats ingesting acid beverages seemed to respond with increased serum triglycerides and lowered HDL cholesterol concentration. Thus, soft drinks might increase the acid load, thereby possibly contributing to increased hepatic desaturase activities and lipoprotein formation.

Key words: Colas, acid load, sucrose, fatty acids, desaturase indexes, liver, serum, rat.

INTRODUCTION

Intake of sucrose sweetened and acid soft drinks may give an acid load, caused not only by the preformed acid in the drinks, but also by the sugar content. Previous studies have shown that the fructose moiety of sucrose can increase the urinary excretion of uric acid (Taylor and Curhan, 2008). Furthermore, sucrose may increase the serum triglyceride concentration and lower serum high density lipoprotein cholesterol (HDL) concentration (Høstmark and Blom, 1985; Archer et al., 1998). Soft drink intake has been shown to correlate positively with serum triglycerides and negatively with HDL (Dhingra et al., 2007; Merchant et al., 2007). Colas contain phosphoric acid (Jensdottir et al., 2006; Kapicloglu et al., 2000).

In a cross sectional epidemiological study we recently found a positive association between intake of colas and serum triglycerides, irrespective of the presence or absence of sugar (Høstmark et al., 2009). This observation raised the question of whether the acid content of colas might partly be causally implicated in the serum lipid effect. One main objective of the present work was to study this possibility experimentally, using a diet trial in rats.

The serum triglyceride concentration in fasted animals is mainly carried in very low density lipoproteins (VLDL), which are synthesized and secreted in the liver (Murray et al., 2000). VLDL-triglycerides, cholesterol esters and phospholipids preferably contain monounsaturated fatty acids, that is, palmitoleic (C16:1 n-7) and oleic (C18:1 n-6) acid (Nakamura and Nara, 2004). The rate limiting enzyme for the synthesis of these fatty acids is stearoyl-CoA desaturase (SCD). Mice lacking SCD have reduced hepatic lipogenesis and lower plasma triglyceride concentration (Dobrzyn and Ntambi, 2005a). Accordingly, one mechanism by which cola intake might increase

*Corresponding author. E-mail: a.t.Høstmark@medisin.uio.no. Tel: +47 22 85 82 84. Fax: +47 22 85 82 80.

serum triglycerides could be stimulation of desaturase activities in the liver, caused by some of the components in cola, such as sucrose and/ or phosphoric acid. Sucrose feeding of rats was previously found to increase hepatic Delta9, Delta6 and Delta5 desaturase activities (Brenner et al., 2003). Our hypothesis was that an acid load might possibly contribute to enhance hepatic desaturase activities and lipid synthesis. We accordingly have measured liver and serum triglycerides, urinary acid excretion and indexes estimating hepatic delta9-desaturase, that is, the ratios (16:1n-7)/(16:0) referred to as ds9_1, and (18:1n-9)/(18:0) referred to as ds9_2, and (20:4n-6)/(18:2n-6) estimating delta5 desaturase (ds5), in rats given beverages containing phosphoric acid (colas, with and without sugar and phosphoric acid dissolved in water), acetic acid, or water.

MATERIALS AND METHODS

This diet trial was conducted in accordance with the laws and regulations controlling experiments/ procedures in live animals in Norway, that is, the Animal Welfare Act of 20 December, 1974 No 73, chapter VI, sections 20 - 22 and the regulation on Animal Experimentation of 15 January, 1996. Norway has signed and ratified by The European Convention for the protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 18 March, 1986. The Norwegian legislation conforms in all respects with the basic requirements of this Convention and guidelines prepared in pursuance of it.

Feeding

Thirty male rats (Mol: Wist, L1, Skensved, Denmark) were kept in-house for a one week acclimatisation before the diet trial. Throughout the study period the rats were fed ad libitum maintenance rat pellets RM1 from Special Diets Services, England (2.7% crude fat, 14.4% crude protein, 4.7% crude fibre and 6.0% crude ash). The animals were divided into six groups with five rats in each group and given different beverages (all given ad libitum):

(1) Sucrose-cola (Coca Cola Company, 8022N11838 or 8043N10022 or 8060N10012).
(2) Cola light (Coca cola company, 8032N11018 or 8032N11019 or D062T or D195R) 3) Phosphoric acid (3.1g/L) (ortho-phosphoric acid 85%, Merck, Germany).
(4) Phosphoric acid (6.2g/L) (ortho-phosphoric acid 85%, Merck, Germany).
(5) Acetic acid (1.92 g/L) (Acetic acid 100%, VWR International) and 6) distilled water (control).

The concentrations of phosphoric acid were chosen to resemble levels used in colas. Acetic acid concentration was equimolar to the lowest concentration used for phosphoric acid. Solution 1 - 6 had the following pH: 2.6; 2.6; 2.0; 1.9; 3.0 and 6.5. Data for body weight, intake of food and consumed volumes of the various beverages, volume of excreted urine and amount of faeces were collected at zero time, after three weeks, and after six weeks when ending the diet trial. For practical reasons the rats were kept for 18 h in the individual metabolism cages (3700D000, Scanbur Techniplast).

