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The reduction of the diabetic syndrome in the C57Bl/KsJ (db/db) diabetic mouse by diet-restriction and exercise

Horst R. Rudrich

THE REDUCTION OF THE DIABETIC SYNDROME IN THE C57BL/ KsJ (db/db) DIABETIC MOUSE BY DIET-RESTRICTION AND EXERCISE

A Thesis
Presented to the
Faculty of
California State
University, San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology

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by
Horst R. Rudrich
October 1985

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ABSTRACT

Normal male and female C57Bl/KsJ (+/db) mice and diabetic (db/db) mice were subjected to diet-restriction and diet-restriction plus exercise. The exercise was conducted on a mouse treadmill achieving 70% maximum heart rate for twenty minutes per day for six days per week. Normal female mice showed no statistical difference from normal male mice in any of the physiological parameters measured except females had decreased average body weights throughout the study ($p<0.05$).

Diabetic male mice were statistically compared to normal male mice. Diabetic mice fed *ad libitum* drastically increased ($p<0.01$) their body weight, while diet-restriction and diet-restriction plus exercise groups normalized their body weight to that of normal male mice fed *ad libitum*. Diet-restriction and diet-restriction plus exercise in diabetic mice caused a drastic decrease in food and water consumption compared to normal mice. Fasting serum glucose and insulin, the Glucose/Insulin ratio, and percent body fat were all elevated in diabetic mice and there was no additional improvement with exercise. However, exercise reduced the percent increase in body weight ($p<0.01$) in diabetic mice equal to that

of normal mice. The metabolic efficiency to incorporate new body weight from ingested food was lowered ($p<0.05$) in the diabetic to that of normal mice fed *ad libitum*. Hepatic glycogen stores were depleted by exercise, and hepatocytes were thinned with condensed nuclei. In addition to the improved physiological parameters, the diabetic mice were active and alert in diet-restriction and diet-restriction plus exercise groups.

It is concluded in this study that exercise plus diet-restriction further reduced several parameters of the diabetic syndrome in diabetic mice than diet-restriction alone.

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INTRODUCTION

The diabetic mutant mouse (db/db) of the strain C57BL/KsJ is a model for type II diabetes mellitus, or noninsulin dependent diabetes mellitus (NIDDM). In humans this disorder encompasses 80% of all diabetic people and usually manifests itself in adults 40 years old or greater. The mutant diabetes shows full penetrance as an autosomal recessive allele (db), and it is located near the locus for misty coat color (m) to mark the diabetic gene in the mouse stock. The homozygous diabetic mice (db/db) are obtained as the F1 generation from a cross between two heterozygous nondiabetic parents (m+/+db) in which "+" represents wild-type or normal phenotype. Approximately 15% of the offspring are diabetic mice from such a cross and the mutant diabetic mice are infertile (2).

The diabetic mouse (db) exhibits the following characteristics as concurrent manifestations of the syndrome for diabetes: obesity, hyperglycemia, polyphagia, polydipsia, polyuria, and glucosuria (1,2). Tissues have reduced insulin sensitivity and responsiveness which result in hyperinsulinemia and depleted pancreatic insulin. There is nephropathy (3) due to increased glomerular filtration which is associated with the

elevated blood pressure. Excessive adipose tissue accumulates within the body cavity and subcutaneously with a predominance in the axillary and inguinal areas (1,2). The diabetic mice are inactive and unalert compared to their normal heterozygous littermates and they have a shortened life span of under one year.

The diabetic mice are distinguishable from their normal littermates at four weeks of age by their excess weight gain, hyperglycemia, and hyperinsulinemia (2,3). At five weeks of age the diabetic mice outweigh the normal mice, averaging12 grams(g) to 10g, respectively. The average weight of diabetic mice increases drastically with age compared to that of normal mice. At eight weeks of age diabetic mice outweigh normal mice 30-34g to 19-20g, respectively (1,4). The difference continues to increase with age. Normal mice stabilize their weights at three to five months, while diabetic mice continue to gain weight to 40-50g (1,2,3,4).

Polyphagia in diabetic mice is illustrated by their two-fold increase in food consumption (5.7-8 $g \cdot day^{-1} \cdot mouse^{-1}$) compared to that of the normal mice (2.8-3.6 $g \cdot day^{-1} \cdot mouse^{-1}$) when fed *ad libitum* (3,4,5). In addition, the diabetic animals exhibit greater water consumption than do normal animals, averaging up to 100 $ml \cdot wk^{-1} \cdot m^{-1}$ versus 20-30 ml ·

$wk^{-1} \cdot m^{-1}$, respectively (3). The largest increase in water intake occurs during the early stages of the diabetic state which is associated with the onset of glycosuria and polyuria (3).

Hyperglycemia and hyperinsulinemia in the diabetic mouse are well documented in the literature. However, serum glucose and insulin values vary depending on whether the mice had been fasted or not. Fasting serum glucose levels in the normal mouse are stable at about 70-80 mg/dl from four weeks of age onward. The diabetic mouse, however, already shows elevated glucose levels of 130 mg/dl at four weeks of age (3) and these levels continue increasing to 400-500 mg/dl (3,4,6) at 10 to 12 weeks. Serum insulin levels for normal mice are also stable at 20-50 µU/ml from four weeks of age onward (2,4,7). Diabetic mice have elevated insulin levels of 150 µU/ml at 4 weeks of age (7) and during the first few months of life insulin may range from 150-215 µU/ml (2,4,7). The insulin levels of diabetic animals decline to near normal levels at 20-25 weeks of age, a response asssociated with pancreatic ß-cell atrophy and degranulization (2). In the pancreas, insulin levels are slightly increased at 4 weeks of age; however, the levels decline to one half the normal amount at 8-12 weeks of age (4,6).

