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Effect of cola intake on insulin resistance in moderate fat-fed weaning male rats

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Abstract

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 a long-term cola intake for insulin resistance in weaning male Sprague Dawley rats consuming a moderate fat diet. Thirty male pubs born from 6 female rats were randomized into cola or water drinking groups. The rats of the cola group were freely provided with 33 energy percent fat diets and cola for 28 weeks, while the rats of the control group had the same diet with water instead of cola. The daily caloric intake did not differ between groups, while the rats in the cola group consumed more carbohydrates. However, the mean body weight of the cola group was lower than that of the control group from the second week of the study. Whole body glucose disposal rates measured by euglycemic hyperinsulinemic clamp were higher in the cola group. Compared to the control group, glycogen contents and fraction velocity of glycogen synthase of the quadriceps muscle in the cola group were higher by 39.4% and 40.3%, respectively. Uncoupling protein (UCP)-2 and GLUT 4 contents of soleus and quadriceps muscles were higher in the cola group than the control group. In conclusion, insulin action improved with increased peripheral glucose utilization in weaning male rats drinking cola, which was partly due to lower body weight. This latter was possibly as a result of increased thermogenesis in muscles.

Keywords: Euglycemic hyperinsulinemic clamp; Glycogen synthase; GLUT 4; Body weight; UCP-2; Cola

1. Introduction

Fat and simple sugar intake in 1995 increased about 2.8 fold from those in 1970 according to the National Nutrition Survey Report in Korea [2], although the absolute amount of the intake was still much lower than that of westerners. This increase of fat and simple sugar intake was due to the westernization of eating behavior, represented by increased consumption of fast foods and carbonated beverages such as cola. Meanwhile, the prevalence of type 2 diabetes mellitus has been continuously and rapidly increasing in Korea, and recent statistics show this prevalence to now affect over 8% of population [1]. Increased prevalence of type 2 diabetes is possibly influenced by the westernization of eating patterns.

The cause of type 2 diabetes mellitus has not been revealed, though it is known that a major characteristic of type 2 diabetes is insulin resistance. However, the mechanisms of insulin resistance are not completely understood either [3]. The dietary composition of macronutrients such as fat, fructose and sucrose may be an important environmental factor for the development and/or exacerbation of the insulin-resistant state [4,5]. The mechanism of nutrient-induced insulin resistance is complex. They are involved in stimulation of hepatic glucose production and suppression of peripheral glucose utilization. However, it has been shown in studies that high fat and sucrose diets induce insulin resistance in different pathways. Kraegen et al. [6] demonstrated that a high fat diet (59% of total energy from safflower oil) produced insulin resistance in liver and adipose tissues before the skeletal muscles in adult male Wister rats. However, Tobey et al. [7] observed hepatic but not muscle insulin resistance after 7 days of high fructose feeding (66% of total energy from fructose) in Sprague Dawley rats. In addition, high sucrose diets for 4 or 8 weeks produced hepatic insulin resistance before peripherals such as skeletal muscles, independent of obesity [8]. Very few studies have reported that cola intake is involved in the induction of insulin resistance and obesity, even though cola has

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been blamed as a factor for them [9,10]. The purpose of this study was to determine the effect of long-term cola intake with 33 energy percent (En%) fat diets on insulin resistance in weaning male Sprague Dawley rats.

2. Methods and materials

2.1. Experimental animals and diets

All animal surgical and experimental procedures were performed according to the guidelines of the Animal Care and Use Review Committee at Hoseo University, Korea. Thirty male pubs 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°C; 12 hr light and dark cycle) environment. They were randomly assigned to two treatments; one group freely consumed water (n = 15), and the other group had free access to cola (The Coca-Cola Company, Atlanta, GA) instead of water (n = 15). Both groups consumed semipurified diets, made by modified methods for experimental diets [11]. The composition of the moderate fat diet was 47 En% carbohydrates, 20 En% protein and 33 En% fats. 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.