Blood and tissue sampling

After a 4 - 6 h fast venous blood was collected from the right dorsa-lateral tail vein at zero time, after three weeks and after six weeks (end of study), using heparin-moistened syringes. Blood samples were centrifuged at 1750 x g for 10 min and the supernatant was collected and frozen at -70°C. The animals were killed by an overdose of pentobarbital (10 mg/ml) intraperitoneally. For all animals, a biopsy was taken from the distal part of the median (cystic) lobe. About 1/3 of the lobe was sampled, immediately frozen in liquid nitrogen and then stored at -70°C.

Determination of serum total cholesterol, HDL cholesterol and triglycerides

Serum total cholesterol was determined with Cholesterol CHOP-PAP kit (12016630 122, Roche/Hitachi), and HDL-cholesterol using an enzymatic HDL-Cholesterol kit (Biomed Labordiagnostik GmbH, Germany). Triglycerides in serum was determined using Triglycerides GPO-PAP kit (12016648 122, Roche/Hitachi).

Determination of liver triglycerides

Using ultrasound, for each rat a homogenate was made of 200 mg hepatic tissue in 2 ml of a 5% bovine serum albumin solution in 0.9% NaCl. The homogenate was subsequently centrifuged (1750 x g) for 10 min and the supernatant was collected for measurement of triglycerides (vide supra) and for extraction of fatty acids (vide infra).

Determination of fatty acids

The fatty acid profile was determined in liver total lipids, and in the phospholipids fraction. The lipids were extracted using n-butanol and phospholipids isolated from the lipid extracts using Varian Bond Elut NH2, LCR columns (Varian, Walnut Creek, CA). Diheptadecanoyl-glycerophospho ethanolamine and butylated hydroxytoluene (Sigma Chemical, United Kingdom) were added as internal standard and antioxidant, respectively. Phospholipids were transmethylated and fatty acid methyl esters quantified as mg fatty acid/g tissue, using gas liquid chromatography on a SP2330 column (Supelco Inc., Bellefonte, PA). A normal human serum sample was included to assess analytical performance. The results of the measurements are presented as weight percentage of total fatty acids. When describing fatty acids, e.g. arachidonic acid, C20:4 n-6, the first figure indicates chain length, the second figure is the number of double bonds, and n-6 refers to the position of the first double bond when counting from the terminal methyl group. The following 14 fatty acids in phospholipids were analyzed: Myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1n-7), stearic acid (18:0), oleic acid (18:1n-9), linoleic acid (18:2n-6), linolenic acid (18:3n-3), eicosenoic acid (20:1 n-9), eicosadienoic acid (20:2 n-6), arachidonic acid (20:4 n-6), eicosatrienoic (20:3n-3), EPA (20:5n-3), erucic acid (22:1n-9), DHA (22:6n-3). The day to day coefficient of variation (n=28) for 18:0, 18:1n-9, 18:2 n- 6, 20:4n-6, 20:5n-3 and 22:6n-3 was 5.2, 6.2, 6.6, 9.6, 10.0, and 11.6 %, respectively. The main focus in the present work was on palmitic-, palmitoleic-, stearic-, oleic-, linoleic- and arachidonic acid, serving as components of the desaturase indexes.

Estimates of fatty acid desaturases

To estimate Delta9-desaturase, we used the (16:1 n-7)/ (16:0) and (18:1 n-9)/ (18:0) ratios (referred to as ds9_1 and ds9_2) in total lipids and in phospholipids from rat livers. Delta-5-desaturase (ds5) was estimated by the (20:4 n-6)/ (18:2 n-6) ratio. Corresponding indexes based upon fatty acids in total lipids and in the phospholipids fraction correlated positively: for ds9_1: r=0.966; for

Table 1. Body weight and food intake in rats ingesting various acid beverages.

	Body weight (g)			Food intake (g)	
	Initial[a]	After 3 wk	After 6 wk	After 3 wk	After 6 wk
Cola with sucrose	172.8 ± 2.4	306.4 ± 8.1	396.2 ± 11.0	15.5 ± 2.0	15.4 ± 1.1[b]
Cola light	168.2 ± 3.0	303.8 ± 4.7	392.2 ± 7.7	23.1 ± 0.9	22.5 ± 0.6
Phosphoric acid_1	175.5 ± 4.6	310.1 ± 5.0	409.5 ± 5.2	19.3 ± 0.8	22.3 ± 1.0
Phosphoric acid_2	172.6 ± 3.2	299.0 ± 4.2	396.3 ± 6.1	22.7 ± 2.0	23.8 ± 1.4
Acetic acid	172.2 ± 2.2	307.6 ± 7.3	408.4 ± 9.7	24.5 ± 2.2	24.1 ± 1.2
Water	171.7 ± 4.9	315.4 ± 11.1	418.1 ± 15.4	23.0 ± 0.8	24.4 ± 1.4

[a]Mean ± SEM, n=5. [b]p <0.01 vs. all other groups (ANOVA with Bonferroni correction). pH of the colas was 2.6. Concentration phosphoric acid_1= 3.1 g/L giving pH=2.0, and phosphoric acid_2= 6.2 g/L pH=1.9. Acetic acid was diluted in H_2O to a final concentration of 1.92 g/L, pH 3.0.