Glucagon, a counter-regulatory hormone of insulin, is secreted by the pancreatic alpha cells. It causes the breakdown of glycogen and the mobilization of glucose into the bloodstream, and is also elevated in the diabetic mouse. The literature contains conflicting results of immunoreactive glucagon concentrations ranging from not significantly elevated at 113% of normal values in 7-9 week old diabetic mice (7) to significantly increased levels of 200% (8) or 300-400% (9) in adult diabetic mice. The elevated glucagon levels contribute to hyperglycemia in the diabetic mouse by doubling the hepatic glucose secretion (10). Glucagon stimulates some insulin secretion which facilitates uptake and storage of glucose in hyperglycemic states. However, insulin resistance overwhelms this regulatory mechanism by not facilitating the uptake of serum glucose and thereby causing hyperglycemia. Somatostatin is a hormone produced by the pancreatic ∂ cells and suppresses both insulin and glucagon. It is decreased by one half the normal amount in the diabetic mice which also contributes to the maintenance of the elevated levels of these hormones (11).

Hormonal control of carbohydrate metabolism is most pronounced in the liver, an organ which serves as an immediate and vital source for

nutrient storage and mobilization (2,4,7,8,11,12). Hyperinsulinemia in the diabetic mouse causes increased insulin-dependent enzyme concentrations and activities within hepatic and adipose tissues. This further causes greater glucose absorption and storage. Insulin-dependent glycogenic enzymes (glucokinase, glycogen synthetase) are elevated in 8-10 week old diabetic mice (7,8). The activity of several insulin-dependent glycolytic enzymes (glucose-6-phosphate dehydrogenase, citrate lyase, acetyl-coA-synthetase, and pyruvate kinase) are also slightly elevated at this early age (2,11,12). The increased amounts of serum glucose plus increased enzyme activities cause increased lipid formation via hepatic lipogenesis. Another contributing factor to the elevated serum glucose levels of the diabetic mouse is the animals characteristic inactivity (1,2,5) which reduces its need for glucose as a metabolic fuel.

Glucagon-sensitive enzymes are elevated in diabetic animals at 8-10 weeks of age (2,4,7,8,10,11). Glycogenolysis, which is the breakdown of glycogen, is also elevated causing greater glucose mobilization. This is reported to be due to increases in glucose-6-phosphatase and phosphorylase enzyme activity levels (4,7,8). Gluconeogenesis utilizes noncarbohydrate substrates such as lactate, glycerol and amino acids in

increased proportions as metabolic fuels (glucose sparing action) for the formation of glucose. Hepatic concentrations of gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-diphosphatase are increased in diabetic animals. This results in increased gluconeogenic activity which in turn causes increased hepatic glucose secretion contributing to hyperglycemia.

At later ages, glycogenic and glycogenolytic enzyme activities are elevated in the diabetic mouse (2,4,12) which suggests a fast turnover rate of hepatic glycogen deposits. Glycolytic enzymes are elevated at 9 weeks (4,12) but reduced below normal at18 weeks (4), possibly due to increased insulin resistance which hinders glucose utilization. In the developing diabetic syndrome, glucagon seems to override insulin since it uses alternative fuel sources during the condition of hyperglycemia (8,9,13). Diabetic animals have a 1.5-fold increase in glucagon binding capacity compared to normal animals which may lead to greater glucagon sensitivity (9).

Relative rates of glycogenesis and glycogenolysis which build-up and breakdown glycogen stores, respectively, can be indirectly approximated by measuring hepatic glycogen levels. This would provide an indirect

measurement of hepatic carbohydrate metabolism. However, there is some discrepancy on the actual amounts of glycogen contained in the livers of normal and diabetic mice. In 8-9 week normal animals hepatic glycogen content is reported to vary from 22-30 mg/gram of wet weight of liver (2,8,12) with uniform distribution throughout the liver. In diabetic mice the levels are reportedly elevated to 50-60 mg/g liver (2,12), although Stearns (8) found the increase limited to only 30.1 mg/g liver. In the diabetic mouse, glycogen deposits were more massed near hepatic arteries and portal veins (2). Glycogen deposits are very dense with uneven dispersal patterns in the hepatic periportal cells of diabetic mice livers (8,14). In hepatic centrilobular cells, the deposits are larger and more dispersed (14). However, glycogen storage patterns in hepatocytes of diabetic mice vary with age. At 9 through 12 weeks of age glycogen deposits show rosette alpha patterns but at 21 weeks they have a granular appearance. It has been suggested that this may be due to a deterioration in carbohydrate metabolism (8).

It appears, then, that hepatic glycogen stores are elevated in the diabetic mice due to hyperglycemia, hyperinsulinemia and the associated high glycogen synthetase activity observed early on in the diabetic state

(14). The diabetic mouse has an enlarged liver: 1.29g for a 20g normal mouse to 4.5g for a 40g diabetic mouse (2). This is due to increased glycogen and fat deposits and is probably the result of the diabetic mouse's hyperinsulinemia which leads to increased storage capabilities. Simultaneously, insulin resistance in the diabetic mouse causes insufficient blood glucose handling resulting in hyperglycemia.

Excess fat deposition is rather apparent in the diabetic mouse. Lipogenesis is elevated in adipose and hepatic tissues (2,12,14,15,16) and there are elevated serum levels of triglycerides, cholesterol (16,17), and FFA (18). Chan (16) reports twice the normal amount of activity in lipogenic enzymes which use lactate, glucose, acetate, and glycerol as fuel sources (15,16). There is also a high esterification rate of fatty acids to form fat deposits which is typical in NIDDM (12,15).

