

Bittersweet: The Hormonal Implications and Effects of Aspartame, Saccharin, and Chromium Propionate upon Body Mass

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Recent clinical evidence emphasizes weight control as an important factor in disease prevention, and the quest for weight management now dominates the American health consciousness. Many dietary supplements and substitutes have been synthesized to accommodate this growing trend, although the safety and usefulness of these substances is often questioned. The purpose of this scientific study was to evaluate $[\text{Cr(III)}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$ (or Cr3), a compound comparable to that found in most weight loss medications, as well as aspartame and saccharin, common artificial sweeteners, to determine their effectiveness in promoting weight loss or maintenance in Sprague-Dawley rats. Additionally, effects upon glucose management and coronary health were evaluated through various assays. Results suggest that while none of the substances tested were effective in promoting weight loss, saccharin and high doses of chromium may be effective in improving glucose management and some factors of coronary health.

1. Introduction

Recent clinical evidence emphasizes weight control as an important factor in disease prevention, and awareness of this evidence among Americans contributes to their quest for weight management. The preoccupation with body weight has led to the synthesis and evaluation of various weight management supplements; sugar substitutes, also known as artificial sweeteners, are among this group. Seven sweeteners are currently approved by the Food and Drug Administration. They are categorized as either nutritive, providing energy in the form of calories, or non-nutritive, calorie free. Sucrose and fructose are approved nutritive sweeteners and are recognized as fairly safe

substances. The approved non-nutritive sweeteners include acesulfame-k, aspartame, neotame, saccharin, and sucralose.¹ These substances are by far more controversial, with many possible effects of intake, including cancer, phenylketonuria, and other abnormalities.² Sugar alcohols, such as sorbitol and xylitol, although not considered sugar substitutes, are new sweetening alternatives currently available and approved by the Food and Drug Administration as well.³

Experimental data from various trials assessing weight gain or loss due to sugar substitute use are few and demonstrate conflicting results; there are currently no conclusive data. In 2002, Raben, Vasilaras, Moller, and Astrup studied the long-term supplementation of the diet of overweight human subjects with non-nutritive sweeteners. The subjects' body masses were reduced by an average of 1.0 kg in a period of ten weeks, presumably due to reduced calorie intake.⁴ Although the study is one of the best-defined to date, Raben et al.'s findings are debated due to the confounding of caloric intake and carbohydrate consumption across groups.⁵

Findings of studies with aspartame have been conflicting. Some studies, such as that conducted by Blundell and Hill, suggest that the use of aspartame may actually increase appetite and lead to increased food consumption and weight gain.⁶ Some researchers attribute these findings to a psychological phenomenon leading to the loss of appetite control, not to calorie compensation. Overall, previous sugar substitute research indicates that more controlled trials are needed and that the physiological consequences of sugar substitute intake should be evaluated.

Chromium picolinate is another popular substance in the battle against obesity and can be found in most weight-loss medications. Large amounts of chromium picolinate, however, are believed to cause cancer, cell damage, and genetic mutations.⁷ However, due to the benefits of chromium use and its potential role in many key biological pathways, a safer, synthetic compound, Cr3, has been developed as an alternative source of chromium supplementation.⁸ Chromium has experimentally lowered LDL ("bad cholesterol") levels and increased insulin sensitivity in rats with Type 2 diabetes.⁹ Any weight loss promoted by Cr3 would further decrease the insulin resistance observed in diabetics and allow diabetic individuals to more properly utilize the functioning insulin available in their bodies, thus decreasing the need for insulin injections or medications. Decreased insulin resistance from Cr3 has also experimentally decreased the formation of colorectal tumors in rats.¹⁰

Recent research has provided a tentative explanation involving hormone changes to account for the loss of body fat. A key physiological link was discovered in the hormone leptin. Leptin, commonly known as the "hunger hormone," is a fundamental hormonal factor, produced in fat cells, in the

signaling pathway influencing satiety. The importance of fat cells and the substances they secrete continues to be explored;¹¹ however, it has been observed that an absence of or lowered levels of leptin are characteristic of obese mice and rats.¹² Other research supports the theory that overeating may be due, in part, to elevated leptin levels.¹³ In previous experiments involving the intake of chromium compounds, a decrease in leptin concentration has been noted as a factor warranting further exploration.¹⁴ If leptin levels facilitate weight gain or loss, therapies targeting this hormone for weight management could be developed.

