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Potentiation of Diabetes By Testosterone in Male Rats

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Diabetes mellitus is what many people refer to simply as diabetes. It affects thousands of people directly and is an indirect cause of blindness, renal failure, myocardial infarction, and stroke. The symptoms of diabetes are increased thirst, appetite, fatigue, urination, and weight loss (Wilson, J., 1985). A common symptom for male diabetics is impotence. It is estimated that 37-55% of male diabetics become impotent (Brotherton, J., 1976). Diabetes is gentically inherited and is caused by a lack of functioning beta cells of the pancreas. Deficient beta cells result in a lack of insulin and therefore a low insulin to glucose ratio. Diabetes has been referred to as a disease of starvation in the midst of plenty. The tissues of the body are literally starved for glucose because without insulin, the glucose cannot be used by the tissues. The glucose content in the blood increases while the utilization of glucose by peripheral tissues decreases. Diabetes also results in a decrease in muscle anabolism and an increase in ketogenesis. The use of ketone bodies for energy, however, is decreased in diabetes. Diabetes causes adipose tissue triglycerides to be mobilized at an increased rate. These triglycerides are converted to free fatty acids, thus increasing the level of free fatty acids in the plasma (Berne, 1988). It is this last symptom of diabetes that will be the topic of this experiment.

Free fatty acids are a common source of energy for the body. They can go through the citric acid cycle in order to produce ATP for energy or they can be converted into ketone bodies and used for energy (Fox, Stuart Ira, 1984). Excess free fatty acids are carried by the blood to the liver. In the

liver, free fatty acids are re-esterified and secreted back into circulation as very low density lipoproteins (VLDL) (Crepaldi, G. 1985). Estrogens in diabetics show an increase in VLDL whereas testosterone shows a decrease in VLDL. This may be due to testosterone indirectly inhibiting the uptake of free fatty acids into the liver (Brotherton, J., 1976). Testosterone regulates the activity of lipoprotein lipase and the lipase inversely regulates VLDL synthesis (Crepaldi, G., 1985).

Testosterone itself also has an effect on fatty acid mobilization. It is possible that testosterone acts synergistically with diabetes and therefore enhances the symptoms of diabetes. It is the purpose of this study to investigate the effects of testosterone on diabetic male rats.

This research project was based on the results of a previous experiment. Due to the high rate of impotence found in male diabetic patients, testosterone was given to diabetic male rats, in an effort to reverse the diabetic effects. Testosterone, being an anabolic sex steroid, was expected to increase body weight and hopefully reverse the abnormal sex behavior. The opposite of the expected results were observed in the previous experiment. The animals lost weight, at a rate faster than the diabetic animals not receiving testosterone, and the abnormal sex behavior was not reversed. In other words, the exogenous testosterone appeared to make the animals more diabetic. Since this was completely unexpected, possible explanations were sought, and thus the basis for this experiment.

In this experiment, four subject groups of rats were set up. Group 1, STZ, was a diabetic group receiving oil injections, and group 2, STZ + T, was a diabetic group receiving testosterone injections. Group 3 served as

the control group and received oil, and group 4 was normal and received testosterone injections. The subcutaneous injections were given daily and the amount given was based on the individual animal's body weight. All animals were weighed on March 23 and groups 1 and 2 were given intraperitoneal streptozotocin, STZ, injections at a dose of 50 mg/kg of body weight. The STZ rendered those animals diabetic. Animals from groups 3 and 4, were injected only with a citrate buffer, in order to maintain consistency. The animals were all weighed every other day thereafter, in order to adjust the testosterone dosage for changing body weight. Both STZ and testosterone dosages are listed in table 1. Groups 1 and 3 received daily subcutaneous sesame seed oil injections, while groups 2 and 4 received daily subcutaneous testosterone injections. The testosterone was diluted with the sesame seed oil so that the dose was 0.5 ug/gm of body weight. Animals were injected daily beginning on March 24 and proceeding to April 9, 1989. For one week during the experiment, food and water intake was measured. Then, on April 10 the animals were sacrificed by decapitation and the following were collected: blood, brain, heart, liver, pituitary, spleen, kidneys, adrenal glands, epididymal fat pad, gastrocnemius muscle, seminal vesicles, and testis. The blood samples were used to measure glucose levels of the animals, using the Sigma glucose test. All organs were weighed for wet weight. Then all organs, except for pituitaries and brains, were dried in an oven in order to obtain dry weights.

RESULTS

The body weights of all the animals were recorded every other day. As was previously mentioned, the diabetic animals that were receiving testosterone injections, lost more body weight than any other group. The normal rats receiving testosterone gained more weight than any other group. These results are easily seen in graph 1. On day 1 the four groups were not significantly different in body weights ($p=0.3377$), but on day 5 of the experiment, the body weights between the diabetic and control groups were significantly different ($p=0.0001$). By day 9 of the study, all four groups were significantly different from each other ($p=0.0001$). This is consistent with the data which was recorded in the previous study (graph 2).

The food and water intake was measured for five days. The average food intake of the diabetic rats was 190% higher than the non-diabetic rats. The water intake of the diabetic animals (groups 1 and 2) was 505% higher than the non-diabetic animals. Group 4, Ctrl + T, had a slightly higher water consumption, than the control (group 3), but it was not significant. The increased water and food consumption of the diabetic animals was expected due to the clinical fact that diabetics normally consume large quantities of fluids and increased amounts of food. The diabetic rats were also observed to be urinating more than the non-diabetic rats. These results are depicted in graphs 3 and 4.

Glucose tests were run on the blood samples. This is also used clinically to diagnose diabetes. The average glucose level for group 1, STZ, was 498.3 mg/ml and for group 2, STZ + T, was 536.5 mg/ml. The average glucose levels for group 3, Ctrl, and group 4, Ctrl + T, were 56.2

mg/ml and 67.4 mg/ml respectively. The diabetic groups, 1 and 2, have a significantly higher plasma glucose concentration. This can be seen in graph 5.

The organ weights were measured for both wet and dry weights. For the wet weights, the heart, liver, and adrenal glands did not have any significant differences. The following organs were significantly different ($p<0.05$): pituitary, kidneys, spleen, skeletal muscle, epididymal fat pad, testis, and seminal vesicles. The dry organ weights were not significantly different for the heart, liver, kidneys, adrenal glands, and testis. The spleen, skeletal muscle, epididymal fat pad, and the seminal vesicles were all significantly different except between the two diabetic groups (STZ and STZ + T). These results can be seen in graphs 6-16.

DISCUSSION

The increased rate of body weight loss in the diabetic, testosterone treated rats, group 2, may be due to synergistic actions of diabetes and testosterone on free fatty acids (FFA). Free fatty acids are a main source of energy in the body. They contribute approximately 41-49 % of the energy used by the striated muscle alone (Reiser, Peter. 1967). In diabetes, due to the fact that the glucose utilization by striated muscles is inhibited by the lack of insulin, the muscles depend more heavily on free fatty acids as their energy source (Brotherton, J. 1976). There are several mechanisms which cause an increase in the concentration of free fatty acids in the blood. These are due to the actions of both testosterone and diabetes on free fatty acids.

