Nutritional Sciences 6(4), November, 2003, pp. 195 - 200

Effects of Cola Intake on Glucose Metabolism and Oxidative Stress in Weanling Male Rats Fed a Moderate Fat Diet*

Soo Bong Choi, Mi Kyung Choi[1], Chun Hee Park[1], Dong Wha Jun[1], Won Kuk Lee[1] and Sunmin Park[1§]

Internal Medicine, KonKuk University, Kyohyun-Dong, Chungjoo-Si, ChungBuk-Do, 380-062, Korea

[1]Food and Nutrition, Hoseo University, Sechul-Ri, Asan-Si, ChungNam-Do, 336-795, Korea

In recent years, the prevalence of type 2 diabetes mellitus has dramatically increased in Korea as the diet has rapidly become westernized. We determined the effect of long-term cola intakes on glucose metabolism and oxidative stress in weanling male Sprague Dawley rats consuming a moderate fat diet. Thirty male rats, born from 6 female rats, were randomized into cola or water drinking groups. For 28 weeks, all rats were provided with an ad lib solid diet having 33 percent of its metabolisable energy as fat. In addition, rats of the cola group were provided with ad lib cola instead of water. The daily total caloric intake did not differ between groups. The rats in the cola group consumed a higher proportion of carbohydrates, and their mean body weight and fasting serum insulin level were lower than that of the control group. Whole-body glucose disposal rates measured by an euglycemic hyperinsulinemic clamp were higher in the cola group. However, lipid peroxide levels in kidney tissue were higher in the cola group than in the control group. Superoxide dismutase activity in kidney tissues was lower in the cola group compared to the control group, while glutathion peroxidase and catalase activities were not significantly different between the two groups. In conclusion, long-term cola intakes decreased insulin resistance, but increased oxidative stress in kidney tissue due to decreased SOD activities, which may lead to kidney damage. Thus, moderate changes in insulin resistance may not affect the status of oxidative stress, and vice versa.

Key words : lipid peroxides, superoxide dismutase, serum glucose, serum insulin, insulin resistance

INTRODUCTION

There was a 2.8-fold increase in per capita consumption of fats and simple sugars in Korea between 1970 and 1995 according to the National Nutrition Survey Report[1], although the absolute amounts consumed of these dietary components remained much lower than in Western countries. This increase in fat and simple sugar intake was largely due to the westernization of eating behavior, represented by the increased consumption of fast foods and carbonated beverages such as cola. Meanwhile, the prevalence of insulin resistance syndrome such as type 2 diabetes mellitus, hypertension, and dyslipidemia has been rapidly and continuously increasing in Korea, with recent statistics showing that this syndrome now affects over 8% of the population.[2] This increased prevalence of type 2 diabetes is possibly related to the increased Westernization of eating patterns.

Insulin resistance syndrome includes conditions such as glucose intolerance, hypertension and dyslipidemia. Although the mechanisms of insulin resistance are not completely understood,[3] insulin resistance syndrome may generate oxidative stress in cells, and vice versa. The mechanism of nutrient-induced insulin resistance and oxidative stress is complex and not understood. Very few studies have reported that cola intake is involved in the development of insulin resistance syndrome, even though cola has been blamed as a factor for them.[4),5]

Several lines of evidence have also emerged supporting the role of oxidative stress in the development of insulin resistance and diabetic complications.[6),7] This could involve an increase in the production of reactive oxygen species and a decrease in antioxidative defense systems in an insulin-resistant state. The increased production of reactive oxygen species was recently proposed as the single unifying mechanism to induce the pathogenesis of nephropathy.[8),9] Thus, we conducted an experiment to determine the effect of long-term cola intakes on glucose metabolism and oxidative stress in weanling male Sprague Dawley rats consuming a moderate fat diet.

*This research was supported by Korea Science and Engineering Foundation.

Accepted : October 23, 2003

§ To whom correspondence should be adressed.