Given the importance of further research on both non-nutritive sugar substitutes and chromium compounds, this experiment was designed to evaluate the efficacy of saccharin, aspartame, and Cr3 in promoting weight loss. Other hormonal changes potentially promoted by these substances were also studied to determine if additional pathways and physiological effects could be associated with the weight gain or loss.

2. Materials and Methods

2.1 $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]NO_3$, Cr3, and Artificial Sweeteners

Saccharin was obtained from ACROS organics and aspartame from Sigma Chemical Company. The nitrate salt of $[Cr_3O(propionate)_6(H_2O)_3]^+$ was prepared using the methodology of the Earnshaw study.¹⁵

2.2 Rats

Forty-eight six week old male Sprague-Dawley rats were obtained from Charles River Laboratories and randomly split into six experimental groups of eight rats each. The rats were allowed one week in the University of Alabama animal care facility to acclimate before the procedure began. During the ten-week experimental protocol, all rats were allowed to feed ad libitum on a standard commercial rat food. In addition to rat food, the control group's diet consisted solely of tap water. The second experimental group received a 0.1% solution of saccharin in water, while the third group received a 0.1% solution of aspartame in water. The remaining three experimental groups received tap water as well as daily gavage administrations of 5 microliters of an aqueous solution containing 1 mg Cr/kg body mass $[Cr_3O(propionate)_6(H_2O)_3]^+$, 5 mg Cr/kg body mass $[Cr_3O(propionate)_6(H_2O)_3]^+$, or 10 mg Cr/kg body mass $[Cr_3O(propionate)_6(H_2O)_3]^+$ respectively. The control group was also gavaged with 5 microliters of tap water daily. Water intake was measured daily for the control group and the two groups with treated drinking water. Food intake and body mass were measured every four days for all rats. At five and ten weeks of treatment, fasting blood samples averaging 1.5 mL were collected from each rat and

centrifuged to collect plasma. At five weeks, the plasma was assayed for leptin, insulin, and glucose concentrations. At ten weeks, the plasma was assayed for leptin, insulin, glucose, HDL, and triglyceride concentrations. Four rats, two in the aspartame group and two in the 5 mg Cr/kg body mass $[\text{Cr}_3\text{O}(\text{propionate})_6(\text{H}_2\text{O})_3]^+$ group, were removed from the study due to technical difficulties. After ten weeks, the rats were sacrificed by carbon dioxide asphyxiation. The heart, epididymal fat, perineal fat, subcutaneous fat, liver, kidneys, spleen, testes, and pancreas of each rat were harvested and weighed. A portion of the liver and one kidney per rat were dried for potential future iron and chromium assays, and an additional portion of the liver as well as the other kidney were preserved for future research. All procedures with rats were approved by the Institutional Animal Care and Use Committee of The University of Alabama.

2.3 Statistical analysis

Statistical analyses were performed by analysis of variance; the numerical values, figures, and tables are presented as the mean \pm the standard deviation. The level of significance for the statistics was $P \leq 0.05$.

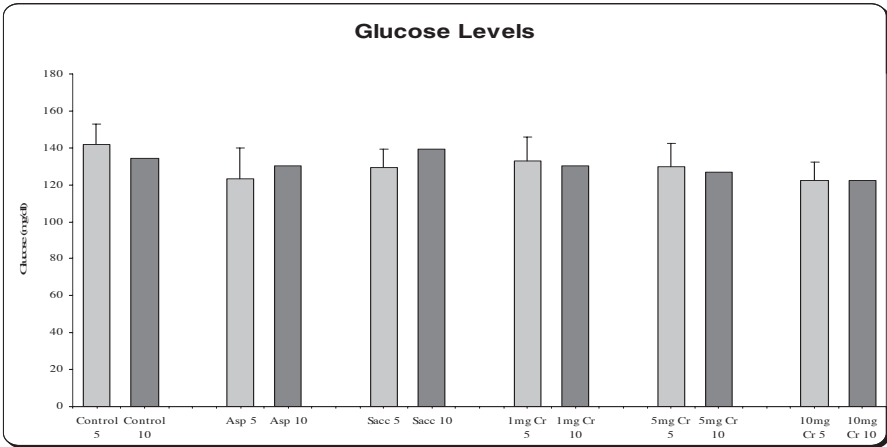
3. Results

The food intake and body mass of the control group and the other five experimental groups were statistically equivalent throughout the study, with little variance among groups (Appendix Figures 1 & 2). Body mass gain and food intake were both typical for rats of this age, meaning that neither artificial sweeteners nor chromium had a substantial effect. Water intake, although equivalent throughout the study for the control and aspartame groups, was greatly increased amongst the saccharin group (Appendix Figure 3).