Normally testosterone causes an increased mobilization of fatty acids from adipose tissue. The fatty acids then bind to albumin in the blood and are carried to the skeletal muscles, where they will be used as an energy source. The free fatty acids are then used to increase the muscle mass (Berne, 1988). This process is normally aided by insulin. The lack of insulin, as in diabetes, inhibits the muscles from taking up the free fatty acids from the bloodstream. Therefore, the concentration of FFA in the blood would increase due to the exogenous testosterone (Brotherton, J. 1976).

Testosterone also regulates the local activity of lipoprotein lipase. This lipase accelerates the mobilization of lipids into free fatty acids. The increase in testosterone, due to the exogenous injections, would cause and increase in the activity of the lipoprotein lipase, and thus the increase of free fatty acids in the blood. Lipoprotein lipase activity is also inversely related to the synthesis of VLDL (Crepaldi, G. 1985). The increased testosterone accounts for the decreased VLDL in the plasma. Estrogens, which are not known to affect the lipoprotein lipase, show an increase in VLDL (Brotherton, J. 1976). These increased levels of free fatty acids, due to testosterone, may enhance the effects of diabetes.

The potentiation of diabetes by testosterone, has been noted in other studies. S. G. Paik noted that testosterone enhances the induction of diabetes in genetically susceptible mice, and that estrogens inhibit the induction of diabetes by streptozotocin (Paik, S. G., 1982). Another study by R. A. Kava, stated that in men and women with matched body percent fat, men were more susceptible to developing diabetes. This sexually

dimorphic response to diabetes may be due to the ratio of androgens to estrogens in the genetically susceptible animal (Brotherton, J., 1976). This may prove to be the reason that males more commonly develop diabetes.

Diabetes in itself also causes an increase in the plasma level of free fatty acids through several mechanisms. One mechanism by which diabetes causes increased plasma free fatty acids is a higher turnover rate of free fatty acids. Insulin inhibits the mobilization of free fatty acids in two ways. First, the action of insulin to move glucose into the cell produces a substance called glycerophosphate. Glycerophosphate is used by the body to re-esterify free fatty acids. The second mechanism is also due to the actions of insulin. Insulin inhibits many adipokinetic hormones. Some of these hormones are epinephrine, norepinephrine, adrenocorticotrophin, glucagon, and cortisol. Without insulin these substances would enhance the rate of mobilization of free fatty acids into the blood. The lack of insulin would produce an increased concentration of free fatty acids by mobilizing them and not allowing them to be removed by the normal re-esterification process (Reiser, Peter, 1967).

Another action of diabetes, would be a decrease in the rate of carbohydrate metabolism. Thirty percent of the dietary carbohydrates are stored in adipose tissue. In diabetics, the carbohydrates are not easily stored, which causes a decrease in the synthesis of fatty acids by the adipose tissue. The fatty acids that are stored there, are hydrolyzed into free fatty acids in order to be used as an energy source by the body. This may lead to the common symptom in diabetics of metabolic acidosis.

A lack of insulin also greatly affects the synthesis of proteins. Insulin normally stimulates adipose tissue and muscles to convert amino acids into proteins. In the testis, insulin also causes an increase in amino acid uptake and conversion into proteins (Reiser, Peter, 1967). It seems likely that insulin is necessary for muscle anabolism. Testosterone, therefore in diabetics, may be blocked from increasing muscle mass due to the lack of insulin. This may account for the decrease in testis weight, epididymal fat pad weight, and skeletal muscle weight in the diabetic animals, especially those receiving testosterone (group 2).

CONCLUSION

The lack of insulin acts to indirectly inhibit the re-esterification of fatty acids. This causes them not to be removed from the blood, therefore increasing the plasma concentraion of free fatty acids. Insulin also causes the inhibition of many hormones. These hormones normally mobilize fatty acids into the blood. Since the hormones are not inhibited in diabetics, the increased rate of mobilization results in increased levels of plasma free fatty acids. Diabetes also decreases the storage rate of free fatty acids allowing them to build up in the blood. The lack of insulin may also cause a decrease in muscle anabolism. Testosterone, which normally builds muscle, may not be able to do so without insulin. Testosterone also mobilizes fatty acids causing increased levels in the blood. The actions of the body caused by the lack of insulin, in addition to the actions of testosterone may be synergistic. They cause an increased level of free fatty acids in the blood. One study has shown, that normal

tissues can be made to behave like tissues from diabetic animals by raising the free fatty acids concentration of the medium which bathes the tissues (Reiser, Peter, 1967). Exogenous testosterone may raise the plasma free fatty acid concentration of the blood enough to potentiate diabetes in the living tissues of the male rat. The endogenous testosterone of the male may explain why males are more prone to developing diabetes.