METHODS AND MATERIALS

1. Experimental animals and diets

Thirty male rats were born from 6 female Sprague Dawley rats weighing 241.1±8.6g. After weaning, the animals were housed individually in stainless steel cages in a controlled (23℃; 12 hour light and dark cycle) environment, and were randomly assigned to two treatment groups; one group was given continuous access to water (n=14), while the other group had unrestricted access to cola (The Coca-Cola Company, Atlanta, GA) instead of water (n=14). Both groups consumed semi-purified diets, which were made using a modified method for experimental diets.[10] The composition of the moderate fat diet was 47% carbohydrates, 20% protein and 33% fats in terms of energy. The sources of carbohydrates, protein, and fats were starch, casein, and shortening, respectively. The animals were allowed free access to diets for 28 weeks. Blood glucose levels, food intake, and body weight were measured weekly at an assigned time.

2. Euglycemic hyperinsulinemic clamp studies

Indwelling catheters were inserted into the jugular vein and carotid artery during the twenty-seventh week of the trial period.[11] After 5-6 days of insertion, euglycemic hyperinsulinemic (EH) clamp studies were performed on the rats in an awake, unstressed, and fasting state. Hyperinsulinemia was achieved with a constant infusion of human insulin (12 mU/kg/min) and euglycemia was maintained at a variable rate of infusion of 25% glucose solution from 90 to 120 minutes, which was adjusted every 10-15 minutes.[12] The glucose infusion rate was calculated and expressed in terms of mg of glucose per kg of body weight per minute. The glucose disposable rate is an index of whole-body response to exogenous insulin. After the EH clamp study, the rats were sacrificed by decapitation. Tissues were rapidly removed and frozen in liquid nitrogen, and were stored at -70℃ until further analyses were performed.

3. Biochemical measurements

Serum glucose levels were analyzed by a Glucose Analyzer II (Beckman, Fullerton, CA). Serum insulin levels were measured using commercial radioimmunoassay kits (Linco Research, St Charles, MO).[13] The advanced glycated end products of subcutaneous tissue samples were measured using fluorescence methods.[14] The supernatant from the subcutaneous tissue, which was homogenated with phosphate buffered saline, was removed. Chloroform and methanol (2 : 1, vol : vol) were added to the precipitates and kept at 4℃ overnight. After removing the organic solvent, the defatted tissue was incubated at 37℃ for 48 hours with collagenase type 7 and proteinase K in PBS. After centrifugation at 10,000xg for 10 minutes at 4℃, half of the supernatant was used for fluorescence determination (at an excitation level of 370nm and an emission level of 440nm). The remaining supernatant was used for determining hydroxyproline content by colorimetric measurement.

In order to determine the oxidative status of kidney tissues, the levels of the lipid peroxidation products, thiobarbituric acids reactive substances (TBARS) (mainly malondialdehyde), were determined fluorometrically according to the modified method of Ohkawa[15] using a fluorometer at an excitation level of 515nm and an emission level of 550nm. 1,1,3,3-tetraethoxypropane was used as the standard. This TBARS assay is a common method for measuring lipid peroxidation, which can represent oxidative stress in several situations such as in an insulin resistant state.[16] Glutathione peroxidase (GSH Pxase) activity was measured by a modified method of Gunzler *et al.*,[17] using t-butyl hydroperoxide as substrate. The results were expressed in nmols of NADPH oxidized per minute per mg of protein of kidney tissue. Superoxide dismutase (SOD) activity was determined by monitoring the auto-oxidation of pyrogallol, according to the method of Flohe.[18] One unit of SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol autoxidation by 50% and is given in mgs of protein of kidney tissue. Catalase activity was determined using the method described by Aebi.[19] The rate of decomposition of the substrate H_2O_2 by the catalase was monitored at 240 nm in an UV/visible spectrophotometer (Pharmacia, Uppsala, Sweden). Units were expressed as nmols of H_2O_2 decomposed per minute per mg of protein of kidney tissue.

4. Statistical analysis

All results were expressed as mean±standard deviation. Statistical analysis was performed using the SAS statistical analysis program.[20] The effect of the cola was examined by a two-sample t-test. $P<0.05$ was considered to be statistically significant.