At the five-week midpoint of the experiment, increased levels of chromium demonstrated increasingly lowering effects on concentrations of glucose, insulin, and leptin (Appendix Table 1). All experimental groups possessed lower glucose levels than the control, with the largest dose of chromium (10 mg Cr/kg) having the lowest (see below).

Figure 4

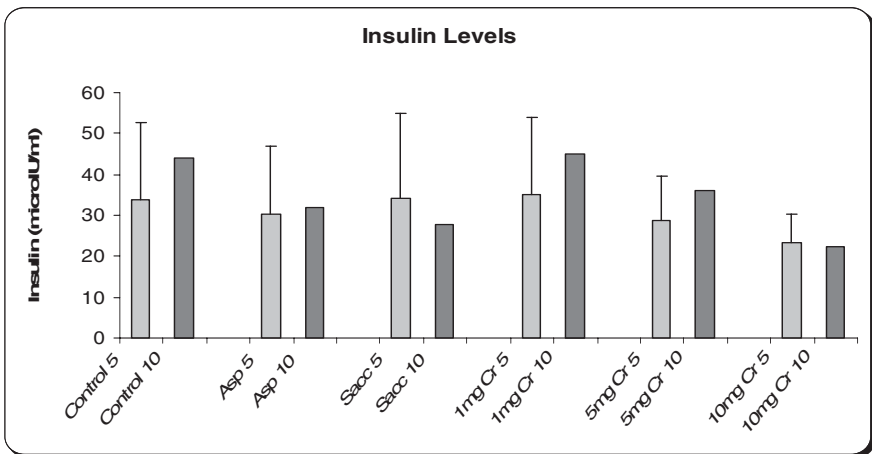
Glucose assay results for all groups at the midpoint of five weeks and end-point of 10 weeks.



Insulin levels were similar for the control and saccharin group but slightly increased for the group receiving the smallest dose of chromium (1mg Cr/kg). This result for the chromium group is highly irregular when compared to previous experimental data and is believed to be due to statistical error. The insulin levels for the aspartame and higher dose chromium groups all demonstrated some decrease, but the most significant change was observed amongst the 10 mg Cr/kg chromium group (see below).

Figure 5

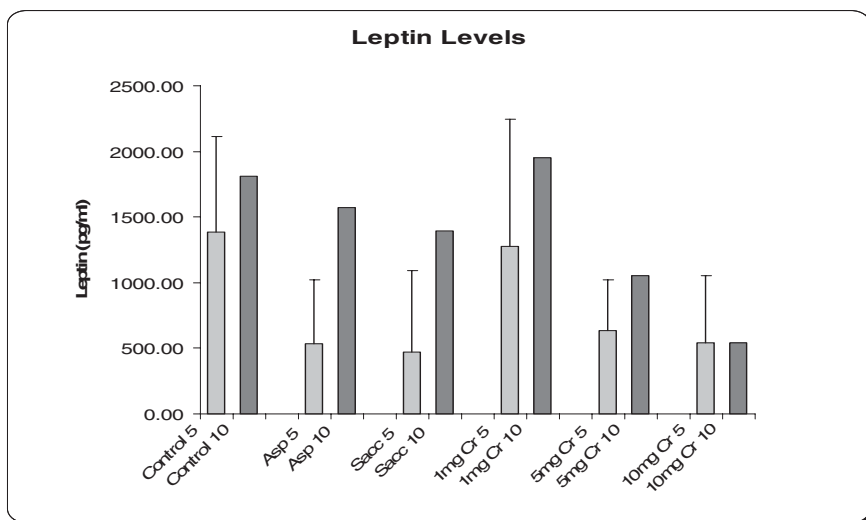
Insulin assay results for all groups at midpoint and endpoint.



Leptin levels were lower than the control for all groups, with the saccharin group demonstrating an especially low result (Figure 6).