Studies of epididymal fat tissue by Steinmetz (18) indicate that there is a decline in lipolysis due to a defective adenyl cyclase system. This system normally produces intracellular cyclic-AMP and decreases the activities of lipase enzymes. Cyclic-AMP is produced within the cytoplasm of the adipocyte or hepatocyte in order to promote a cascade of enzyme phosphorylation reactions to breakdown lipids and glycogen. These

enzymes perform their activities at a much reduced rate in diabetic animals when compared to those of normal animals. An additional study utilizing *in vitro* epinephrine perfusion of diabetic animal adipose tissue resulted in less lipid mobilization (18) suggesting a defective lipolysis response.

Since carbohydrates and fats are proportionally less utilized as fuel sources in diabetic mice compared to normal mice, amino acids are the main substrate for gluconeogenesis. Thus, diabetic mice do not mobilize stored lipids or utililize serum glucose (12,19) but rather accumulate fat stores and maintain high serum glucose concentrations. Supplementing the diabetic mice with a high protein-noncarbohydrate diet has reduced wasting of muscle proteins and reduced hyperglycemia. There is a subsequent increase in glucose tolerance in the diabetic mice and their life span is extended to one year (20). However, this treatment was limited because diabetic mice still retain a high fat proportion, have delayed pancreatic ß cell atrophy and hyperglycemia (20,21).

In contrast to elevated insulin-stimulated lipogenesis in the diabetic mouse, insulin-stimulated glucose utilization is reduced. There seem to be two modes of insulin resistance in the diabetic mouse. The first is

insulin insensitivity by the reduction of insulin receptors on tissue membranes which decreases insulin binding. Secondly, insulin resistance may be due to a post-receptor defect in which the response of tissues to bound insulin does not stimulate serum glucose uptake and further raises serum insulin levels. A major site of insulin insensitivity in the diabetic mouse is the liver cell (3,8,22). Peripheral tissue insulin resistance is indicated in fibroblastic skin cultures from diabetic mice which have a 30-50% reduction in ^{125}I-insulin binding with 45-48% fewer receptor sites (23). Similarly, insulin resistance has been demonstrated in obese humans by decreased insulin binding in their tissues (24).

The post-receptor defect which causes a decline in insulin response in NIDDM and in the diabetic mouse is still unknown; however, some mechanisms have been postulated. Insulin normally decreases cytoplasmic cyclic-AMP levels (13) to stop glycogen breakdown and this mechanism may be defective in the diabetic mouse. Alternately, insulin's second messenger, which has not as yet been determined, may be defective or secreted in inadequate amounts (25). Thus a decreased insulin response leads to hyperglycemia which leads to hyperinsulinemia (23,26). As a result of the hyperinsulinemia the cells reduce the number of insulin

receptors on their surface (26).

Diet-restriction studies on diabetic mice have yielded encouraging results by relieving some diabetic symptoms. Diabetic mice on diet-restriction in which food is available for only two-three hours daily, or fed to maintain body weights similar to that of normal mice (match-pair feeding) only slightly outgained normal mice with the same treatment. They reduced their body weight to that of normal mice fed *ad libitum* (3,5,19). Serum glucose levels were still nearly double that of normal mice, but they stabilized at 180mg/dl at twelve weeks of age. Immunoreactive serum insulin levels of diet-restricted diabetic mice dropped from levels observed in diabetic mice fed *ad libitum*, 150μU/ml to 30μU/ml, respectively (3). Normal mice had less than 5μU/ml serum insulin illustrating the persistent hyperinsulinemic state of the diabetic mice. Fasting and high protein-noncarbohydrate diets did not normalize insulin secretion but did increase pancreatic insulin levels (4,20,21) and decreased pancreatic islet atrophy.

Diet-restriction in diabetic animals resulted in the absence of nephropathy (3). In the liver, gluconeogenic enzyme concentrations were not elevated as drastically, and glycolytic enzyme concentrations were

elevated due to increased insulin sensitivity (4). However, high protein-noncarbohydrate diets did not change gluconeogenic enzyme levels (2). Leiter (21) reports that hepatic glycogen levels were halved due to enhanced mobilization.

Diabetic mice retain about a five-fold increase in excess carcass lipid compared to normal mice (19) due to accelerated lipogenesis (3,14). Cox (19) and Leiter (21) have reported only slight decreases in percent body fat from high protein-noncarbohydrate and diet-restricted diets by match-pairing. Even though they have demonstrated a reduction in the incorporation of fat as new body weight, diabetic mice still reduce skeletal and muscular growth in favor of fat deposition (19). They are able to incorporate more new body fat from ingested food than normal mice (3,5). Finally, Lee (3) observed that diet-restricted diabetic mice were more active and alert which showed improved behavioral changes. Humans studies have displayed similar results (24,27,28).

By placing chemically-induced diabetic rats and NIDDM humans on an exercise regime, researchers have produced a partial reversal of diabetic pathology. In obese mice, exercise alone does not reduce body weight when the animals are fed *ad libitum* (29,30). Similar results have been

observed in human studies (31) although there is some indication that body fat may be reduced (31,32,33). Exercise lowers serum glucose (29,31,34,35) by increasing muscle glucose uptake in the presence or absence of insulin (36,37). Storage capacities for glycogen are also enhanced by exercise although these deposits are depleted during an exercise bout (31). Serum FFA levels are increased in humans during and shortly after exercise because of their utilization as a metabolic fuel (38). Serum triglyceride and cholesterol levels are decreased due to utilization and packaging into high density lipoproteins thus reducing the chance of atherosclerotic vascular disease (33,39). For these reasons, exercise is recommended in the treatment of diabetes mellitus.