RESULTS

1. Energy intake and body weight

Average daily energy intake during the experimental period is presented in Table 1. Rats in the control group consumed their total energy as 47% carbohydrates, 20% protein and 33% fat. Since the rats in the cola group received about one third of their total calories from cola, which provided 27.6±1.2 kcal of simple sugars per day, they actually consumed their total energy as 58.5% carbohydrates, 15.5% protein, and 25.6% fat. However, the daily total caloric intakes were not significantly different between the two groups. Mean initial body

weight of all rats was 45.2+4.8g, which was the same for both the cola and control groups (Table 2). Figure 1 shows the weekly changes in body weights during the entire experimental period. The mean body weight of the cola group was lower than that of the control group from the second week onwards. Serum osmolality was no different between the cola and control groups, indicating that the cola-fed rats were not in a state of dehydration.

Table 1. Average daily dietary intakes for 28 weeks

	Control group (n=13)	Cola group (n=12)
Energy (Kcal)	125.1±9.5[1)]	126.4±10.3
Fluid intake (mL)	22.5±4.5	63.5±10.1***
Caffeine intake (mg)	-	7.0±0.8
Complex carbohydrates (g)	14.7±1.3	11.6±1.5***
(En% of total energy)	47	36.7
Simple sugar (g)	-	6.9±0.3
(% of total energy)	-	21.8
Protein (g)	6.3±0.5	4.9±0.4**
(% of total energy)	20	15.5
Fat (g)	4.6±0.3	3.6±0.2**
(% of total energy)	33.0	25.6

1) Mean+SD.
The value in the cola group is significantly different from that in the control group at **p<0.01 and ***p<0.001.

Table 2. Body weight before and after the treatment

	Control group (n=13)	Cola group (n=12)
Initial body weight (g)	46.9±9.5[1)]	44.6±10.3
Final body weight (g)	375.1±36.8	331.6±27.3**
Serum osmolality (mOsm/kg)	290±17	294±19

1) Mean+SD.
The value in the cola group is significantly different from that in the control group at *p<0.05 and **p<0.01.

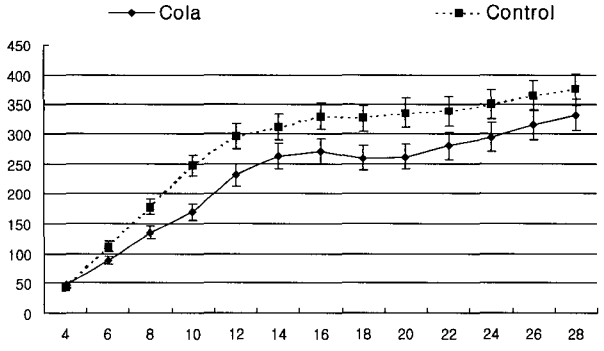

Fig 1. Weekly changes of body weight
* The value in the cola group is significantly different from that in the control group at p<0.05.
Body weight was measured every week, however, in the graph, each point represents the average values of the previous and the indicated week.

2. Glucose metabolism

The fasting serum glucose levels of the rats in both the cola and control groups were maintained in the normal range; nevertheless, the fasting serum glucose levels of the cola group were significantly lower than the control group ($p<0.05$) after 6, 14, 18, 20, 24 and 26 weeks (Figure 2). Fasting serum glucose and insulin levels, before and after the experimental period, are shown in Table 3. At the end of the experimental period, the fasting serum insulin levels of the cola group were significantly lower than those of the control group. Advanced glycated end products, representing in vivo long-term glycemic control, tended towards lower levels in the cola group compared to the control group, but these were not statistically significant (p=0.08).

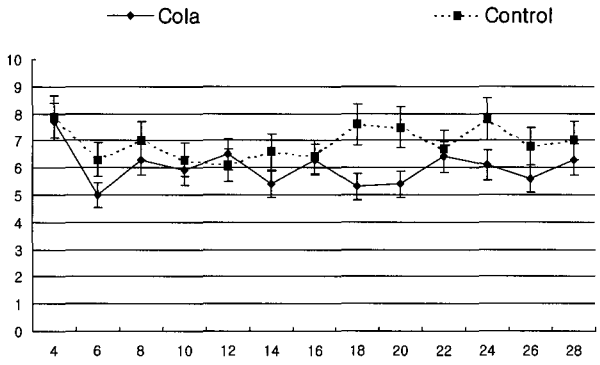

Fig 2. Weekly changes of blood glucose
* The value in the cola group is significantly different from that in the control group at p<0.05. ** p<0.01.
Blood glucose levels were measured every week, and in the graph, each point represents the average values of the previous and the indicated week.