The endpoint results indicated little variance in glucose levels and displayed trends similar to those at the midpoint. Glucose concentrations were lower than that of the control in all groups excluding that of the 1mg Cr/kg group. Overall insulin levels observed at the endpoint were higher than those at the midpoint. The two sugar substitutes groups and the 5 and 10mg Cr/kg groups were consistent in demonstrating insulin-lowering effects in comparison with the control at 10 weeks; furthermore, the effects were more dramatic at the endpoint. Leptin concentrations were overall much higher at the endpoint but demonstrated trends comparable to those at the midpoint when experimental group results were compared to those of the control (see below).

Figure 6
Leptin assay results for midpoint and endpoint.



Additional endpoint tests included a plasma HDL, triglyceride, and total cholesterol assay (Appendix Figures 7, 8, and 9). The most beneficial changes in HDL and total cholesterol levels were observed in the 1mg Cr/kg group. Conversely, there was the least beneficial change in triglyceride level amongst this group. The 5mg Cr/kg and 10mg Cr/kg groups exhibited the most consistent beneficial change in all three tests. The sugar substitutes were not beneficial in promoting total cholesterol lowering or increased HDL but did facilitate lowered triglycerides.

In regard to body composition, no visible differences were noted in the organ weight and composition of any rats with the exception of an observed

trend of gray liver color present amongst the aspartame group (Appendix Table 2). In addition, there were no statistically significant effects upon the quantities of epididymal, perineal, and subcutaneous fat amongst any group of rats.

4. Discussion

Aspartame has been shown by Beck et al. to experimentally decrease body mass.¹³ Thus, aspartame was meant to serve as a positive control for this experiment. However, the previous research's findings were not duplicated although the same methodology was utilized. No statistically significant change in food consumption, water intake, or body mass was observed between the aspartame and control group. Leptin levels, however, did decrease as observed in the previous aspartame study. In addition to leptin, glucose and insulin levels were also assayed and found to be lower for the aspartame group than those of the control. Although a slight increase was observed in HDL, aspartame produced no significant change in total cholesterol. Triglycerides concentrations, however, decreased.

The saccharin group also failed to demonstrate a deviation from the control in food intake or body mass. Water consumption, however, was much higher, possibly due to the rats' taste preference. The use of saccharin did positively affect leptin and glucose levels, but insulin levels remained consistent with data from the control group. This demonstrates that a decrease in glucose and leptin levels alone is not sufficient to positively affect insulin. The results for the use of this sugar substitute were similar to those for aspartame with respect to triglycerides, total cholesterol, and HDL, although the results were lower for triglycerides concentration and less effective for total cholesterol and HDL. Saccharin was also consistently ineffective in decreasing body fat from the aforesaid locations.

The largest pharmacological doses of Cr3 used to date were administered in this experiment. Because of the magnitude of the dose, it was uncertain whether the positive effects noted in other studies, such as decreased cholesterol, body fat, glucose levels, and insulin levels, would occur. Remarkably, the increasingly large doses continued to be increasingly effective at lowering glucose, insulin, and leptin levels, although seemingly anomalous results were observed at the 1mg Cr/kg dose. All rats appeared to be physically unaffected negatively by the large doses, and all organs and tissues recovered were normal.

5. Conclusion

Neither Cr3 supplementation at any concentration nor non-nutritive sugar substitute use led to outwardly discernable differences in the rats, or in their food or water consumption, other than the increased drinking fluid consumption by the saccharin-treated group. However, the physiological responses to the use of these substances differed. Overall the substances which produced the most consistently advantageous results upon factors relating to body mass, coronary health, and glucose management were saccharin and the two highest doses of chromium used, the 5mg Cr/kg and 10mg Cr/kg doses. Thus, this research demonstrates that none of these substances alone promotes change in body mass or could be used to promote weight loss but that some may improve certain aspects of overall health.

Acknowledgments

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Appendix

Figure 1

Average food intake for each group during the ten-week experiment.