2.2. Euglycemic hyperinsulinemic clamp

Indwelling catheters were inserted into the jugular vein and carotid artery during the twenty-seventh week of cola or water intake [12]. 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 25% glucose solution infusion at 90 to 120 min, which was adjusted every 10–15 min [13]. 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 the 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 in −70°C until further analysis was performed.

2.3. 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) [14]. Serum osmolality was determined by freezing-point depression using a Fiske osmometer (Boston, MA).

In order to determine the glycogen contents in the liver and soleus and quadriceps muscle tissues, these tissues were homogenized and deproteinized with 1.5 N perchloric acid. The glycogen was digested into glucose with α-amyloglucosidase in acid buffer and the glucose amount was measured by a glucose analyzer II. The glycogen contents were expressed as glucose levels derived from glycogen in soleus and quadriceps muscle and liver tissues [15]. Interstitial fat from soleus and quadriceps muscles was removed, and triacyglycerol in muscles was extracted with chloroform-methanol (2:1, vol/vol). Subsequently, the extracted triacyglycerol was resuspended in pure chroloform [16]. Triacyglycerol concentration was determined using a Trinder kit (Sigma, St. Louis, MO). Glycogen synthase activity was measured by modified method of Thomas et al. [17] and Frontoni et al. [15] After centrifugation of muscle homogenates, infranate was incubated with physiologic concentration of substrate (0.3 mM UDPG-[^{3}H] glucose) with the absence or presence of 10.0mM glucose-6-phosphate (G-6-P). Glycogen synthase activity was determined by the radioactivity in glycogen synthesized from the incubation. Maximal glycogen synthase activity was observed in the presence of 10 mM G-6-P whereas independent form activity was assayed in the absence of G-6-P. Total glycogen synthase activity is the sum of the G-6-P dependent and independent activities. Total glycogen synthase activity is expressed as nanomoles per mg protein per minute and as fractional velocity (FV), a percentage of the ratio of independent form and total activities.

Total membranes from soleus and quadriceps muscles were prepared by the methods of Walker et al. [18] GLUT4 content in total membranes was measured by Western blotting with goat GLUT4 antibody (Chemicon, Temecula, CA). The second antibody was antigoat IgG conjugated with horseradish peroxidase. Immune complexes were detected using an enhanced chemiluminesence detection system followed immediately by several sequential exposures to X-ray film (Eastman-Kodak, Rochester, NY). A muscle standard (an unrelated crude membrane fraction) was run on every gel for comparison of samples from different immunoblots. Quantification was performed with a scanning laser densitometer (BioRad, Richmond, CA). Uncoupling protein (UCP)-2 contents in skeletal muscle homogenate were measured by immunoblots with goat UCP-2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) [19], and quantification was performed with the same method for GLUT4 assay.

The advanced glycated end product of the subcutaneous tissue was measured using fluorescence methods [20]. The supernatant from subcutaneous tissue homogenate with phosphate buffered saline was eliminated. Chloroform and methanol (2:1, vol:vol) were added to pellets and kept at

Table 1
Average daily dietary intakes for 28 weeks

	Control group (n = 14)	Cola group (n = 14)
Energy (Kcal)	125.1 ± 9.5	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
(En% of total energy)	—	21.8
Protein (g)	6.3 ± 0.5	4.9 ± 0.4**
(En% of total energy)	20	15.5
Fat (g)	4.6 ± 0.3	3.6 ± 0.2**
(En% of total energy)	33.0	25.6

Data presented as Mean ± SD.
** The value in the cola group is significantly different from that in the control group at $\alpha = 0.01$; *** $\alpha = 0.001$.

4°C overnight. After removing the organic solvent, the defatted tissue was incubated at 37°C for 48 hr with collagenase type 7 and proteinase K in PBS. After centrifugation at 10,000 × g for 10 min at 4°C, half of the supernatant was used for fluorescence determination at excitation 370 nm, emission 440 nm. The rest was used for determining the content of hydroxyproline by colorimetric measurement.