Table 2. Fluid intake and urinary volume in rats ingesting various acid beverages

	Fluid intake (ml/18 hours)			Urinary volume (ml/18 hours)	
	Initial	After 3 wk	After 6 wk	After 3 wk	After 6 wk
Cola with sucrose	24.6±2.8[a]	46.9±12.9[b]	83.4±7.7[d]	64.2±21.4[f]	55.8±7.6[h]
Cola light	24.0 ± 1.3	71.7 ± 6.6[c]	44.4 ± 10.2[e]	81.0 ± 18.0[g]	23.3 ± 8.6
Phosphoric acid_ 1	21.8 ± 2.1	11.4 ± 2.8	21.4 ± 2.2	4.3 ± 0.2	5.8 ± 0.4
Phosphoric acid_2	19.9 ± 3.4	11.3 ± 1.9	17.1 ± 1.8	4.5 ± 0.5	5.3 ± 0.2
Acetic acid	9.0 ± 5.0	18.4 ± 4.2	19.2 ± 2.4	5.3 ± 0.4	5.6 ± 0.4
Water	21.1 ± 1.8	17.6 ± 3.7	23.3 ± 1.7	5.9 ± 0.5	8.2 ± 1.1

[a]Mean ± SEM, n = 5. [b]p < 0.01 vs. corresponding values for phosphoric acid 1 and 2 (ANOVA/Bonferroni); [c]p < 0.001 vs. corresponding two phosphoric acid groups, acetic acid and water; [d]p < 0.001 vs. all corresponding other groups; [e]p ≤ 0.05 vs. corresponding phosphoric acid 2 and water; [f]p 0.02 vs. all corresponding other groups, except cola light; [g]p < 0.002 vs. all corresponding other groups, except sugar-cola; [h]p 0.001 vs. all corresponding other groups. pH of the colas was 2.6. Concentration phosphoric acid_1= 3.1 g/L giving pH = 2.0, and phosphoric acid_2 = 6.2 g/L pH =1.9. Acetic acid was diluted in H_2O to a final concentration of 1.92 g/L, pH 3.0.

ds9_2: r=0.853; for ds5: r=0.868, all with p < 0.001.

Statistical analysis

The influence of the various beverages upon serum lipids and desaturase indexes in phospholipids was assessed by correlation analyses (Pearson), by one- way ANOVA with Bonferroni correction for multiple comparisons, or by repeated measures ANOVA. When appropriate, non-parametric test (Mann-Whitney) was applied to estimate significance of differences. We used SPSS 15.0 for the analyses and sigma plot 2001 to produce the figures. A significance level of 0.05 was accepted. The results are given as mean values ± SEM.

RESULTS

Body weight and food intake in rats ingesting various acid beverages

There were no significant group differences in body weight during the experiment (Table 1). Mean food intake, measured after 3 and 6 weeks, was lower (p <0.01) in the sugar-cola group than in all of the other groups, which had similar food intake values.

Fluid intake and urinary volume in rats ingesting various acid beverages

For fluid intake (Table 2), there was a main effect of time (F10.9, p = 0.003; repeated measure ANOVA) and an interaction between time and type of beverage (F=11.0, p < 0.001). All groups had similar fluid intake at zero time. After 3 and 6 weeks, there seemed to be higher fluid intakes in rats drinking colas than in the other groups. After 3 weeks statistical significance (ANOVA/ Bonferroni correction) was found only for the difference between the sugar cola group and the two groups given phosphoric acid; cola light rats had higher fluid intake than all other groups, except the sugar cola group. After 6 weeks, the sugar cola rats had significantly higher fluid intake than all other groups (p 0.001), and cola light rats had higher intake than rats given acetic acid and phosphoric acid_2.

Also for urinary volume (Table 2), there was a main

Table 3. Serum triglycerides (TG) and HDL cholesterol (HDL) in rats voluntarily ingesting various acid beverages.