Most important to the treatment of diabetic patients is the finding that exercise increases insulin sensitivity of peripheral tissues. This apparently occurs by increasing the number of insulin receptors as indicated by studies using liver membranes and monocytes (31,32,34,35, 39-42). Insulin responsiveness may also be enhanced by increasing receptor affinity (41,43).

The present study uses a combined diet-restriction and exercise program on normal (+/db) and diabetic (db/db) mice to determine if diet

plus exercise reduces the diabetic syndrome of the diabetic mice more than that of diet alone. Exercise should enhance the metabolic need of the mice and deplete glycogen stores thus reducing their metabolic efficiency to incorporate more new body weight from ingested food. The diabetic mice should become more insulin-sensitive or insulin-responsive as indicated by the glucose/insulin ratio. The influence of diet-restriction and exercise on body weights, percent increase in body weight, food and water consumption, serum glucose, serum insulin, percent body fat, and hepatic glycogen levels are investigated.

MATERIALS AND METHODS

Experimental Animals

Experimental animals were obtained from a breeding stock of C57BL/KsJ (db/m) mice which originated at the Jackson Laboratories (Bar Harbor, Maine). Diabetic males and heterozygous littermates (normal) were retained for experimentation. Eight db males were also obtained directly from the Jackson Laboratories.

Animals were housed in a Scherer environmental chamber at 23.5°C, on a light:dark cycle of 14:10 hours. Three or four mice where housed per stainless steel cage. Cages were equipped with raised wire mesh floors with collection pans for retrieval of crumbs from waste products. Glass water bottles with metal tips were attached to the cage fronts and mice were handled through sliding tops so as not to disturb the bottles.

Experimental Design

Treatments were started on animals at five weeks of age and continued to age 8 weeks (three weeks total). This regimen was selected because the diabetic syndrome develops rapidly at these ages. Animals were assigned to one of three experimental groups: 1) Control (C)

2) Diet-restricted (D) 3) Diet-restricted plus exercise (DE). Each group consisted of three +/db females, three +/db males, and three db/db diabetic males. Mice at five weeks of age exhibit a wide range of weights (44); thus animals were assigned to groups by distributing heavy, moderate, and light individuals to each group to insure similar beginning weight ranges.

Mice were fed Purina Rat Chow according to each treatment protocol (see below). Tap water was provided *ad libitum*. Food and water intakes were measured weekly by weighing the difference between the initial and residual food and water amounts per cage. Fallen crumbs were collected from the bottom of the cages to more accurately measure food intake. Food consumption per mouse was calculated by using the proportion (each mouse's body weight per total body weight of all mice in that cage) to calculate each mouse's food consumption per total weekly food consumption per cage. Water consumption was calculated by dividing the weekly total water consumed per cage by the number of mice.

Ad libitum fed mice served as controls (C). Diet-restricted mice (D) were limited to four hours of feeding per day; from 8:00 am to

12:00 pm. daily. The diet-restricted, exercise group (DE) was limited to the same four hour feeding schedule and was also subjected to an exercise regimen as described below.

Exercise Protocol

The (DE) group was exercised at 2:30 pm. daily. This schedule allowed proper digestion (45) and ensured the normal elevation of serum glucose levels. Weights of all mice were taken just prior to an exercise bout. The exercise protocol was modified from a recommended protocol for moderately-conditioned adult diabetic humans (46) which suggests twenty minutes of exercising per day at 70% of the maximal heart rate for six days a week. During the first week the (DE) mice were only exercised for three days, every other day, to prevent muscle soreness and fatigue. In subsequent weeks they were exercised daily. The mice were run for twenty minutes on a customized treadmill (Figure 1) at a rate of 6.8 meters/minute. A five minute warm up and cool down period at 55% maximal heart rate (60% dial reading) was included at the beginning and end of each session. The treadmill consisted of a ROLL-A-CELL (New Brunswick Scientific Corp.) equipped with an inverted sandpaper belt as a running surface (47) and an elevated partitioned box capable of holding

FIGURE 1. Mouse treadmill made from a ROLL-A-CELL (New Brunswick Scientific Co.) with an inverted sandpaper belt as a running surface.

FIGURE 2. Experimental set-up for recording mouse ECGs. In the foreground is a Gilson 5/6 Polygraph Recorder, and in the background is the treadmill mounted with a partitioned box capable of running ten mice simultaneously.

ten mice (48,49). A calibration curve for the speed indicator was derived by counting revolutions for five minutes at various speeds to determine the velocity at each speed [velocity = perimeter (m)/time (min)]. No grade was incorporated into the protocol because of the negligible increase in metabolic rate of a mouse moving on an incline plane (50).

Electrocardiograms (ECG) were performed on three 5-6 week old +/db males and three females to determine heart rates at various treadmill speeds. Mice were shaved on their medial forelimb surfaces to allow for attachment of two ECG electrodes and a third was slipped on the base of the tail. Heart rates were recorded on a Gilson 5/6H recorder on the multipurpose galvanometer module (Figure 2). Heart rates were determined at resting, 70%, and 98% (dial reading) running speeds and after one minute of hazing. Hazing was performed by harmlessly chasing the mice around their cage to achieve maximal heart rate. Calculation of the 70% maximum heart rate is determined by the formula given in the 1980 guidelines for graded exercise testing and exercise prescription (51):