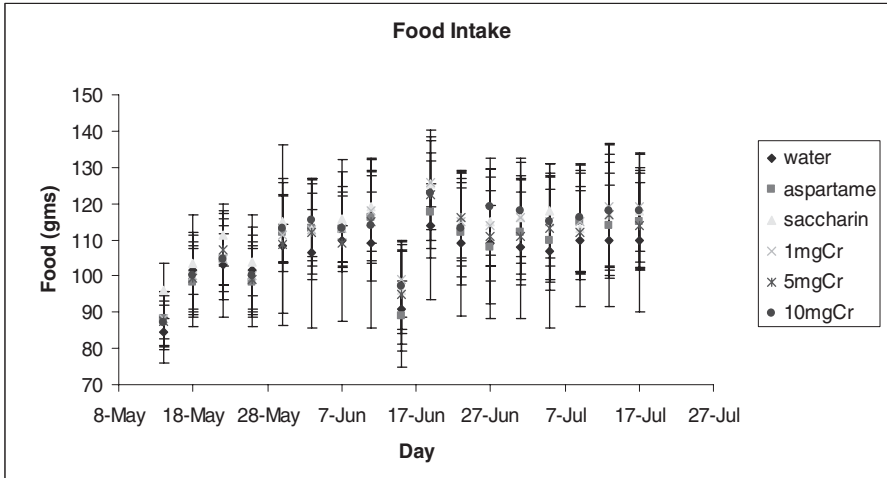


Figure 2

Average body mass for each group during the ten-week experiment.

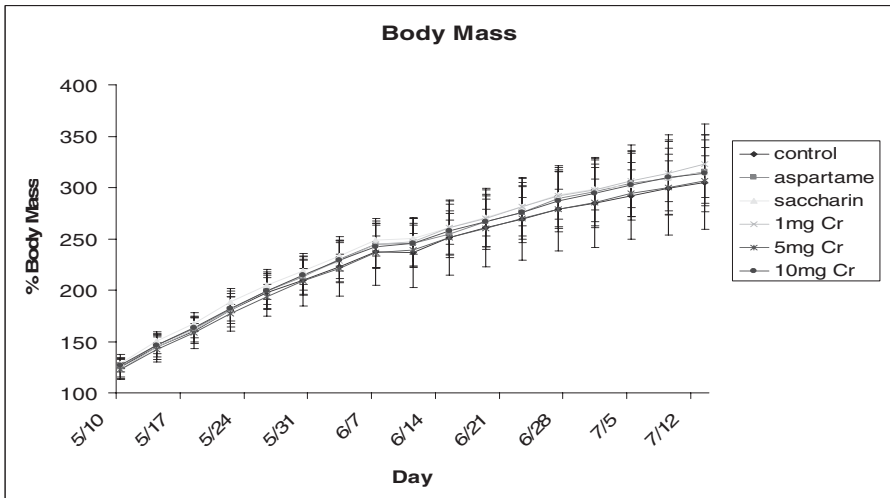


Figure 3

Average water consumption of control and groups with treated drinking water.

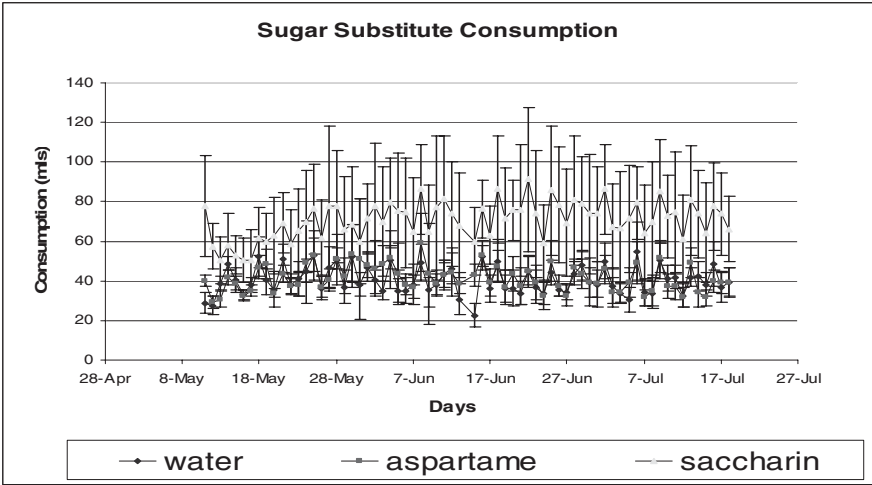


Table 1

Effects of Cr3 and artificial sweeteners on plasma variables of male Sprague-Dawley healthy rats after 5 and 10 weeks of administration. Values are means +/- the standard deviation; eight rats per group. For each variable for rats receiving Cr, means with different superscripts are significantly different from each other (P < 0.05). For rats receiving the artificial sweeteners, asterisks indicate variables significantly different from those of the control (P < 0.05).