	Initial	After 3 wk	(3-0 wk)	After 6 wk	(6 – 0 wk)
			TG (mmol/L)		
Cola with sucrose	1.36 ± 0.19[a]	1.61 ± 0.34	0.25 ± 0.21[b]	2.19 ± 0.36	0.83 ± 0.18
Cola light	1.19 ± 0.09	2.40 ± 0.16	1.20 ± 0.21	2.08 ± 0.22	0.88 ± 0.22
Phosphoric acid_1	0.89 ± 0.09	1.78 ± 0.17	0.89 ± 0.19	1.47 ± 0.14	0.58 ± 0.08
Phosphoric acid_2	1.07 ± 0.25	1.97 ± 0.33	0.90 ± 0.09	1.69 ± 0.18	0.62 ± 0.10
Acetic acid	1.47 ± 0.21	1.94 ± 0.19	0.47 ± 0.18	1.83 ± 0.13	0.36 ± 0.17
Water	1.61 ± 0.06	1.68 ± 0.08	0.07 ± 0.13[c]	1.73 ± 0.15	0.12 ± 0.20
			HDL (mmol/L)		
Cola with sucrose	1.32 ± 0.10	1.28 ± 8.16	-0.04 ± 0.16	1.06 ± 0.16	-0.26 ± 0.09
Cola light	1.13 ± 0.11	0.92 ± 0.06	-0.20 ± 0.06	0.97 ± 0.11	-0.16 ± 0.09
Phosphoric acid_1	1.26 ± 0.07	1.20 ± 0.13	-0.06 ± 0.07	1.06 ± 0.10	-0.20 ± 0.08
Phosphoric acid_2	1.15 ± 0.13	1.01 ± 0.17	-0.15 ± 0.06	0.98 ± 0.16	-0.17 ± 0.06
Acetic acid	1.18 ± 0.13	0.95 ± 0.03	-0.23 ± 0.06	0.66 ± 0.07	-0.52 ± 0.09
Water	1.15 ± 0.03	1.15 ± 0.03	0.00 ± 0.04	1.09 ± 0.04	-0.05 ± 0.07

[a]Mean values ± SEM, n=5 in all groups. [b]p<0.05 vs. cola light; [c]p<0.05 vs. cola light, phosphoric acid_1 and phosphoric acid_2. pH of the colas was 2.6. Concentration phosphoric acid_1= 3.1 g/L giving pH=2.0, and phosphoric acid_2= 6.2 g/L pH=1.9. Acetic acid was diluted in H_2O to a final concentration of 1.92 g/L, pH 3.0.

effect of time (F = 6.7, p = 0.016) and an interaction between time and type of beverage (F = 6.0, p =0.001). After 3 weeks, both cola groups had a several fold higher urinary volume than all other groups which had similar urinary volumes. After 6 weeks, only the sugar-cola group had significantly higher (p < 0.001) urinary volumethan the corresponding groups. The fluid intake seemed to increase until 6 weeks in the sucrose cola group, whereas there was a reduced intake from 3 to 6 weeks in the cola light group. In accordance with this, from 3 to 6 weeks there was an appreciable decrease in the urinary volume in the cola light group, but no significant decrease in the sucrose cola group.

Conceivably, urinary volume should reflect the fluid intake and this suggestion was corroborated by the strong positive correlation between the two variables (r=0.988, p < 0.001; results not illustrated). After 6 weeks, the two groups given phosphoric acid had significantly lower pH in the urine than the other groups (p<0.01) which had similar pH values.

Serum triglycerides (TG) and HDL cholesterol

For serum triglycerides (Table 3), there was a main effect of time (F = 39.5, p < 0.001) and a significant interaction between time and type of ingested fluid (F = 3.6, p = 0.015). For all groups, except the water group, there seemed to be an increase in serum TG until three weeks; then: a slight decrease, except for the cola group in which the triglyceride concentration continued to increase throughout the experimental period. After 3 weeks all groups, except those given cola, water or acetic acid had higher triglyceride values than at zero time (p < 0.05, t-test); after 6 weeks the same groups plus the cola group had increased triglycerides. After 3 weeks, only cola light and the two groups given phosphoric acid had higher triglyceride values (p < 0.05, ANOVA/ Bonferroni) as compared with rats given water; after 6 weeks there were no significant group differences in the serum triglyceride concentration. The TG increase during 3 weeks was higher in the cola light group than in the sugar cola group (p = 0.01), whereas the TG increase in the two cola groups did not differ after 6 weeks. For HDL there was a main effect of time (F = 18.9, p < 0.001) but no significant interaction between time and type of ingested fluid. In all groups except those given water, the HDL values after 3 weeks were lower than at zero time, however without attaining statistical significance except in the acetic acid group. For each of the groups ingesting some kind of acid fluids there were no significant group differences in the time related HDL reduction as compared with rats given water.

However, when comparing the changes in serum lipids in the control group with the changes in the pooled acid groups (that is, all groups given beverages containing either phosphoric acid or acetic acid, grouped together) there was a significant increase in the serum TG concentration both after 3 weeks (z = -2.616, p = 0.009, Mann-Whitney, Table 4) and after 6 weeks (z = -2.478, p = 0.013) and also a reduction in the HDL concentration after 3 weeks (z = -2.172, p = 0.030) and after 6 weeks (z = -2.032, p = 0.042). Thus, rats ingesting acid beverages seemed to respond with increased serum triglycerides

Table 4. Changes in serum triglycerides and HDL in the control group and the pooled acid groups.