2.4. Statistical analysis

All results are expressed as mean ± standard deviation. The effect of cola was examined by a two-sample t-test. $P < 0.05$ was considered to be statistically significant.

3. Results

Average daily energy intake during the experimental period is present in Table 1. Rats in the control group consumed energy from 47 En% carbohydrates, 20 En% protein and 33 En% fat. Since the rats in the cola group received about one third of the calories from cola, they were daily provided by 27.6 ± 1.2 kcal of simple sugars. They ended up consuming 58.5 En% carbohydrates, 15.5 En% protein, and 25.6 En% fat. However, the daily total caloric intake was not different between groups. Mean initial body weight of all rats was 45.2 ± 4.8g, and it was the same for both the cola and control groups. Fig. 1 shows the weekly changes of 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 of the feeding period to the end.

The rats in both the cola and control groups maintained fasting serum glucose levels in a normal range throughout the entire experimental period (Fig. 2). When rats were 6, 14, 18, 20, 24 and 26 weeks old, the levels were significantly lower in the cola group than the control ($p < 0.05$). Fasting serum glucose and insulin levels before and after the experimental period are shown in Table 2. At the end of experimental period, fasting serum insulin levels of the cola group was significantly lower than that of the control group. Serum osmolality was no different between the cola and control groups, indicating rats fed cola were not in a state of dehydration.

Table 3 demonstrates a statistically significant change in whole body glucose disposal rates and basal insulin concentration in cola fed rats compared to the control group. However, as an index of a long-term glucose control, ad-

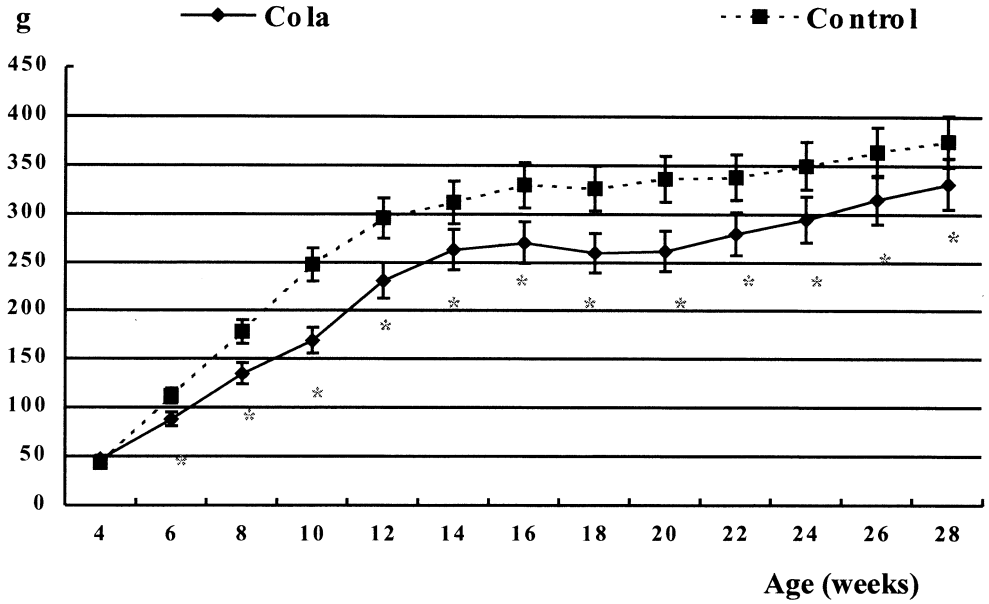

Fig. 1. Weekly changes of body weight. *The value in the cola group is significantly different from that in the control group at $\alpha = 0.05$. Body weight was measured every week, however, in the graph, each point represents the average values of the previous and the indicated week.