(maximum ECG - resting ECG) x .70 + resting ECG = target heart rate

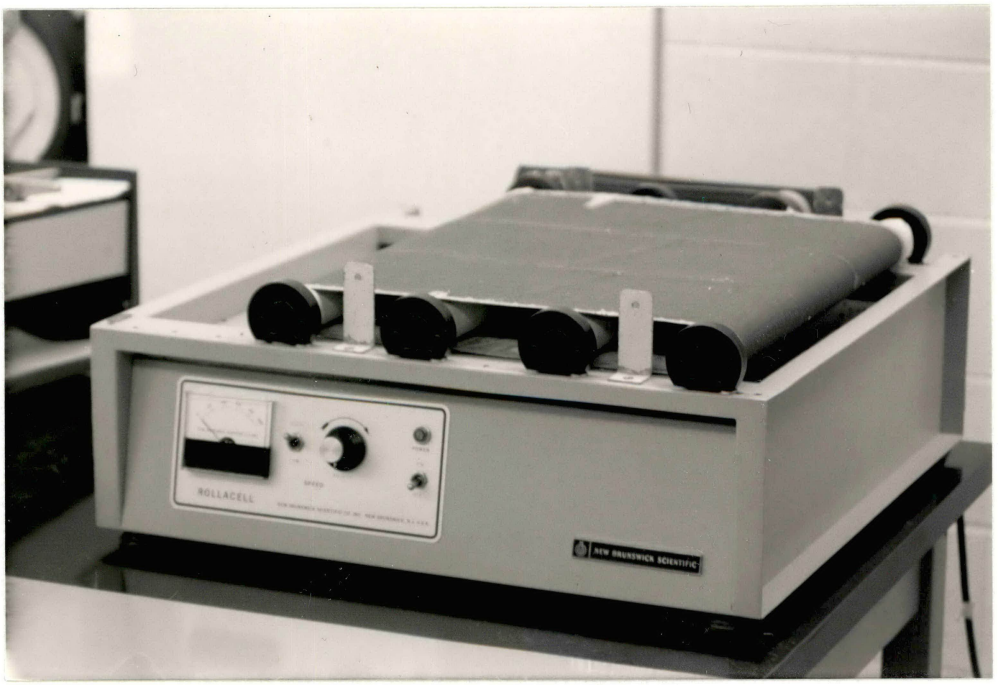
The target heart rate was 742 beats per minute which closely correlated with the heart rate of +/db totals at the 98% dial speed = 738±167 heartbeats/minute (Figure 3).

Blood Collection and Analysis

All mice were sacrificed at 8 weeks of age between 9:00 and 10:30 a.m. to avoid fluctuation in circardian cycles. Animals were fasted for approximately 18 hours prior to blood collection. Blood was collected by cardiac puncture using a 25 gauge needle on a 1.0 ml syringe. Samples were placed in a refrigerator at 4°C to clot. At 11:00 a.m. blood samples were centrifuged at 1700 r.p.m. (800 x g) for 20 minutes at 4°C. Recentrifuging was done on samples when necessary. Twenty microliters (µl) of serum were pipetted into 1.5 ml Eppendorf centrifuge tubes for serum glucose determination and 200 µl into the same type tubes for insulin radioimmunoassay (RIA). Samples were stored at -20°C until assayed.

Serum glucose was assayed by a commercial kit (Sigma Diagnostics) using a colorimetric determination involving enzymatic degradation by hexokinase and glucose-6-dehydrogenase (52). Percent transmittance was read at 520 nm on a spectrophotometer.

FIGURE 3. Representative electrocardiograms of C57BL/KsJ (+/db) normal mice at resting (A), 70% treadmill speed (B), 98% treadmill speed (C), and at maximal heart rate following hazing (D).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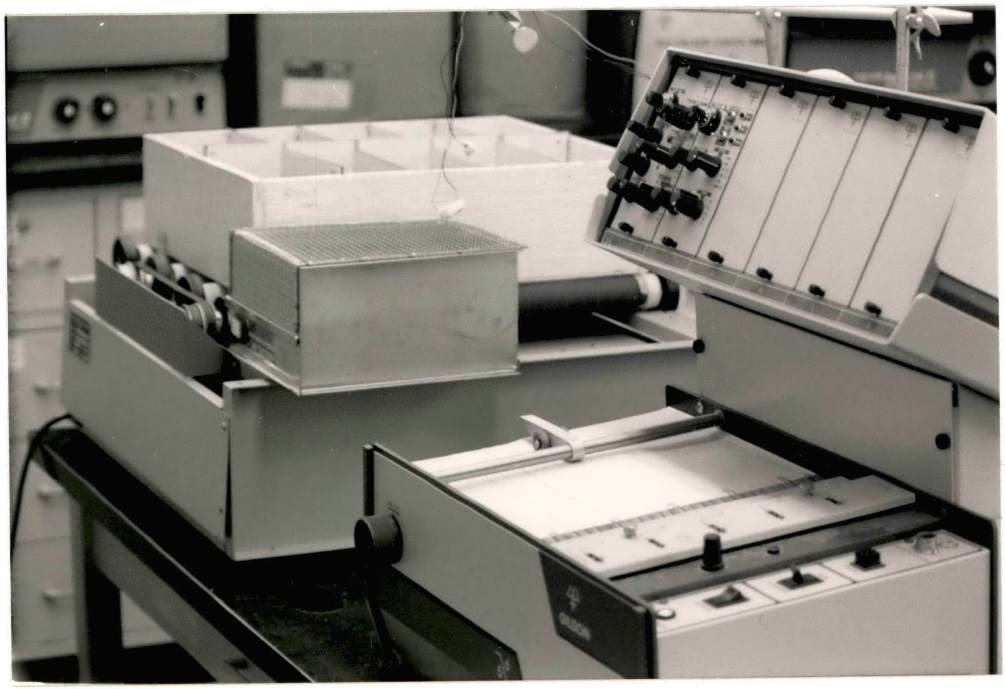