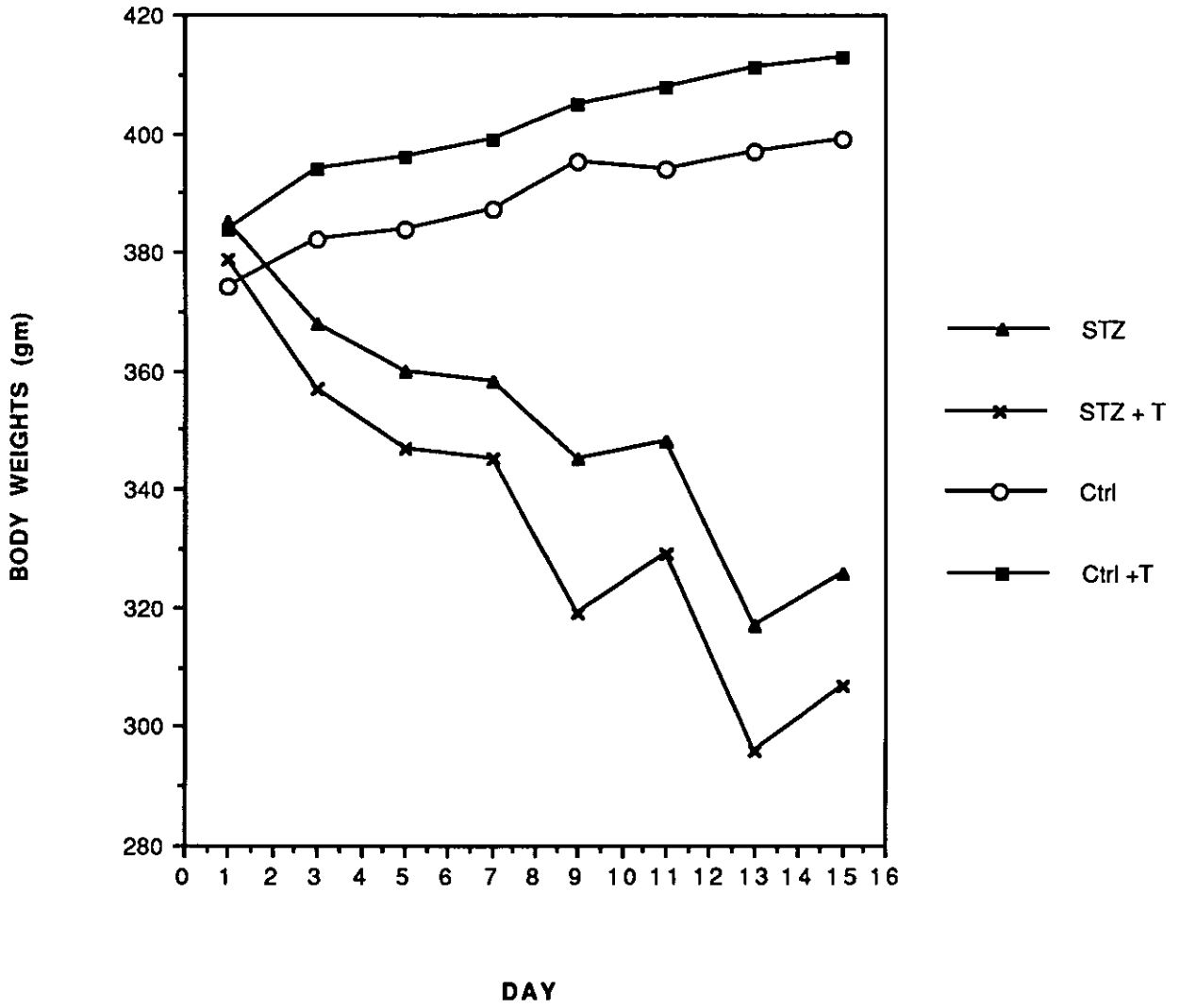
There are many other studies which can stem from this experiment. Examining the direct levels of plasma free fatty acids throughout the experiment would most definately provide useful information. Also studying treatment with nicotinic acid would be interesting. Nicotinic acid inhibits free fatty acid mobilization (Crepaldi, G. 1985). The effects of growth hormone and ACTH may also play a role in the process. Advanced studies on this topic may need to consider these effects. Also, measuring the cholesterol, VLDL, HDL, and LDL levels in the blood would be of interest. Monitoring the activity of the hormones and enzymes which participate may also be of use. Overall much more in depth research is needed to conclusively prove the mechanism of action suggested here.

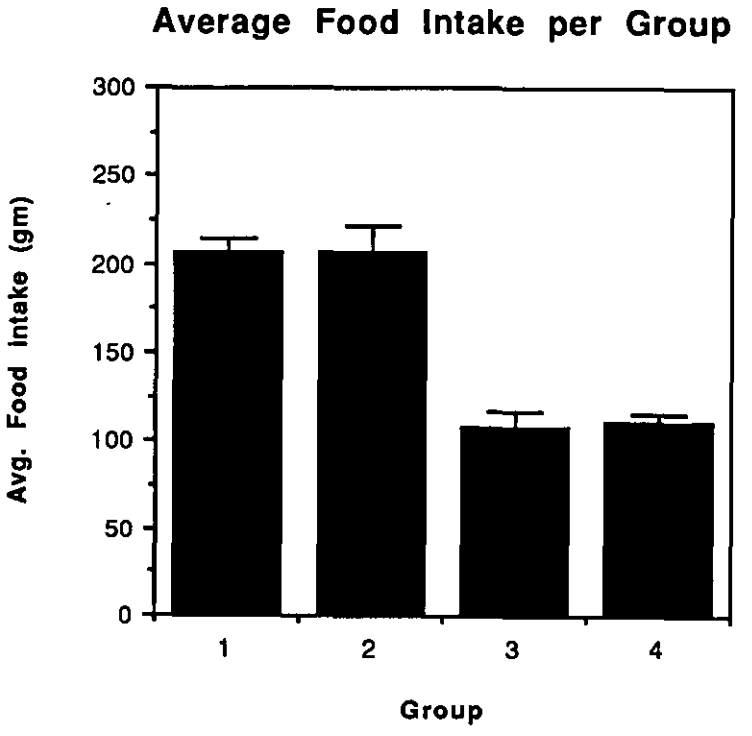
STREPTOZOTOCIN AND TESTOSTERONE DOSAGES

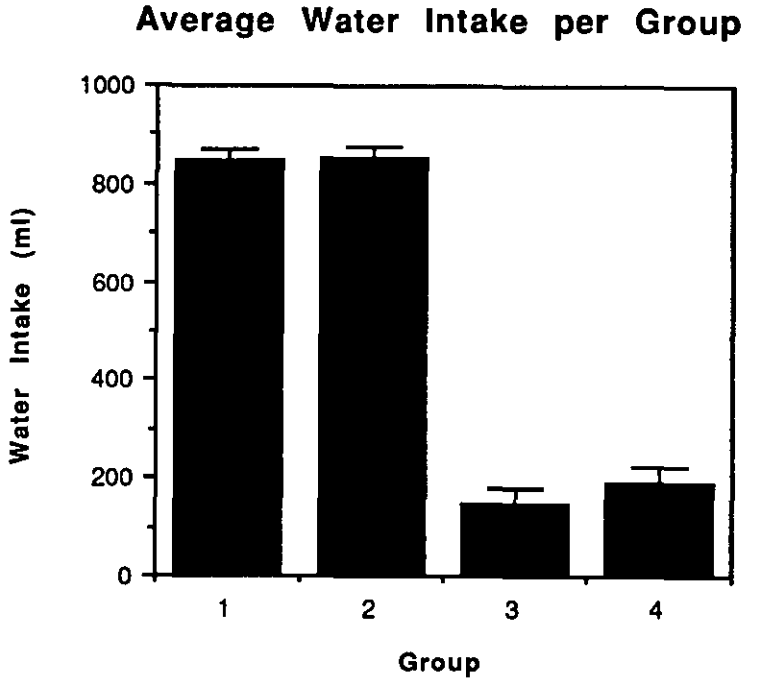
BY BODY WEIGHT

Body Weight (grams)	Streptozotocin (mls)	Testosterone (mls)
280	0.28	0.140
290	0.29	0.145
300	0.30	0.150
310	0.31	0.155
320	0.32	0.160
330	0.33	0.165
340	0.34	0.170
350	0.35	0.175
360	0.36	0.180
370	0.37	0.185
380	0.38	0.190
390	0.39	0.195
400	0.40	0.200
410	0.41	0.205
420	0.42	0.210
430	0.43	0.215
440	0.44	0.220