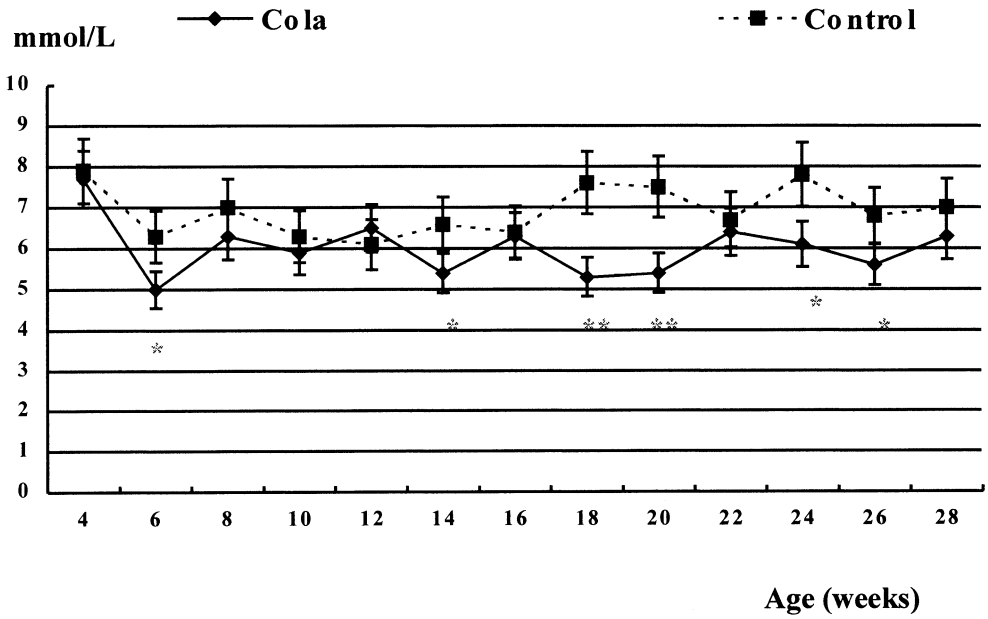

Fig. 2. Weekly changes of blood glucose. *The value in the cola group is significantly different from that in the control group at $\alpha = 0.05$. **$\alpha = 0.01$. Blood glucose levels were measured every week, and in the graph, each point represents the average values of the previous and the indicated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

vanced glycated end product contents of subcutaneous tissues in abdomen at post EH clamp did not differ between the two groups.

Liver and muscle glycogen contents and glycogen synthase activities at post EH clamp are shown in Table 4. Liver glycogen levels were not different between the cola and the control groups at the post clamp. Post clamp quadriceps muscle glycogen levels were significantly higher in the cola group compared to the control, whereas soleus muscle glycogen levels were not significantly different between the two groups. Total glycogen synthase activities in muscles were not different between the cola and control groups at post EH clamp. Fraction velocities of the quadriceps muscle were higher in the cola group compared to the control group. Muscle triacyglycerol levels had an opposite tendency to muscle glycogen contents. Soleus muscle triacyglycerol levels were lower in the cola group than the control group ($p < 0.05$), but quadriceps triacyglycerol levels were not different between the two groups. Table 5 shows UCP-2 and GLUT4 contents after EH clamp. UCP-2 and GLUT4 contents in soleus and quadriceps muscles were higher in the cola group than the control group.

4. Discussion

As Korean society becomes westernized, increased consumption of fast food causes people to get more energy from simple sugar and fat than before. Some researchers call this phenomenon industrialization or 'cola-colonization' [9]. This shift of 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 patho-

Table 2
Body weight and serum glucose and insulin concentrations before and after the treatment

	Control group ($n = 14$)	Cola group ($n = 14$)
Initial body weight (g)	46.9 ± 9.5	44.6 ± 10.3
Final body weight (g)	375.1 ± 36.8	331.6 ± 27.3**
Initial serum glucose (mmol/L)	7.0 ± 0.6	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*
Serum osmolality (mOsm/kg)	290 ± 17	294 ± 19

Data presented as Mean ± SD.
* The value in the cola group is significantly different from that in the control group at $\alpha = 0.05$; ** $\alpha = 0.01$.