Table 3. Serum glucose and insulin concentrations before and after the treatment

	Control group (n=13)	Cola group (n=12)
Initial serum glucose (mmol/L)	7.0±0.6[1)]	7.1±0.4
Final serum glucose (mmol/L)	7.0±0.5	6.7±0.6
Initial serum insulin (pmol/L)	638±238	646±210
Final serum insulin (pmol/L)	839±253	523±230*
Final advanced glycated end products (AU/mg collagen)	1.8±0.3	1.6±0.2

1) Mean+SD.
The value in the cola group is significantly different from that in the control group at *p<0.05.

Table 4 presents whole body glucose disposal rates, and serum glucose and insulin levels, measured by using the euglycemic hyperinsulinemic clamp in awake, unstressed and fasting rats. Before initiating the euglycemic hyperinsulinemic clamp, the fasting serum insulin levels of rats consuming cola were significantly lower than those in rats consuming water. When exogenous

insulin is continuously infused at constant levels, the exogenous glucose infusion rates needed to maintain euglycemia represents the whole body insulin resistance state. Higher glucose infusion rates indicate a more sensitive insulin action and faster glucose utilization. The cola-fed rats had significantly higher whole body glucose disposal rates than rats fed water. Thus, rats in the cola group maintained a higher insulin sensitive state compared to rats in the control group.

Table 4. Glucose disposal rate, and plasma glucose and insulin levels from the euglycemic hyperinsulinemic clamp

	Control group (n=13)	Cola group (n=12)
Glucose disposal rate (mg/kg/min)	35.0±12.8[1)]	52.5±7.7**
Basal glucose (mmol/L)	7.1±0.9	6.8±0.7
Steady-state glucose (mmol/L)	5.2±0.1	5.3±0.1
Basal insulin (pmol/L)	774±195	547±211*
Steady-state insulin (pmol/L)	3321±525	3107±428
Advanced glycated end products after euglycemic hyperinsulinemic clamp (AU/mg collagen)	1.75±0.14	1.68±0.12

1) Mean+SD.
The value in the cola group is significantly different from that in the control group at *p<0.05 and **p<0.01.

3. Lipid peroxides and antioxidant enzymes

The lipid peroxide contents of the kidney were higher in the cola group than in the control group (Table 5). The activities of SOD, the enzyme which removes superoxides, decreased in the cola group compared to the control group. However, the activities of GSH Pxase and catalase did not differ between the two groups.

Table 5. Lipid peroxide contents and antioxidant enzymes in the kidney at the end of experimental period

	Control group (n=13)	Cola group (n=12)
Lipid peroxide in kidney (nmol/mg protein)	1.78±0.21[1)]	2.07±0.18*
Superoxide dismutase in kidney (units/minute/mg protein)	76.9±8.7	65.7±9.4*
GSH peroxidase in kidney (umol NADPH oxidized/minute/mg protein)	625±83	610±78
Catalase in kidney (nmol/minute/mg protein)	1008±107	1095±121

1) Mean+SD.
The value in the cola group is significantly different from that in the control group at *p<0.05.