	Change, 0 to 3 week		Change, 0 to 6 week	
	Control (n=5)	Acid (n=25)	Control (n=5)	Acid (n=25)
TG, mmol/L	0.07 ± 0.13[a]	0.74 ± 0.10[b]	0.12 ± 0.20	0.65 ± 0.08[c]
HDL, mmol/L	0.00 ± 0.04	-0.14 ± 0.04[d]	-0.05 ± 0.07	-0.26 ± 0.04[e]

[a]Mean ± SEM. Control group = rats ingesting water; Acid group = rats ingesting beverages containing acids (phosphoric acid or acetic acid). [b]z = -2.616 (p=0.009, Mann-Whitney); [c]z = -2.478 (p=0.013); [d]z = -2.172 (p=0.030); [e]z = -2.032 (p=0.042).

and lowered HDL cholesterol concentration as compared with those drinking water. However, pooling of data requires special attention, e.g. other cola components might have detrimental effects.

Liver triglyceride concentration

There were no significant group differences in the mean liver triglyceride concentration. Mean values ± SEM (n = 5 in each group) were in the cola, cola light, phosphoric acid_1, phosphoric acid_2, acetic acid and water groups: 1.51 ± 0.16; 1.23 ± 0.09; 1.11 ± 0.07, 1.17 ± 0.08; 1.09 ± 0.11; 1.32 ± 0.04 (results not shown elsewhere, respectively).

Relationship between the three desaturase indexes in liver

We reasoned that fatty acid desaturases might possibly be regulated in a coordinated manner. If so, we would expect a positive correlation between the three desaturase indexes. In the whole material we found positive correlations between the desaturase indexes (ds9_1 vs. ds9_2: r = 0.689, p < 0.001; ds9_1 vs. ds5: r = 0.637, p < 0.001; ds9_2 vs. ds5: 0.680, p < 0.001), results not illustrated.

Liver triglyceride concentration as related to indexes reflecting fatty acid desaturases

As shown in Figure 1, there was a positive relationship between desaturase indexes and liver triglycerides (top panel: ds9_1 vs. liver TG, r= 0.471, p=0.013; middle panel: ds9_2 vs. liver TG: 0.642, p < 0.001; lower panel: ds5 vs. liver TG, r = 0.559, p = 0.002; n = 28). All desaturase indexes were higher in rats ingesting sugar-cola than in all other corresponding groups (p 0.01), which had similar values (Table 5). Urinary acid excretion was significantly higher in rats ingesting sugar-cola than in those given cola light, acetic acid or water (p 0.004).

Association between acid excretion in the urine (amount phosphoric acid ingested) and desaturase indexes in the liver

There was a positive association between urinary acid excretion and liver desaturase indexes (Figure 2a; acid excretion vs. ds9_1: r=0.630, p<0.001; vs. ds9_2: r=575, p=0.001; vs. ds5: r=0.496, p=0.007). In rats ingesting beverages containing phosphoric acid (n=20), either as colas or as ortho phosphoric acid diluted in H_2O, there was a positive association between the amount of phosphoric acid ingested and each of the desaturase indexes (Figure 2b); for correlation with ds9_1, ds9_2 and ds5, respectively the correlation coefficients were: r = 0.676, p < 0.001; r = 0.776, p < 0.001; r = 0.767, p < 0.001.

DISCUSSION

To our knowledge, this is the first report suggesting that an increased acid load may increase hepatic desaturase activities, stimulate formation of monounsaturated fatty acids and triglycerides in the liver and increase the serum triglyceride concentration.

We recently observed in a human population study (Høstmark et al., 2009) that intake of colas was associated with increased serum triglyceride levels and reduced concentration of HDL, irrespective of whether the colas were with or without sugar. The present diet trial seems to suggest that these associations may be causal ones, and that phosphoric acid could be one potential causative factor. Both cola types, as well as phosphoric acid dissolved in water tended to influence the serum lipids similarly. The finding that also ingestion of acetic acid lowered the HDL concentration could imply that acid load in general might influence the serum lipids. Indeed, in the pooled acid group serum triglycerides rose and HDL cholesterol fell significantly compared with rats given water. However, it could be difficult to appreciate the pooled data results since there are other components than acid in colas.

The considerably higher fluid intake and urinary volume in rats drinking colas is presumably attributable to the