Table 3
Glucose disposal rate, plasma glucose and insulin levels at EH clamp

	Control group ($n = 13$)	Cola group ($n = 12$)
Glucose disposal rate (mg/kg/min)	35.0 ± 12.8	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 endproducts at post clamp (AU/mg collagen)	1.75 ± 0.14	1.68 ± 0.12

Data presented as Mean ± SD.
* The value in the cola group is significantly different from that in the control group at $\alpha = 0.05$; ** $\alpha = 0.01$.

Table 4
Soleus and quadriceps muscle triacyglycerol and glycogen levels and glycogen synthase activities

	Control group (n = 13)	Cola group (n = 12)
Liver glycogen (mg/g tissue)	49.3 ± 10.9	50.0 ± 11.6
Soleus muscle glycogen (mg/g tissue)	3.7 ± 1.3	4.3 ± 1.1
Quadriceps muscle glycogen (mg/g tissue)	3.3 ± 0.6	4.6 ± 0.9*
Soleus muscle triacyglycerol (mg/g tissue)	174.3 ± 35.5	132.2 ± 38.6*
Quadriceps muscle triacyglycerol (mg/g tissue)	134.3 ± 48.6	121.1 ± 46.5
Total glycogen synthase activity in soleus muscle (nmol/mg protein/minute)	27.6 ± 3.8	32.5 ± 4.7
Total glycogen synthase activity in quadriceps muscle (nmol/mg protein/minute)	25.5 ± 3.9	29.6 ± 5.2
Fraction velocity in soleus muscle glycogen (% of the ratio of independent form and total activities)	8.8 ± 2.2	9.3 ± 1.2
Fraction velocity in quadriceps muscle glycogen (% of the ratio of independent form and total activities)	7.2 ± 2.1	10.1 ± 1.8*

Data presented as Mean ± SD.
* The value in the cola group is significantly different from that in the control group at $\alpha = 0.05$.

genesis of type 2 diabetes mellitus, is largely ascribed to the decreased 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 [7,21,22]. However, it has not been determined whether cola intake with a moderate fat diet increases insulin resistance, and we studied this question in male weaning Sprague Dawley rats. Our data showed the opposite results to our expectations. The main conclusion to be drawn from the results described in this article is that cola intake with 33 En% fat for 28 weeks in male weaning rats rather improved insulin resistance with increased glucose utilization in quadriceps muscle. A possible contributor of enhanced insulin sensitivity with cola intake was the decrease of weight gain without changing daily total caloric intake.

Cola contains 11.5% simple sugars such as fructose and sucrose, and 0.0105% caffeine. The cola group daily consumed 6.9 ± 0.2 g of sugar and 7.0 ± 0.8 mg of caffeine in our study. Sugar consumption of 22 En% can be considered as moderate, compared to that of other studies in high sugar or fructose diets (over 60 En%) [7,8]. Simple sugar and caffeine have been reported to affect glucose metabolism. High fructose and sucrose intakes induced insulin resistance, as mentioned previously [7,8]. However, not many studies have been performed to determine whether the consumption of sugar beverages including cola instead of water altered glucose metabolism and insulin resistance.