DISCUSSION

As Korean society becomes more westernized, the increased consumption of fast foods results in people obtaining more energy from simple sugars and fats than before. Some researchers call this phenomenon "industrialization" or 'cola-colonization'.[5)] This shift to increasing simple sugar and fat consumption may increase the incidence of insulin resistance syndromes such as type 2 diabetes, obesity, and hypertension. Insulin resistance, a major etiological factor in the pathogenesis of type 2 diabetes mellitus, is largely ascribed to a decrease in glucose utilization in peripheral tissues by insulin.[3)] Thus, high fat and simple sugar consumption, insulin resistance, type 2 diabetes, and obesity create a vicious cycle.[21),22)]

However, this study showed that cola intake associated with a moderate fat diet decreased rather than increased insulin resistance, and decreased rather than increased body weight. Overall caloric consumption in the cola group was the same as that in the control group. These results are not consistent with the results of the Bukowiecki study.[23)] The Bukowiecki study reported that Wistar rats weighing 190-200g consumed more calories in the cola group than the control groups; however, the final body weights of the cola and sucrose groups did not significantly differ from those of the control group, indicating a marked decrease in energy efficiency.

Cola contains 11.5% of simple sugars such as fructose and sucrose, and 0.0105% of caffeine. In our study, the average daily consumption of the cola group was 6.9±0.2g of sugar and 7.0±0.8mg of caffeine. Sugar consumption representing 22% of total energy intake can be considered as moderate, compared to that of other studies of high sugar or fructose diets (with sugar consumption at rates of over 60% of energy).[24)] In one study, after giving 200mg of caffeine and a placebo, an oral glucose tolerance test was performed on 30 non-smoking healthy subjects aged 26 to 32 years who had abstained not only from coffee but also from tea, chocolate and cola for 4 weeks[25)]; the blood glucose levels of subjects taking caffeine were higher at the 2nd, 3rd and 4th hours in comparison to those taking the placebo, although blood insulin levels did not differ between the two. This elevation of blood glucose levels was associated with increased cAMP concentrations through the inhibition of cAMP phosphodiesterase; in addition, high fructose and sucrose intakes induced insulin resistance, as mentioned previously.[4),24)] However, not many studies have previously been performed to determine whether the consumption of sugar beverages including cola instead of water altered glucose metabolism and insulin resistance.

Faure *et al.*[26)] recently reported that rats consuming high fructose diets had increased lipid peroxidation levels and induced greater insulin resistance. Vitamin E supplementation decreased lipid peroxidation levels and

improved insulin sensitivity. Our study showed that the intake of cola, which represented 6.7% of total energy intake (as simple sugars, mostly fructose), increased lipid peroxides in the kidney due to decreased SOD activity. However, cola intake was shown to increase insulin sensitivity in our previous study,[27] where excessive w-6 PUFA intake in 90% pancreatectomized rats increased insulin resistance, and vitamin E supplementation did not improve insulin sensitivity. Facchini *et al.*[28] reported that supplementation of vitamin A (3700 RE/day), C (560 mg/day), and E (90mg/day) decreased serum and red blood cell lipid peroxidation in healthy adults, but that only vitamin A supplementation improved insulin sensitivity and serum glucose levels. Thus, moderately-improving insulin sensitivity did not decrease the lipid peroxide contents of kidney tissues; increased oxidative stress in kidney tissues was not due to insulin resistance and/or serum glucose levels. In conclusion, in this study, long term cola intake combined with a moderate fat diet increased lipid peroxide contents in the kidneys of male Sprague Dawley rats, even though insulin resistance decreased. Long term cola intake increased oxidative stress as an independent pathway of insulin resistance.