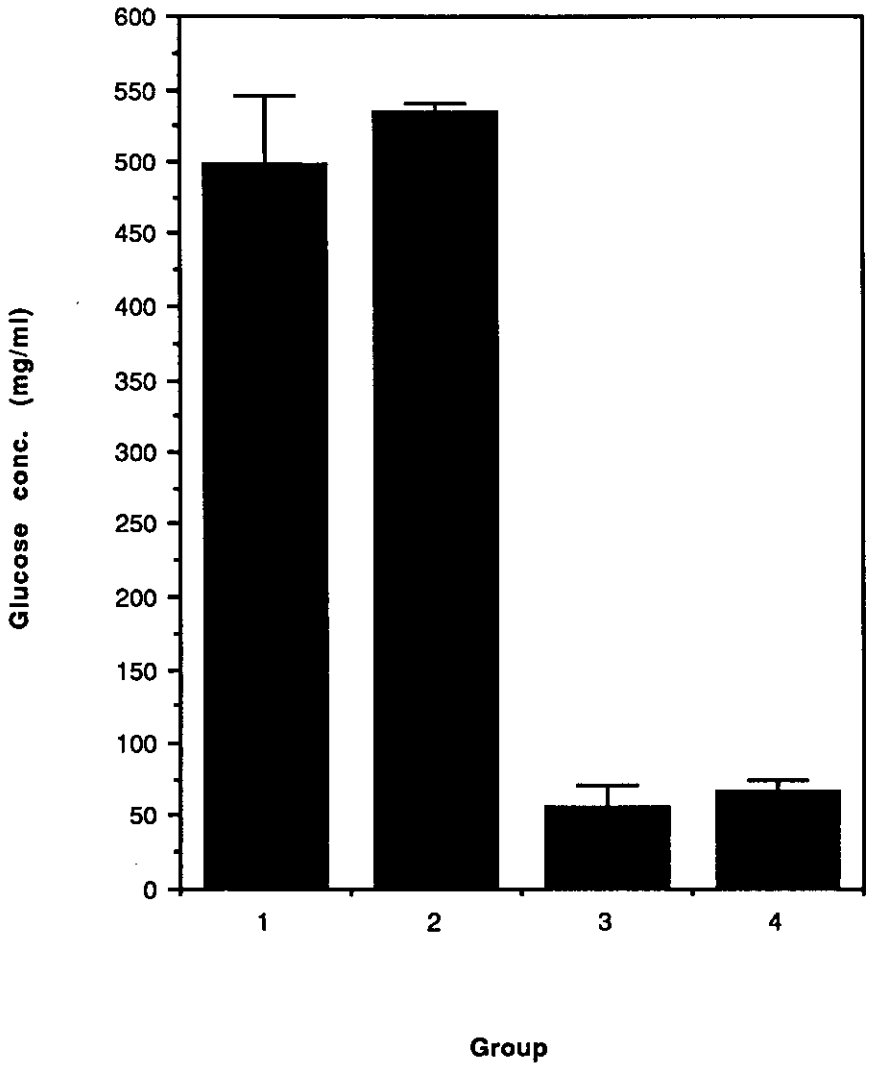
AVERAGE BODY WEIGHTS

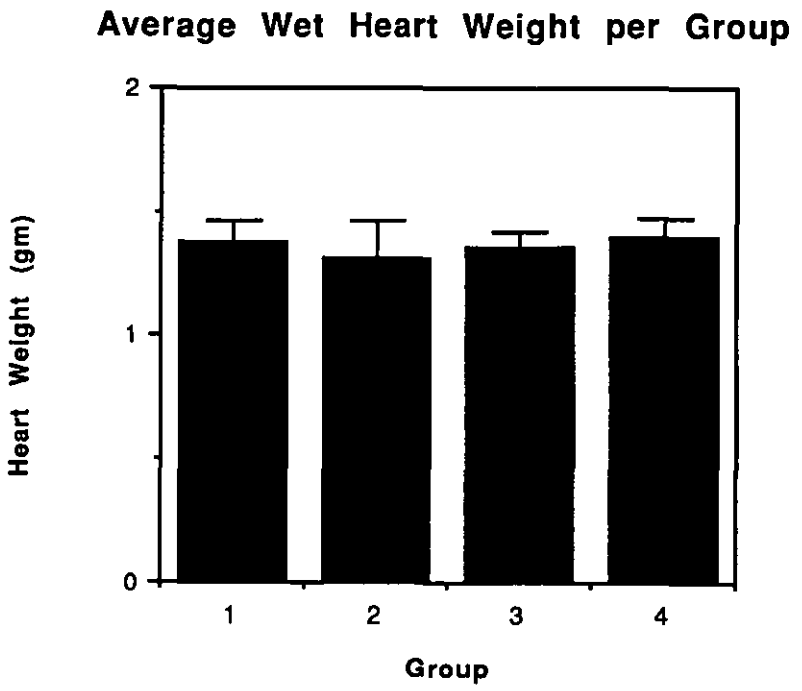


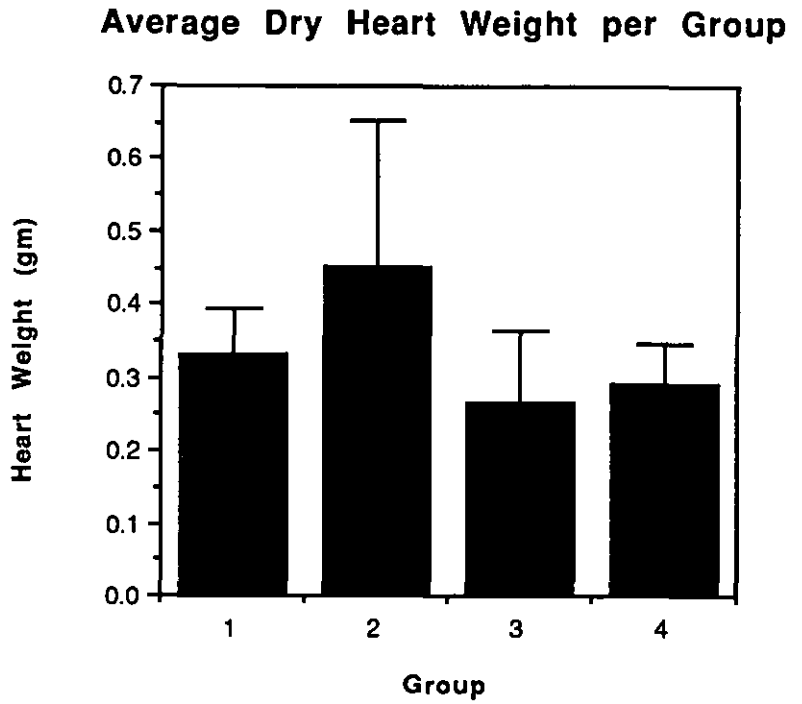

Average Food Intake per Group
Avg. Food Intake (gm)
300
250
200
150
100
50
0
1
2
3
4
Group


Average Water Intake per Group
Water Intake (ml)
1000
800
600
400
200
0
1
2
3
4
Group