	Glucose (mg/dL)	Insulin (µIU/mL)	Leptin (pg/mL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)
Week 5							
Control	142.1 +/- 20.0 ^a	42.8 +/- 18.4 ^a	1389 +/- 179 ^a	ND	ND	ND	ND
1mg Cr	132.8 +/- 9.3 ^b	31.4 +/- 10.7 ^a	1279 +/- 1164 ^b	ND	ND	ND	ND
5mg Cr	129.9 +/- 17.5 ^b	26.7 +/- 11.4 ^a	630.9 +/- 200.0 ^b	ND	ND	ND	ND
10mg Cr	122.6 +/- 9.2 ^c	26.4 +/- 12.8 ^a	557.6 +/- 335.3 ^b	ND	ND	ND	ND
Aspartame	123.5 +/- 9.1 [*]	26.0 +/- 11.7 [*]	534.9 +/- 234.1 [*]	ND	ND	ND	ND
Saccharin	129.1 +/- 14.6	41.5 +/- 29.2	468.8 +/- 364.2 [*]	ND	ND	ND	ND

(continued on next page)

Table 1, continued

	Glucose (mg/dL)	Insulin (μ IU/mL)	Leptin (pg/mL)	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)
Week 10							
Control	133.9 +/- 10.8 ^a	44.1 +/- 19.0 ^a	1814 +/- 723 ^a	82.1 +/- 16.4 ^a	100.1 +/- 9.0 ^a	53.3 +/- 9.2 ^a	33.0 +/- 14.2 ^a
1mg Cr	130.5 +/- 9.3 ^b	45.0 +/- 18.9 ^a	1947 +/- 967 ^b	61.0 +/- 25.1 ^b	90.4 +/- 12.8 ^a	57.0 +/- 6.0 ^b	21.5 +/- 11.8 ^a
5mg Cr	127.0 +/- 12.5 ^a	36.0 +/- 10.8 ^b	1054 +/- 394 ^c	38.4 +/- 18.7 ^c	91.6 +/- 7.3 ^a	55.2 +/- 5.5 ^a	26.8 +/- 9.8 ^a
10mg Cr	122.3 +/- 10.0 ^b	22.4 +/- 7.0 ^c	1075.6 +/- 863 ^c	36.9 +/- 11.5 ^c	89.0 +/- 12.9 ^a	55.1 +/- 5.1 ^a	24.1 +/- 10.7 ^a
Aspartame	130.4 +/- 16.4	31.8 +/- 16.8	1575 +/- 488	60.7 +/- 22.2 ^b	99.0 +/- 12.2	54.90 +/- 7.1	13.1 +/- 14.6 ^b
Saccharin	139.3 +/- 10.7	27.9 +/- 20.7	1395 +/- 619	55.0 +/- 12.7 ^b	96.6 +/- 10.7	51.1 +/- 9.1	27.1 +/- 10.8

Figure 7

Results of endpoint high-density lipoprotein cholesterol assay for all groups.

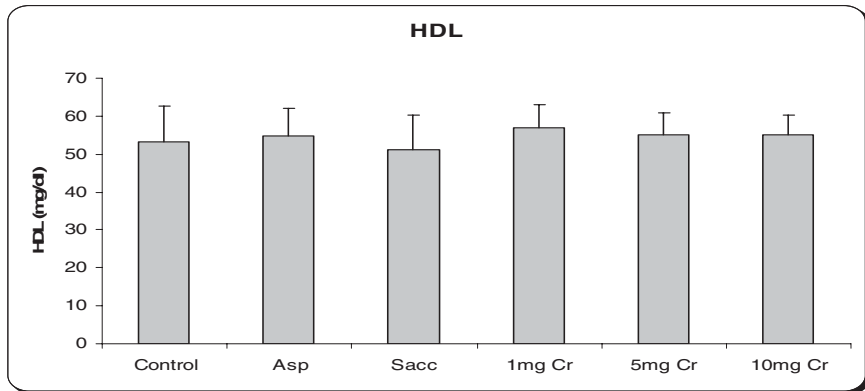


Figure 8

Results of endpoint triglyceride assay for all groups.