Literature Cited

1. Korean Medical Insurance Association, 1992 Annuals for Medical Insurance Statistics, Ministry of Health and Welfare, Seoul, Korea, 1993
2. Ministry of Health and Welfare, 1993 National Nutrition Survey Report in Korea, Ministry of Health and Welfare, Seoul, Korea, 1995
3. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM : A balanced overview. *Diabetes Care* 15 : 318-353, 1992
4. Agheli N, Kabir M, Berni-Canani S, Petitjean E, Boussairi A, Luo J, Bornet F, Slama G, Rizkalla SW. Plasma lipids and fatty acid synthase activity are regulated by short-chain fructo-oligosaccharides in sucrose-fed insulin-resistant rats. *J Nutr* 128 : 1283-1288, 1998
5. Horinger P, Imoberdorf R. Junk food Revolution of the Cola-Kolonisation. *Ther Umsch* 57 : 134-137, 2000
6. Ueno Y, Kizaki M, Nakagiri R, Kamiya T, Sumi H, Osawa T. Dietary glutathione protects rats from diabetic nephropathy and neuropathy. *J Nutr* 132 : 897-900, 2002
7. Obrosova IG, Fathallah L, Stevens MJ. Taurine counteracts oxidative stress and nerve growth factor deficit in early experimental diabetic neuropathy. *Exp Neurol* 172 : 211-219, 2001
8. Nassar T, Kadery B, Lotan C, Da'as N, Kleinman Y, Haj-Yehia A. Effects of the superoxide dismutase-mimetic compound tempol on endothelial dysfunction in streptozotocin-induced diabetic rats. *Eur J Pharmacol* 436 : 111-118, 2002
9. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 414 : 813-820, 2001
10. Report of the American Institute of Nutrition, Ad Hoc committee on standard for nutritional studies, J Nutr 107 : 1340-1348, 1977
11. Waynforth HB, Flecknell PA. Experimental and surgical technique in the rat. Academic press, pp. 120-125, New York, 1994
12. Rossetti L, Farrace S, Choi SB, Giaccari A, Sloan L, Frontoni S, Katz MS. Multiple metabolic effects of CGRP in conscious rats : role of glycogen synthase and phosphorylase. *Am J Physiol* 264 : E1-E10, 1993
13. Morgan CR, Lazarow A. Immunoassay of insulin : Two antibody system, Plasma insulin levels in normal, subdiabetic and diabetic rats. *Diabetes* 12 : 115-126, 1963
14. Oddeti PR, Borgrglio A, Pascal AD, Rolandi R, Adezati L. Prevention of diabetes-increased aging effect on rat collagen linked fluorescence by aminoguanine and rutin. *Diabetes* 39 : 796-802, 1990
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95 : 351-358, 1979
16. Gutteridge JMC, Balliwell B. Measurement and mechanisms of lipid peroxidation in biological systems. *Trends Biochem Sci* 15 : 129-135, 1990
17. Flohe L, Gunzler WA. Assays of glutathione peroxidase. *Meth Enzymol* 105 : 114-121, 1984
18. Flohe L, Otting F. Superoxide dismutase assays. *Meth Enzymol* 105 : 93-104, 1984
19. Aebi H. Catalase in vitro. *Meth Enzymol* 105 : 121-126, 1984
20. SAS Institute, Inc. Guide for personal computers. Cary, NC : SAS Institutes Inc, 1985
21. Storlien LH, Higgins JA, Thomas TC, Brown MA, Wang HQ, Huang XF, Else PL. Diet composition and insulin action in animal models. *Br J Nutr* 83 : S85-S90, 2000
22. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 40 : 1397-1403, 1991
23. Bukowiecki LJ, Lupien J, Follea N, Jahjah L. Effects of sucrose, caffeine, and cola beverages on obesity, cold resistance, and adipose tissue cellularity. *Am J Physiol* 244 : R500-R507, 1983
24. Tobey TA, Mondon CE, Zavaroni I, Reaven GM. Mechanism of insulin resistance in fructose-fed rats. *Metabolism* 31 : 608-612, 1982
25. Pizziol A, Tikhonoff V, Paleari CD, Russo E, Mazza A, Ginocchio G, Onesto C, Pavan L, Casiglia E, Pessina AC. Effects of caffeine on glucose tolerance : a placebo-controlled study. *Eur J Clin Nutr* 52 : 846-849, 1998
26. Faure P, Rossini E, Lafond JL, Richard MJ, Favier A, Halimi S. Vitamin E improves the free radical defense system potential and insulin sensitivity of rats fed high fructose diets. *J Nutr* 127 : 103-110, 1997

27. Park S, Park CH, Choi SB. Effect of cola intake on insulin resistance in moderate fat-fed weaning male rats. *J Nutri Biochem* 13 : 727-733, 2002
28. Facchini F, Coulston AM, Reaven GM. Relation between dietary vitamin intake and resistance to insulin-mediated glucose disposal in healthy volunteers. *Am J Clin Nutr* 63 : 946-952, 1996