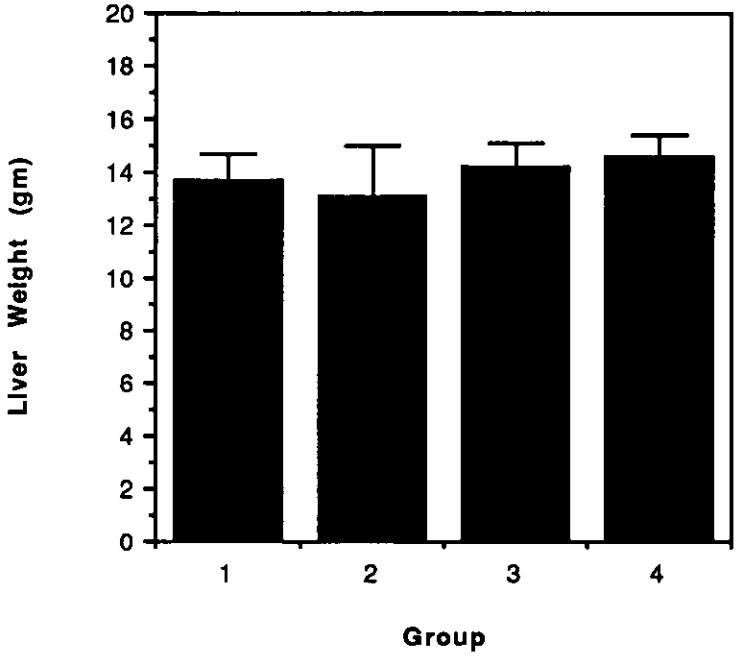
Average Glucose Concentration per Group



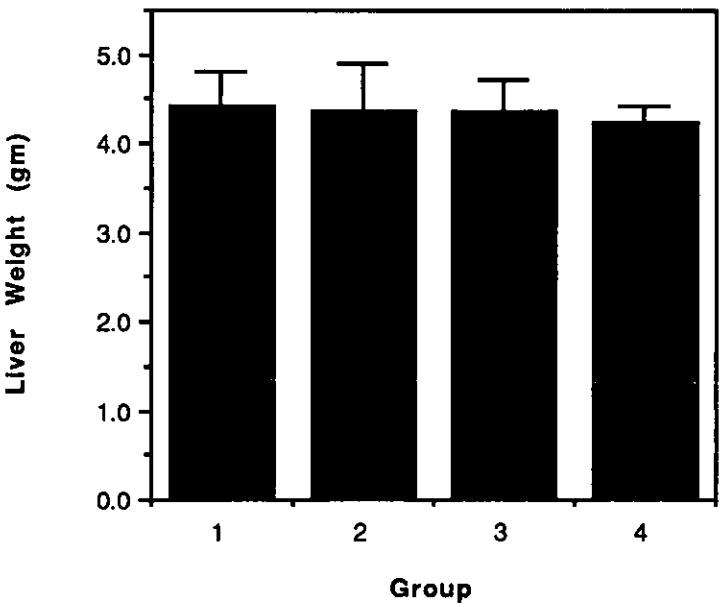

Average Wet Heart Weight per Group
Heart Weight (gm)
2
1
0
1
2
3
4
Group


Average Dry Heart Weight per Group
Heart Weight (gm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
Group