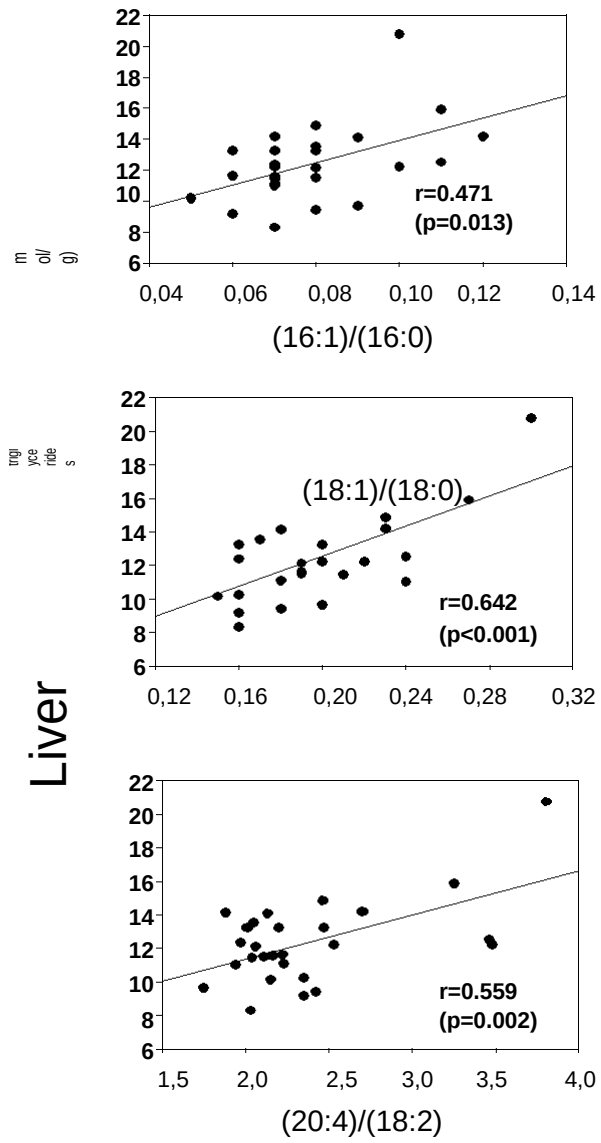

Figure 1. Liver triglyceride concentration (mmol/L) as related to desaturase indexesDelta9-desaturase was estimated by the (16:1n- 7)/ (16:0) ratio (top panel) and the (18:1n-9)/ (18:0) ratio (middle panel), and Delta- 5-desaturase by the (20:4n-6)/(18:2n-6) ratio (lower panel) in liver lipids.

ceffeine content in colas (Goodman and Gilman, 1975). This methyl xanthine may also affect lipid metabolism by inhibiting cyclic AMP phosphodiesterase so as to increase cyclic AMP levels and thereby enhance adipose tissue lipolysis. The cyclic nucleotide stimulates hormone sensitive lipase in adipose tissue followed by increased release of fatty acids which could be used for hepatic triglyceride and very low density lipoprotein (VLDL) synthesis (Murray et al., 2000).

It was previously reported that a positive association between the consumption of cola and apolipoprotein B-100, the main VLDL-apolipoprotein (Carson et al., 1993). The present observations raise the question of whether colas also might stimulate VLDL synthesis via an acid-related increase in the hepatic desaturase activities. Furthermore, the stimulatory influence of dietary sucrose upon lipogenesis might in general at least partly be attributed to an increased acid load.

Sucrose sweetened soft drinks can be a major carbohydrate source. Intake of this disaccharide can increase the serum triglyceride concentration and also lower serum HDL (Høstmark and Blom, 1985; Archer et al., 1998; Dhingra et al., 2007; Merchant et al., 2007). It might be suggested that the serum lipid changes observed in the present trial following intake of sugar - cola was caused by its sucrose content. However, also cola without sugar seemed to have the same effect, suggesting that other factors might be involved. Since intake of all acid fluids tended to affect the serum lipids in the same direction, we suggest that the phosphoric content in colas (Jensdottir et al., 2006; Kapicloglu et al., 2000) might be partly responsible for the serum lipid effect.

As referred to above, the concentration of serum triglycerides and HDL varies inversely with carbohydrate feeding. The present results would be in accordance with this finding. However, our results suggest that an acid load in general may cause this inverse relationship and this observation seems to be a novel one. The data do not, however, clarify whether acid load reduces the HDL concentration secondary to raising serum triglycerides (VLDL), or via a more direct mechanism.

Although intake of acid was the common feature among the groups in the present study, the sugar-cola group ingested sucrose as well and this group had higher hepatic desaturase indexes as compared with the other groups. The finding that sucrose can enhance hepatic desaturase indexes in the rat is in accordance with earlier research (Brenner et al., 2003). In rodents, intake of carbohydrates after fasting is accompanied by increased activity of hepatic Delta9-desaturase, as well as induction of mRNA for the enzyme (Ntambi, 1995). Dephosphory-lation of the transcription factor Carbohydrate Response Element-Binding Protein (ChREBP) and its translocation to the nucleus, has been shown to be involved in the carbohydrate activation of the desaturase (Latasa et al., 2000).

Our results provide an additional explanation for the increased hepatic desaturase activity after sucrose intake, since the fructose moiety of sucrose is known to increase the acid load, as shown by increased urinary excretion of uric acid, calcium and oxalate (Taylor and Curhan, 2008) . In accordance with this, we found that the acid load was positively correlated with liver desaturase indexes, irrespective of whether the acid load was estimated by the amount of phosphoric acid ingested, or by the urinary acid excretion. Conceivably, sugar cola should give more acid than cola light, since the former provides acid both by catabolism of the fructose

Table 5. Liver desaturase indexes and urinary acid excretion, as related to type of drinking fluid.