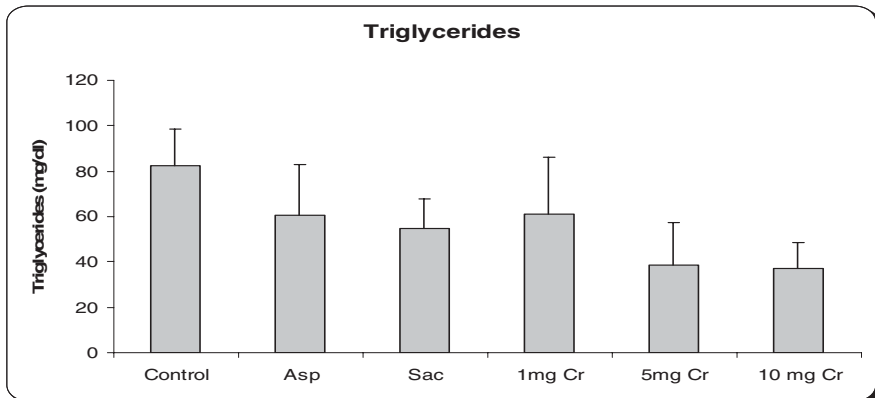


Figure 9

Results of endpoint cholesterol assay for all groups.

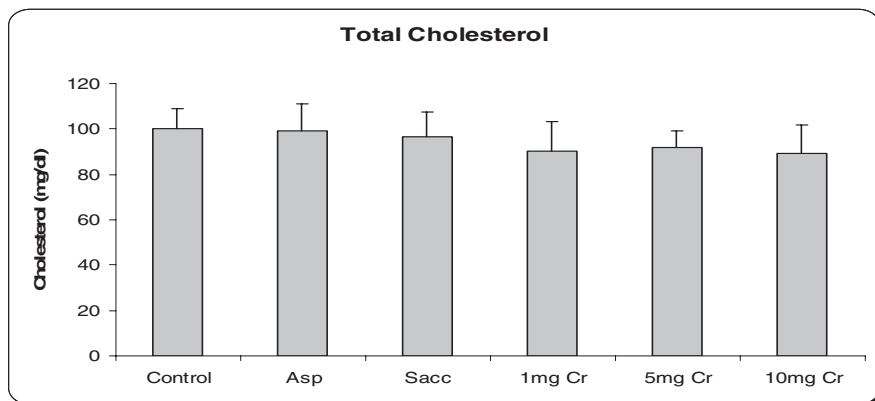


Table 2

Percentage of relative organ mass (tissue mass/body mass x 100%) of male Sprague Dawley rats after 10 weeks of administration of Cr³⁺ and artificial sweeteners. Values are means +/- the standard deviation; eight rats per group.

Tissue	Control	Aspartame	Saccharin	1 mg Cr	5 mg Cr	10 mg Cr
Heart	0.26 +/- 0.031	0.27 +/- 0.035	0.26 +/- 0.022	0.26 +/- 0.045	0.27 +/- 0.032	0.27 +/- 0.031
Liver	3.9 +/- 0.81	3.4 +/- 0.63	3.5 +/- 0.29	3.7 +/- 0.43	3.6 +/- 0.561	3.8 +/- 0.76
Kidney	0.69 +/- 0.12	0.70 +/- 0.083	0.71 +/- 0.083	0.69 +/- 0.097	0.68 +/- 0.036	0.71 +/- 0.069
Pancreas	0.42 +/- 0.17	0.35 +/- 0.082	0.38 +/- 0.083	0.38 +/- 0.086	0.40 +/- 0.056	0.34 +/- 0.093
Testes	0.65 +/- 0.12	0.67 +/- 0.052	0.67 +/- 0.080	0.64 +/- 0.041	0.65 +/- 0.038	0.69 +/- 0.020
Spleen	0.16 +/- 0.046	0.19 +/- 0.029	0.15 +/- 0.022	0.16 +/- 0.016	0.16 +/- 0.037	0.16 +/- 0.017
Epididymal fat	2.1 +/- 0.79	2.0 +/- 0.63	1.9 +/- 0.26	2.3 +/- 0.35	2.0 +/- 0.37	2.0 +/- 0.38
Perineal fat	0.13 +/- 0.052	0.097 +/- 0.018	0.10 +/- 0.041	0.12 +/- 0.049	0.11 +/- 0.038	0.098 +/- 0.021
Subcutaneous fat	2.1 +/- 0.68	2.1 +/- 0.40	2.0 +/- 0.50	2.2 +/- 0.58	1.7 +/- 0.50	1.7 +/- 0.41