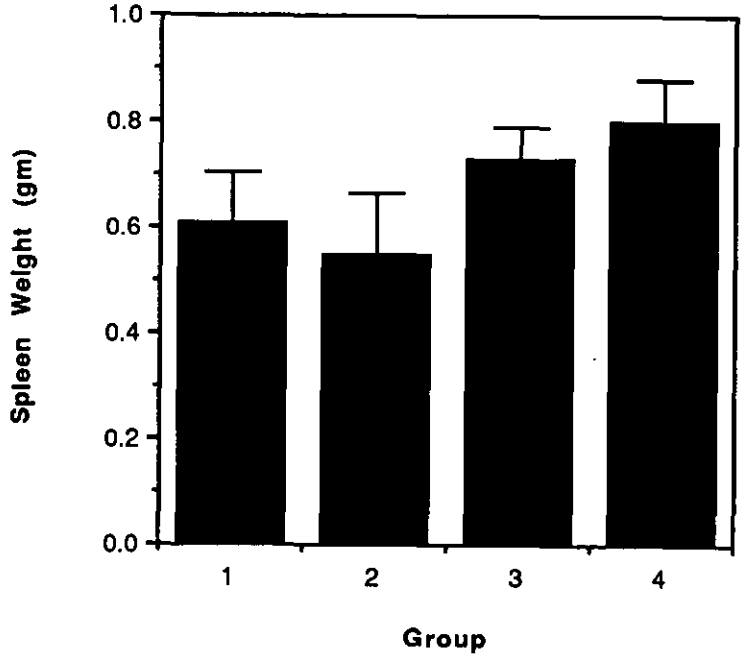
Average Wet Liver Weight per Group



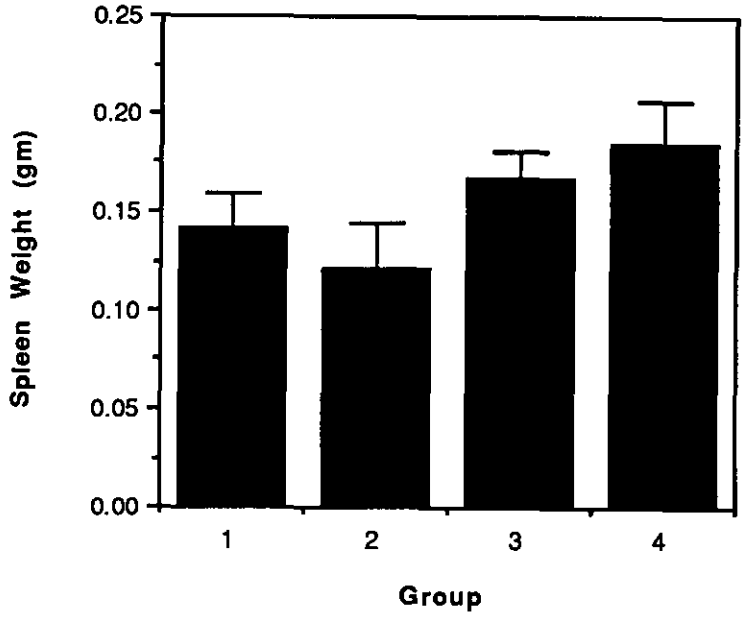
Average Dry Liver Weight per Group

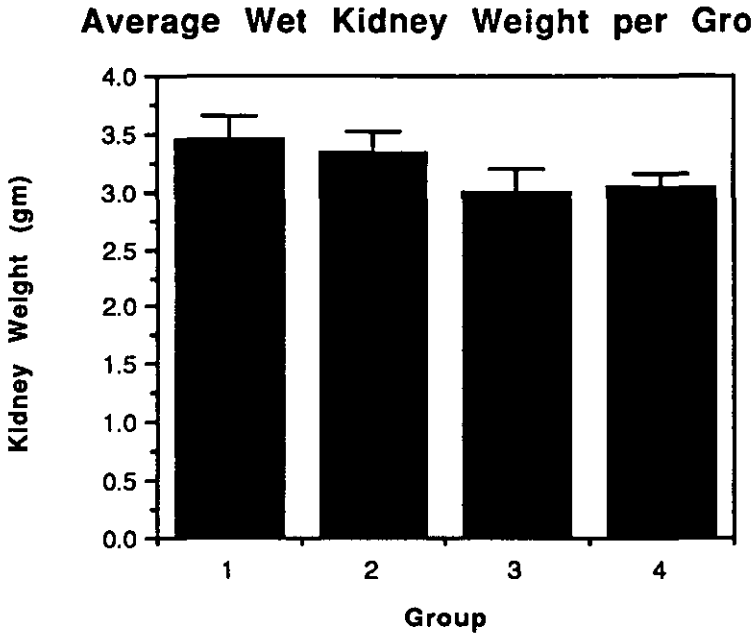


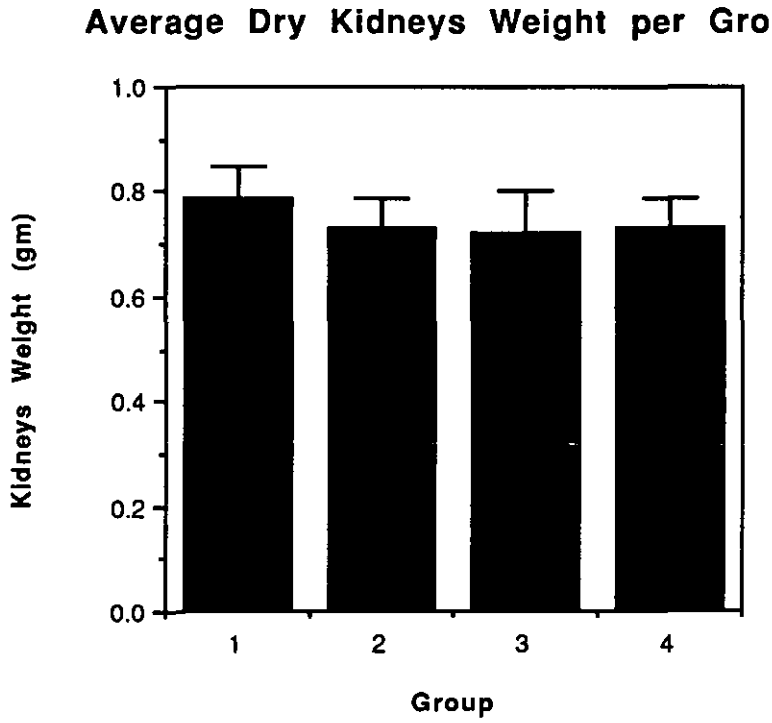
Average Wet Spleen Weight per Group



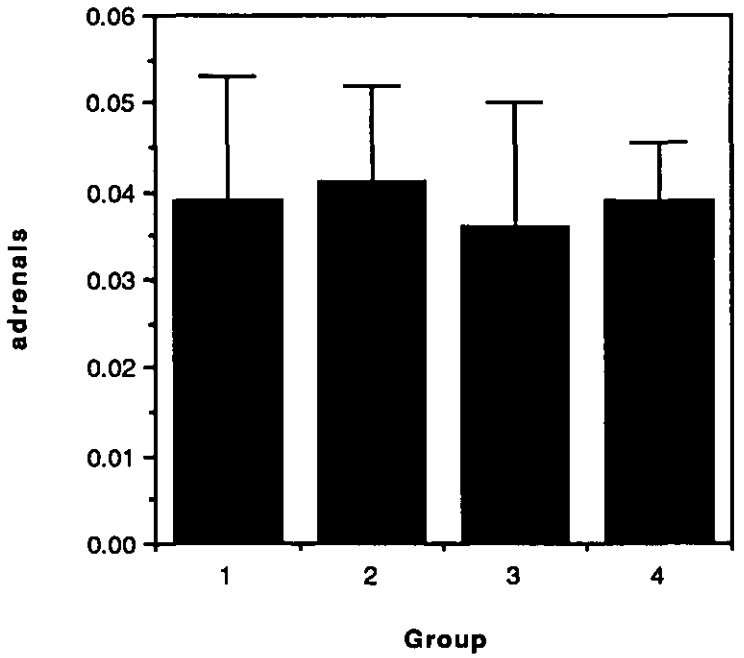
Average Dry Spleen Weight per Group



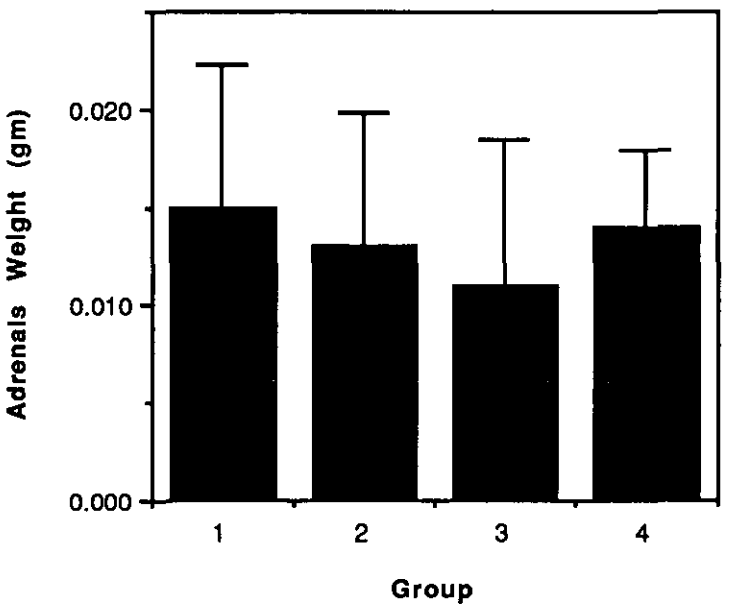

Average Wet Kidney Weight per Group
Kidney Weight (gm)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
Group

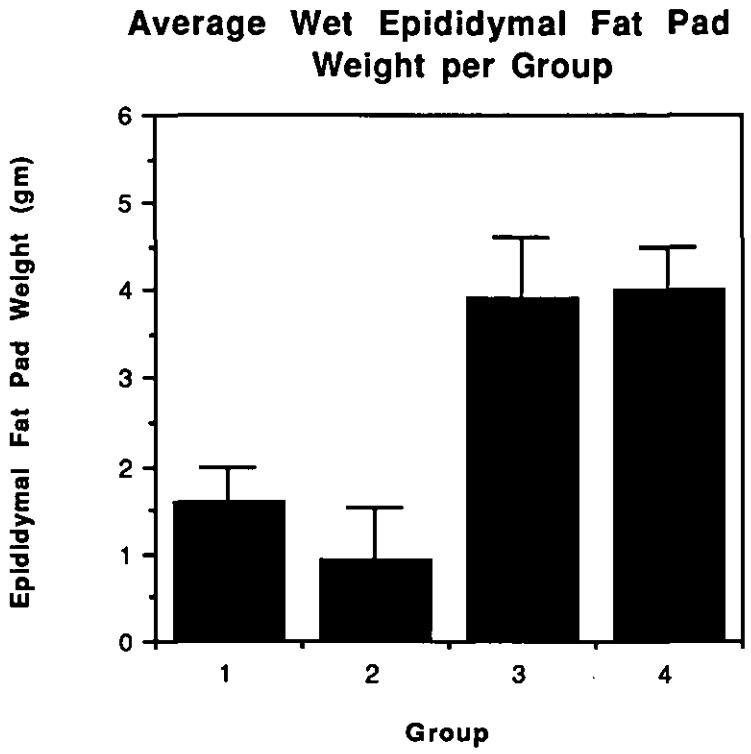

Average Dry Kidneys Weight per Group
Kidneys Weight (gm)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
Group