	Desaturase indexes		Acid excretion [a]	
	(16:1)/(16:0)	(18:1)/(18:0)	(20:4)/(18:2)	
Cola with sucrose	0.24 ± 0.01 [b]	0.92 ± 0.14 [b]	1.62 ± 0.03 [b]	4.93 ± 1.45 [c]
Cola light	0.12 ± 0.01	0.39 ± 0.02	1.34 ± 0.04	0.89 ± 0.35
Phosphoric acid_1	0.15 ± 0.01	0.42 ± 0.01	1.30 ± 0.06	2.59 ± 0.34
Phosphoric acid_2	0.13 ± 0.01	0.39 ± 0.01	1.31 ± 0.04	2.28 ± 0.54
Acetic acid	0.14 ± 0.02	0.37 ± 0.02	1.37 ± 0.02	0.12 ± 0.04
Water	0.15 ± 0.01	0.40 ± 0.02	1.38 ± 0.02	0.59 ± 0.30

[a] µeqv/18 hours; mean values ± SEM, n=5. [b] $p<0.05$ vs. all of the other groups (ANOVA/Bonferroni correction). [c] $p \leq 0.004$ vs. cola light, acetic acid and water. pH of the colas was 2.6. Concentration phosphoric acid_1= 3.1 g/L giving pH=2.0, and phosphoric acid_2= 6.2 g/L pH=1.9. Acetic acid was diluted in H_2O to a final concentration of 1.92 g/L, pH 3.0.