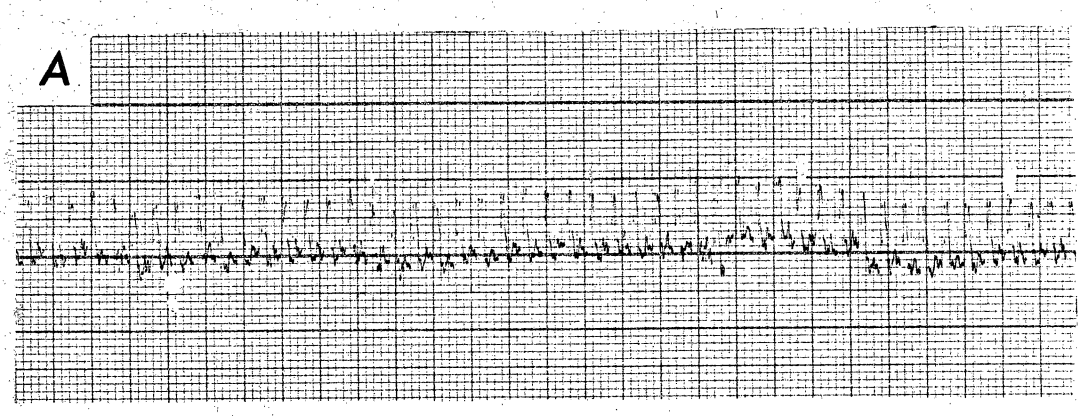
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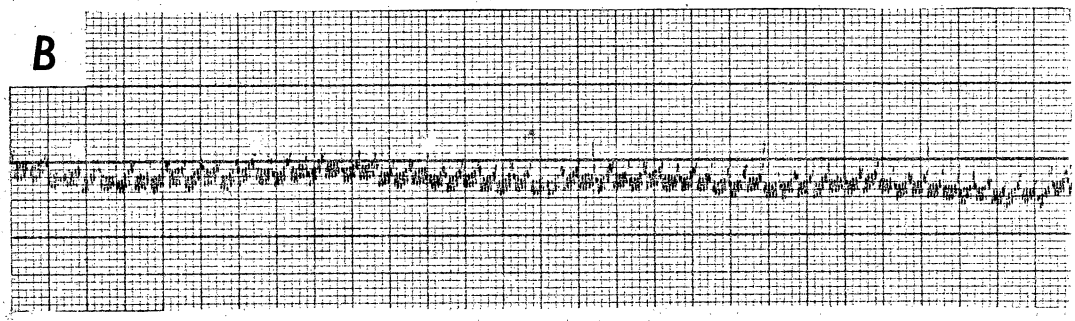
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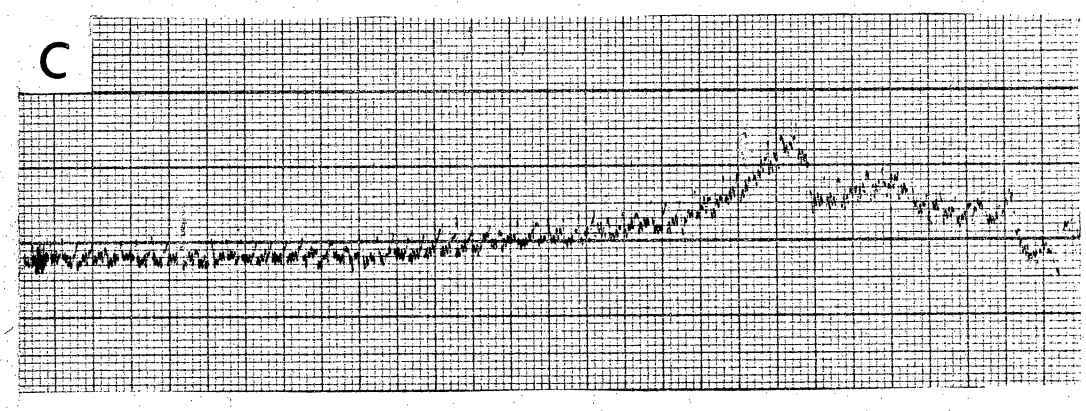
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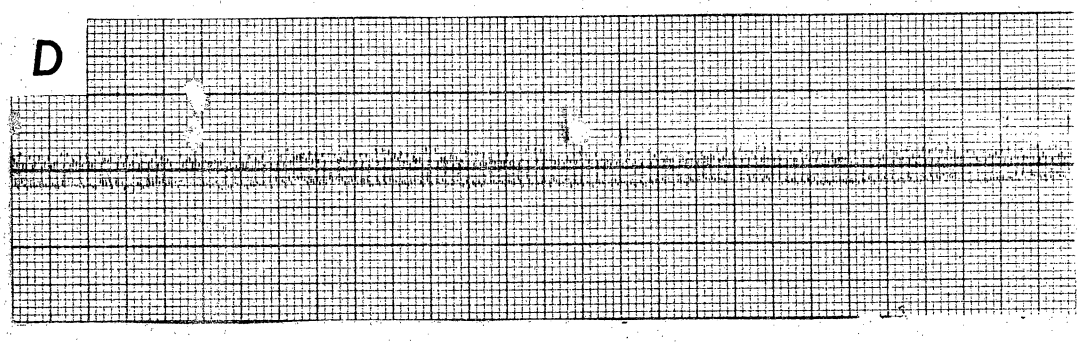

D

Serum insulin was assayed by RIA (Radioassay System Laboratories, Inc.).