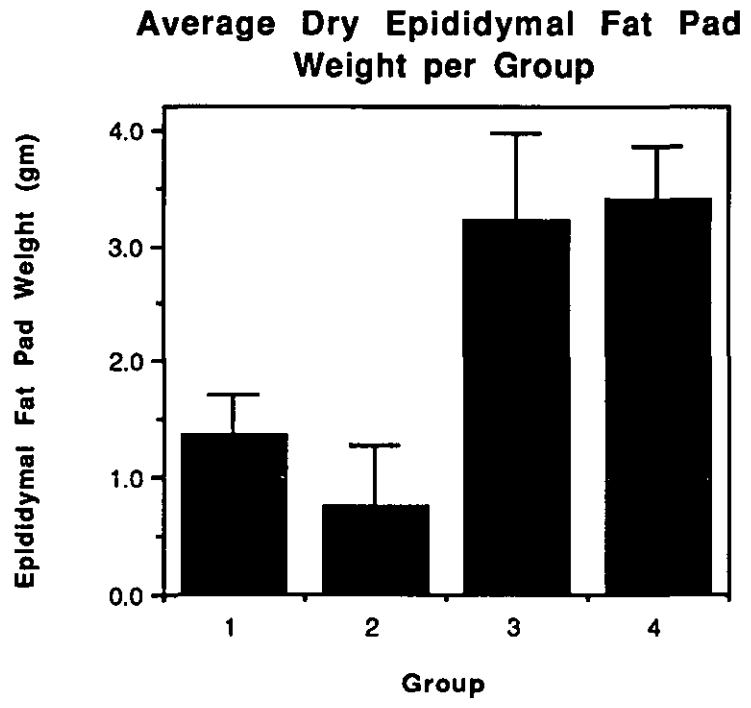
Average Wet Adrenals Weight per Group

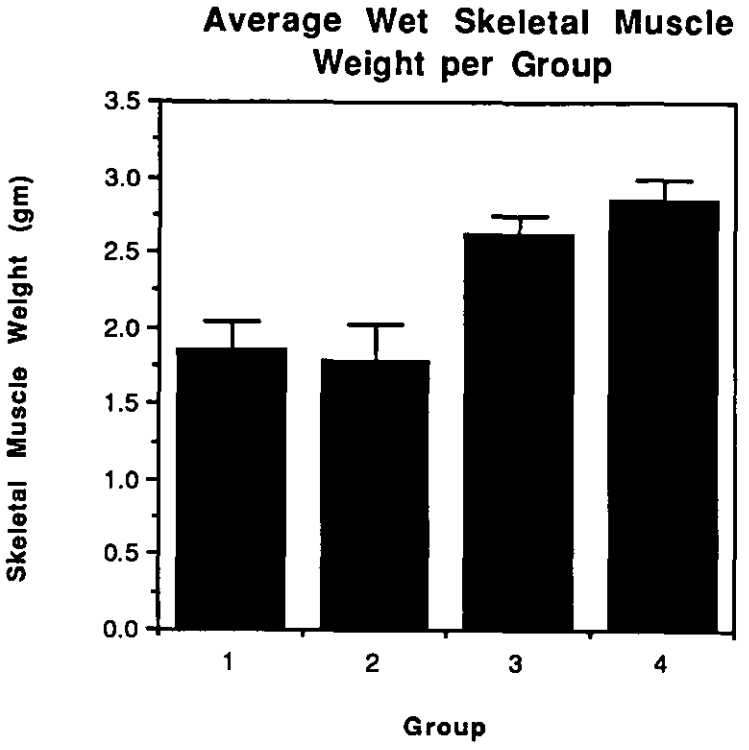


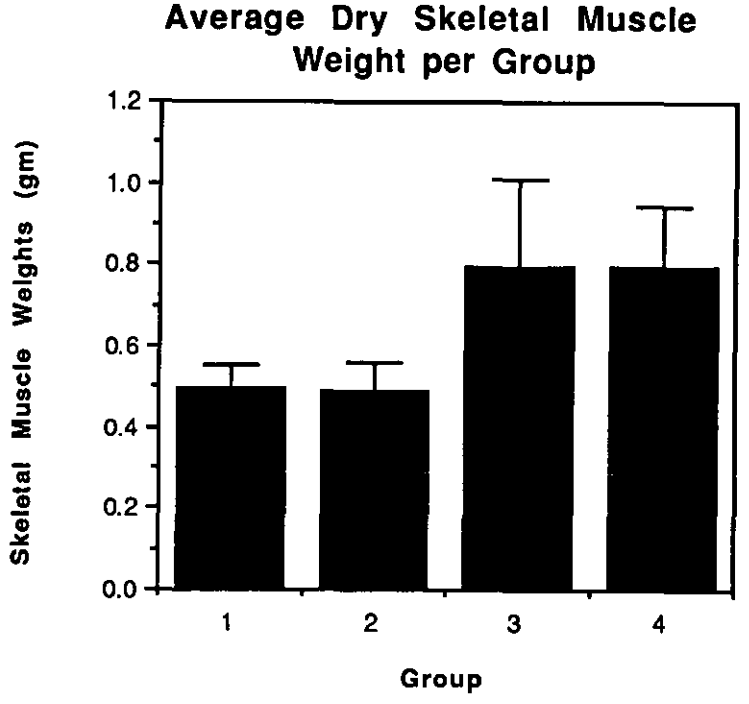
Average Dry Adrenal Weight per Group

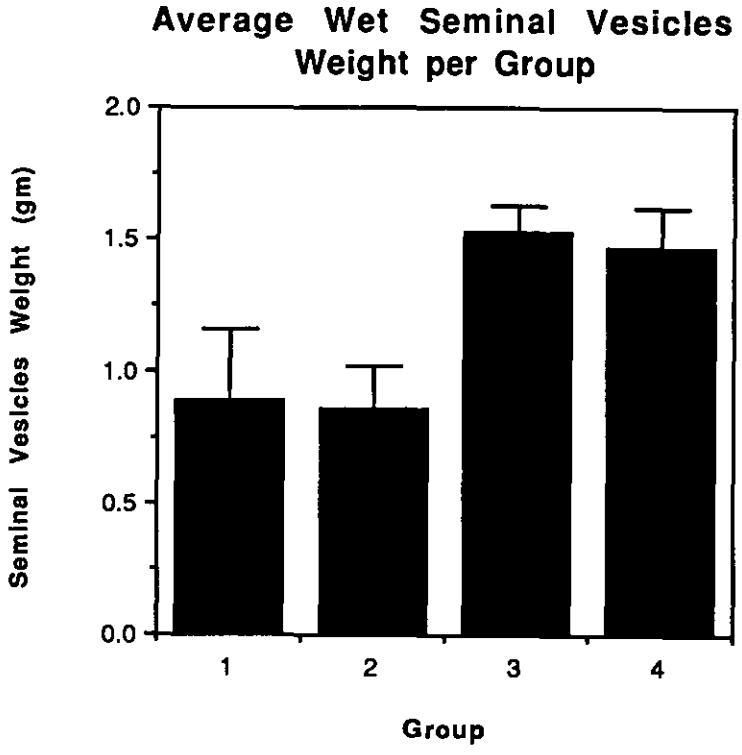


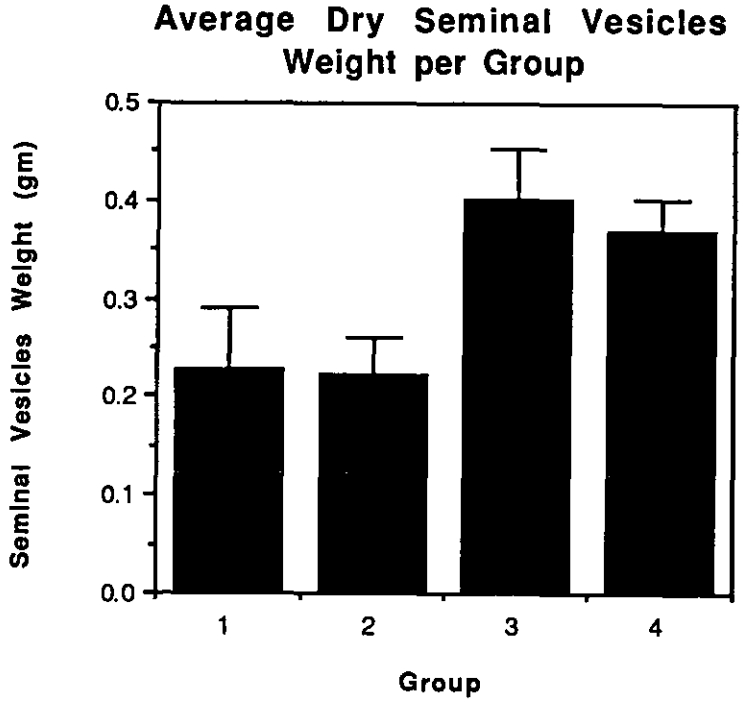

Average Wet Epididymal Fat Pad Weight per Group
Epididymal Fat Pad Weight (gm)
6
5
4
3
2
1
0
1
2
3
4
Group


Average Dry Epididymal Fat Pad Weight per Group
Epididymal Fat Pad Weight (gm)
4.0
3.0
2.0
1.0
0.0
1
2
3
4
Group

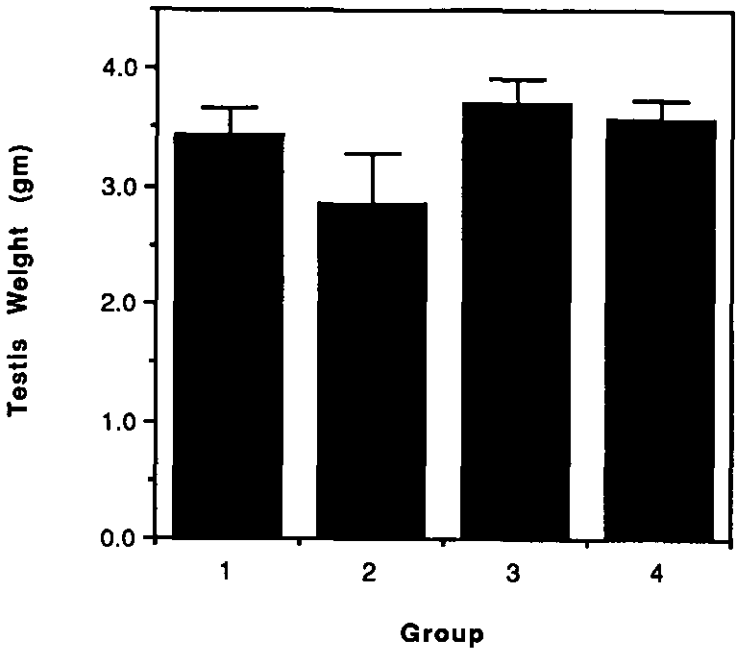

Average Wet Skeletal Muscle Weight per Group