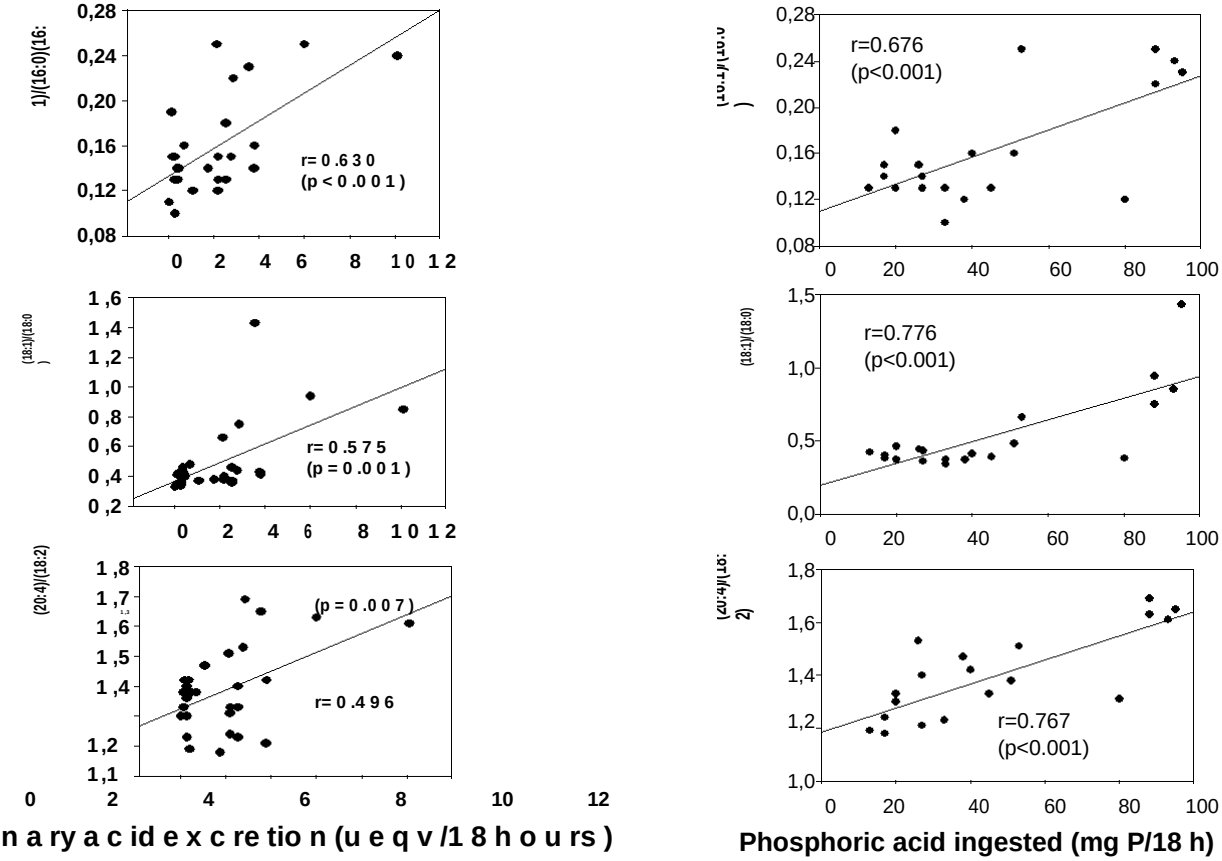

Figure 2. Liver desaturase indexes as related to estimates of the acid load.
Scatter plot of liver desaturase indexes vs. urinary acid excretion calculated from urine pH and the volume collected during 18 hours, at the end of the diet trial (left group of panels) and vs. intake of phosphoric acid, either in colas or as a solution of ortho phosphoric acid in H_2O (right group of panels). Delta9-desaturase was estimated by the (16:1n-7)/ (16:0) ratio (top panels) and the (18:1n-9)/ (18:0) ratio (middle panels), and Delta-5-desaturase by the (20:4n-6)/(18:2 n-6) ratio (lower panels) in liver lipids.

component of sucrose and by acids directly added to colas. Part of the difference between the desaturase effects of the two cola types is probably also attributed to the fact that sucrose cola was ingested in higher amounts than cola light, thereby providing more acid.

High intakes of acid soft drinks with sugar should have a particularly strong stimulating effect on hepatic lipogenesis, due to ample substrate supply, increased insulin release by glucose and possibly acid activation of desaturases. In particular, the fructose moiety of sucrose

is rapidly converted to acetyl-CoA, the precursor of e.g. palmitic acid and stearic acid, which subsequently may be desaturated to palmitoleic and oleic acid by the acid stimulated Delta9-desaturase. In keeping with this reasoning, there was a positive association between hepatic desaturase indexes and triglycerides and sugar-cola ingestion resulted in increased hepatic desaturase indexes.

S**ignificance of desaturase activation by increasing the acid load**

Human epidemiological studies have shown that fatty acid desaturase indexes such as the ratio of C16:1n-7 to C16:0, an estimate of stearoyl-CoA desaturase, may predict cardiovascular mortality (Warensjo et al., 2008) and inhibition of the enzyme may be associated with increased insulin sensitivity (Corpeleijn et al., 2006). Studies in mice lacking stearoyl-CoA desaturase (SCD) seem to offer an explanation of the epidemiological findings (Dobrzyn and Ntambi, 2004a, 2005a,b; Dobrzyn et al., 2005c; Dobrzyn and Dobrzyn, 2006; Dobrzyn et al., 2008a,2008b). Mice lacking the enzyme do not have the ability to form monounsaturated fatty acids which are significant constituents of tissue lipids and serum lipoproteins. These animals have reduced hepatic lipogenesis, lowered plasma triglycerides and increased insulin sensitivity compared with their normal counterparts (Dobrzyn and Ntambi, 2004a; Dobrzyn et al., 2004b, 2008a). These studies indicate that desaturases are important in metabolic Control (Dobrzyn and Ntambi, 2004a, 2005a,2005b; Dobrzyn et al., 2008a) . Anticipating that desaturase inhibition is important also in man, our results could imply that increased intake of sugar-colas might promote major lifestyle diseases, such as obesity, diabetes and cardiovascular diseases via stimulation of liver desaturases. We emphasize that studies in man are required to substantiate this suggestion. In this context it should also be kept in mind a study in hyperlipidemic mice suggesting that inhibition of stearoyl-CoA desaturase 1 may promote aortic atherosclerosis inspite of protecting against diet-induced obesity and insulin resistance (Brown et al., 2008).

Based upon the present results, we suggest that an increased acid load may act as a physiological enhancer of the activity of fatty acid desaturases. To our knowledge, this is a novel observation. Whether a slight increase or decrease in the local proton concentration alters the enzyme activities directly, or proceeds via transcriptional regulation is currently under investigation.

The finding that all of the three desaturase estimates were positively correlated could imply that fatty acid desaturases are regulated in a coordinated manner. This suggestion would seem in accordance with the proximity on chromosome 11 of genes encoding for some of the desaturases, suggesting coordinated transcriptional control (Nakamura and Nara 2004). Possibly, stimulation and inhibition of one desaturase would affect other desaturases as well. In keeping with this suggestion, in the present study all desaturase estimates were higher in livers of rats given sugar-colas than in the other groups.

Lack of stearoyl CoA desaturase in mice has previously been shown to increase insulin sensitivity, inhibit growth, reduce hepatic lipogenesis and steatosis, and reduce serum triglycerides (Dobrzyn et al., 2008a). Based upon the observations made in the present diet trial in rats we suggest that an increased acid load may contribute to increased hepatic desaturase activity, followed by increased formation of monounsaturated fatty acids which govern the synthesis of hepatic triglycerides and probably other compound lipids. These events would eventually result in increased hepatic synthesis and output of VLDL, to be measured for example as an increased fasting serum TG concentration. Our data raise the question of whether frequent intake of large amounts of sucrose and acid soft drinks may promote obesity, diabetes and cardiovascular diseases also by creating a low-grade metabolic acidosis which in turn would increase the activity of fatty acid desaturases.

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