Percent Body Fat

Immediately following cardiac puncture each animal was sacrificed be cervical dislocation. Body weights of animals were measured in air and under water using a Mettler beam balance (±0.01g). Mice were suspended from an alligator clip for weighing while submerged in a 1L beaker for under water measurement. Temperature of the water was recorded for water density corrections.

The density of the mouse body (D_b) is calculated from the Siri equation (53):

$$D_b = \frac{M_a \times M_w}{M_a - M_w - (RV - D_w)}$$

where: D_w = density of the water (corrected for temperature)
M_a = mass of mouse in air
M_w = mass of mouse in water
RV = residual lung volume

Since no values were available in the literature for the RV of mice, RV was estimated by extrapolating the RV for a human. A 150lb.

(68.2kg) human has a usual RV of 1.2L (13,53) which is proportional to 0.352 ml for a mouse. This is also twice the tidal volume of the mouse (54,55) a condition also observed in humans. Since not all the trapped air bubbles in the fur can be worked out by hand massage this also increases the amount of residual air. Therefore the RV estimation was increased to 0.5 ml. Percent body fat is calculated as (53):

$$\% \text{ body fat} = 495(g/ml)/D_b - 450$$

Liver Histology

Sections of liver were extracted from the left-middle lobe and were fixed in 10% phophate buffered formalin overnight. Livers were embedded in soft paraffin wax (m.p. = 53-55°C) and cut on a microtome at 5µm. Ribbons were separated while lying on a warm water bath (50°C) and adjacent sections placed on separate albumin-coated slides for comparison. The first section was stained with Schiff reagent (PAS) for glycogen (56) and counterstained with methylene blue. The second was stained with Hematoxylin-Eosin (57) to examine general cell features. The cell nuclei were measured with a stage micrometer photographed at the same magnification.

Data Analysis

Data were evaluated by analysis of variance (ANOVA).

RESULTS

Treadmill running protocol

The heart rates of normal and diabetic animals subjected to various levels of activity are listed in Table 1. A dial setting of 98% was determined to provide a sufficient workload to bring mice to 70% maximal heart rate (see Materials and Methods). This value was therefore used for the DE treatment group.

Body Weights

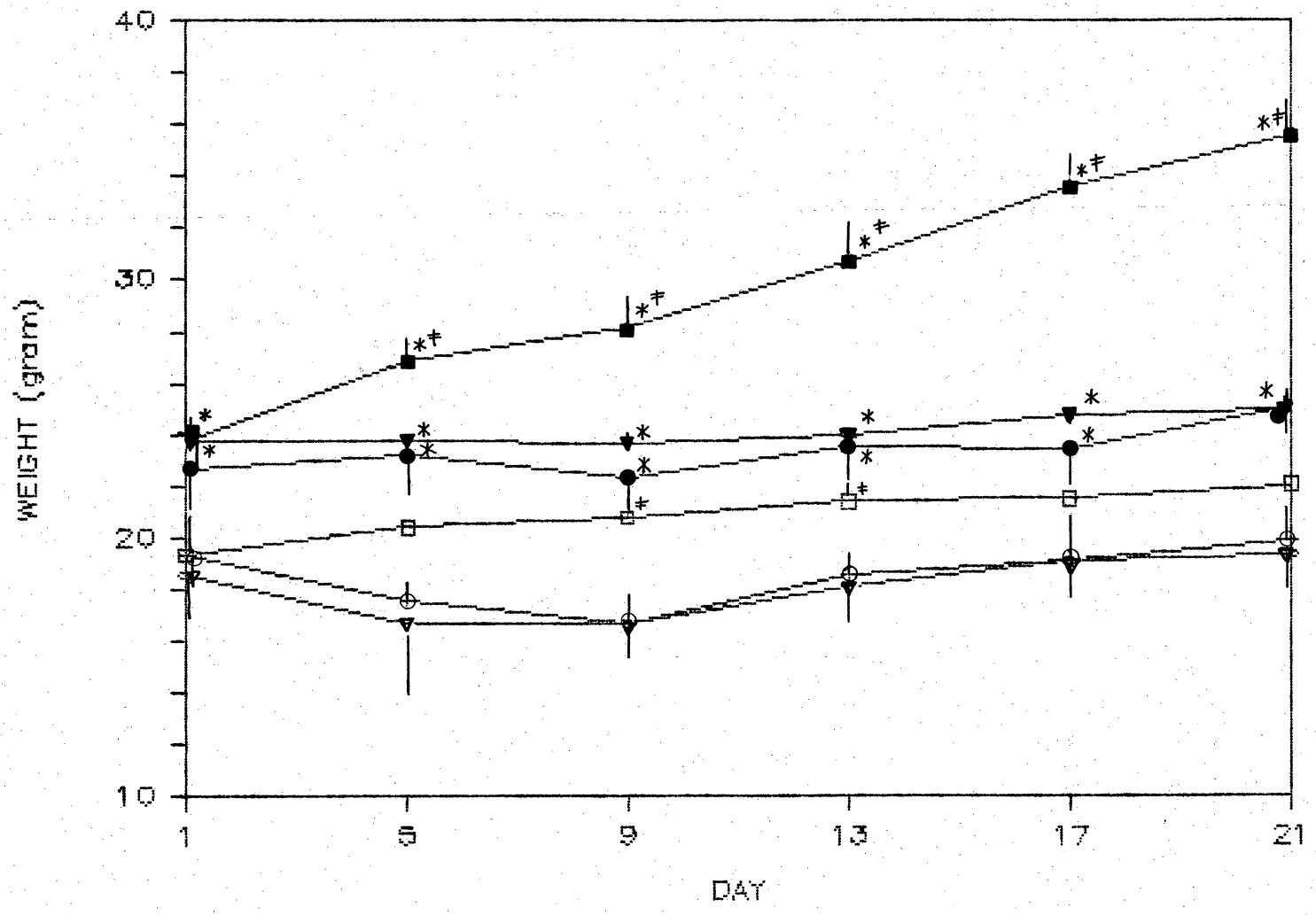
Body weights of male animals in each treatment group are illustrated in Figure 4. Data for +/db (normal) female animals are not shown because they were significantly less than their male counterparts throughout most of the study and were not used in statistical comparison to diabetic male mice. Normal male animals fed *ad libitum* (C) showed a gradual increase in body weight throughout the study, while the diet (D) and diet plus exercise (DE) groups lost weight during the first half of the program, but returned to near normal weights by day 21 (22.01 ±0.22 g, 19,92 ±1.27g, 19.33 ±1.07g, respectively). There were no significant differences in final body weights of normal male animals in any treatment group. Diabetic