Skeletal Muscle Weight (gm)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
4
Group


Average Dry Skeletal Muscle Weight per Group
Skeletal Muscle Weights (gm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
Group

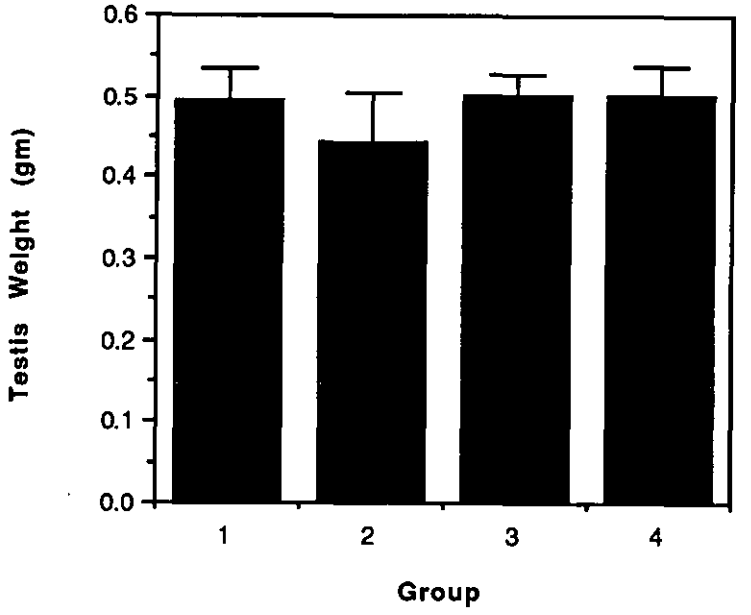

Average Wet Seminal Vesicles Weight per Group
Seminal Vesicles Weight (gm)
2.0
1.5
1.0
0.5
0.0
1
2
3
4
Group


Average Dry Seminal Vesicles Weight per Group
Seminal Vesicles Weight (gm)
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
Group

Average Wet Testis Weight per Group



Average Dry Testis Weight per Group



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