Caffeine is known for increasing blood glucose levels mainly due to increasing the release of catecholamines. Most caffeine studies on glucose metabolism have investigated the acute response, not the prolonged response. After giving 200 mg of caffeine or 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 [23]. The blood glucose levels of subjects taking caffeine were higher at the 2nd, 3rd and 4th hours in comparison to those taking the placebo. However, blood insulin levels did not differ between the two. This elevation of blood glucose levels was involved in increased cAMP concentration by inhibition of intracellular cAMP phosphodiesterase by increased secreation of catecholamines. However, in the other acute response study [24], caffeine ingestion (5 mg/kg) showed different results. In young adult males who had consumed caffeine, serum insulin and c-peptide concentrations were significantly greater during the last 90 min of the oral glucose tolerance test compared to those males who had consumed the placebo. However, the areas under the curve for blood glucose did not differ between the two groups. The data supports the theory that caffeine ingestion may result in a greater increase in insulin resistance. In another study [25], insulin sensitivity measured by a hyperinsulinemic euglycemic glucose clamp was decreased after intravenous caffeine administration (3 mg/kg), compared to placebo (0.9% NaCl) in nonsmoking lean healthy humans. It suggested that decreased insulin sensitivity by caffeine administration was possibly due to elevated plasma epinephrine levels. Most acute caffeine response studies conclude that caffeine administration deteriorates glucose metabolism. These acute caffeine response studies seem to be contradictory to our findings. However, prolonged caffeine ingestion may change glucose metabolism differently compared to acute caffeine ingestion. This duration of caffeine ingestion might be one factor causing the discrepancy between these studies and ours. In addition, other ingredients than caffeine in cola might affect glucose metabolism.

Important results from our study were lower weight gain and increased UCP-2 expression in muscles in the cola group in spite of no differences of caloric intake between the cola and control groups. We will focus the discussion on this observation. The reduced energy efficiency in rats consuming cola was a consistent result in the Bukowiecki study [10]. The study reported that Wister rats weighing 190–200g in the cola group consumed more calories than the control groups. However, the body weight of the cola and sucrose groups, but not the caffeine group, did not significantly differ from that of the control group resulting from a marked decrease in energy efficiency. It suggested the possibility of induction of brown adipose tissue growth, not only by exposing rats to cold but also more simply, by allowing them to drink sucrose beverages [26,27]. Hyperplasia of the brown adipose tissue occurred as a consequence of increased sympathetic activity [28,29]. In our

Table 5
Soleus and quadriceps muscle UCP-2 and GLUT 4 contents

	Control group (n = 13)	Cola group (n = 12)
Soleus muscle UCP-2[a]	103.3 ± 17.4	128.5 ± 19.6*
Quadriceps muscle UCP-2[a]	98.5 ± 22.1	142.7 ± 25.5**
Soleus muscle GLUT4[a]	97.5 ± 16.3	117.6 ± 18.5
Quadriceps muscle GLUT4[a]	95.6 ± 20.6	135.7 ± 22.9*

Data presented as Mean ± SD.

[a] Expressed as % of a muscle standard.

* The value in the cola group is significantly different from that in the control group at $\alpha = 0.05$; ** $\alpha = 0.01$.

study, UCP-2 contents, known as a mediator of thermogenesis in most tissues [30], were increased in soleus and quadriceps muscle. The increase suggested that reduced energy efficiency in the cola group was due to increased thermogenesis in muscles. Arciero et al. [31] reported that caffeine ingestion (5 mg/kg fat-free mass) increased energy expenditure in younger and older people without changing plasma insulin and glucose levels. However, thermogenesis was induced in younger people and it was blunted in older people. The thermic response to caffeine was positively associated with the body weight and waist circumference in younger women, but not in older people. Thus, the physiologic determinants of the thermic response to caffeine differed among different aged women and caffeine ingestion did not affect glucose metabolism.

It is not clear whether losing weight precedes reducing insulin resistance or vice versa. These two phenomena often occur simultaneously, and muscle glucose utilization is also enhanced in both situations. However, weight reduction improves insulin resistance in animal and human studies, and some studies explain decreased insulin resistance in terms of weight reduction. In our study, both the caffeine and sugar in cola possibly contributed to decreased insulin resistance and lower weight gain in the cola group in weaning male rats, compared to the control group. In conclusion, after weaning, a long-term cola intake rather decreased insulin resistance with increased glucose utilization in skeletal muscle of weaning male rats fed 33 En% fat diets. This was partly dependent on lower weight gain, possibly as a result of increased thermogenesis in muscles.

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