TABLE 1. Heart rates of normal(+/db) and diabetic(db/db) mice at rest, subjected to treadmill exercise at 70% and 98% dial settings, and at maximal activity.*

	Heart Rates (Beats/min.)			
	Activity			
	Resting	70%	98%	Maximum
db/db male	562 ±164	638 ±83	647 ±96	811 ±142
+/db female	578 ±128	636 ±87	726 ±199	788 ±176
+/db male	569 ±104	727 ±74	749 ±138	835 ±153
+/db total	574 ±113	679 ±92	738 ±167	814 ±161

* Values represent mean ± standard deviation (n=3).

FIGURE 4. Body Weights of normal (+/db) male and diabetic (db/db) male mice through the three week study started with five week old mice. □ - normal mice fed *ad libitum*, ○- normal mice on diet-restriction, ▽- normal mice on diet-restriction and exercise, ■ - diabetic mice fed *ad libitum*, ● - diabetic mice on diet-restriction, ▼ - diabetic mice on diet-restriction and exercise. * represents differences ($p<0.05$) between normal and diabetic mice given the same type of treatment. ǂ represents the difference ($p<0.05$) between *ad libitum* fed groups and diet-restricted and diet-restricted plus exercise groups of the same phenotype.


40
30
20
10
WEIGHT (gram)
1
5
9
13
17
21
DAY

mice in group C steadily increased their body weights ($p<0.01$) from their original body weights while diabetic mice in D and DE groups similarly increased their body weights only slightly by day 21. Body weights on day 21 for diabetic mice groups C, D and DE are: 35.12 ±1.31g, 25.12 ±0.84g, 25.01 ±0.50, respectively. Initially the diabetic mice groups D and DE were 3-4g ($p<0.05$) heavier than the group C normal male mice, however the growth rates for all three groups were similar throughout the program. At day 21 the diabetic groups were still 2-3 grams heavier ($p<0.05$). Comparisons between similar treatment groups illustrated that the diabetic mice were always heavier than their nondiabetic counterparts (C, $p<0.05$; D and DE, $p<0.01$). Within the diabetic animals the body weight of group C was greater than that of D or DE groups ($p<0.01$), and there was no difference between groups D and DE.

Percent Increase in Body Weight

The percent increase in body weight of each animal was calculated from the difference in weight from day 1 to day 21 divided by the original weight on day 1 (Table 2). Normal male animals showed no significant differences between treatments probably due to the large range of initial weights. However, there was a reduction in the percent increase in body

TABLE 2. Percent increase in body weight[a] of normal (+/db) and diabetic (db/db) mice fed *ad libitum* (group C), subjected to diet-restriction (group D) and those subjected to diet- restriction and exercise (group DE).

	Treatment			
Group	C	D	DE	p values
+/db female	16.8 ±8.5 [b]	5.9 ±4.1	-2.3 ±5.1	NSD
+/db male	14.3 ±9.8 [c]	3.3 ±4.1 [d]	4.8 ±8.8	NSD
db/db male	49.8 ±3.4 [bc]	10.9 ±4.4 [dx]	5.3 ±6.4 [x]	x: $p < 0.01$
p values	a,b,c: $p < 0.01$	d: $p < 0.01$	NSD	

a Values represent means ± standard deviation (n=3).
b,c,d p values of comparisons of various groups with the same treatment.
x p values of comparisons of treatments within the same phenotype group.
NSD (no significant difference)

weight in groups D and DE compared to group C (3.3 ±4.1%, 4.8 ±8.8%, 14.3 ±9.8%, respectively).

The diabetic animals showed a steady reduction ($p<0.01$) in percent increase in body weight from C to D to DE groups. Diabetic animals had a greater percent increase in body weight compared to normal male mice in each treatment group with C and D groups being significant ($p<0.01$). Exercise in addition to diet-restriction (DE group) normalized the percent increase in body weight of diabetic animals to normal male mice (4.8 ±8.8, 5.3 ±6.4, respectively). Group D of diabetic mice normalized its percent increase in body weight to that of normal male mice of group C.

Food and Water consumption

Food consumption measured in the first week of the study was 75% less than that measured in the third week. This was most likely due to the imposed adjustment of the feeding time frame as they were not accustomed to eating in the morning. Normal male and female mice in both D and DE groups ate ≈65% of the amount of group C mice with no difference between D and DE groups (Figure 5). The diabetic mice reduced food consumption to 50% of the amount of group C mice when on the diet (D group) and to 40% by dieting and exercising (DE group).

FIGURE 5. Food consumption in grams per day per mouse of female normal (FE +/db), male normal (MA +/db), and male diabetic mice (MA db/db). The different treatment groups for each phenotype mouse are grouped together for comparison: *ad libitum* (group C), diet (group D), diet and exercise (group DE). Values represent the mean value ± standard deviation over the three week program.

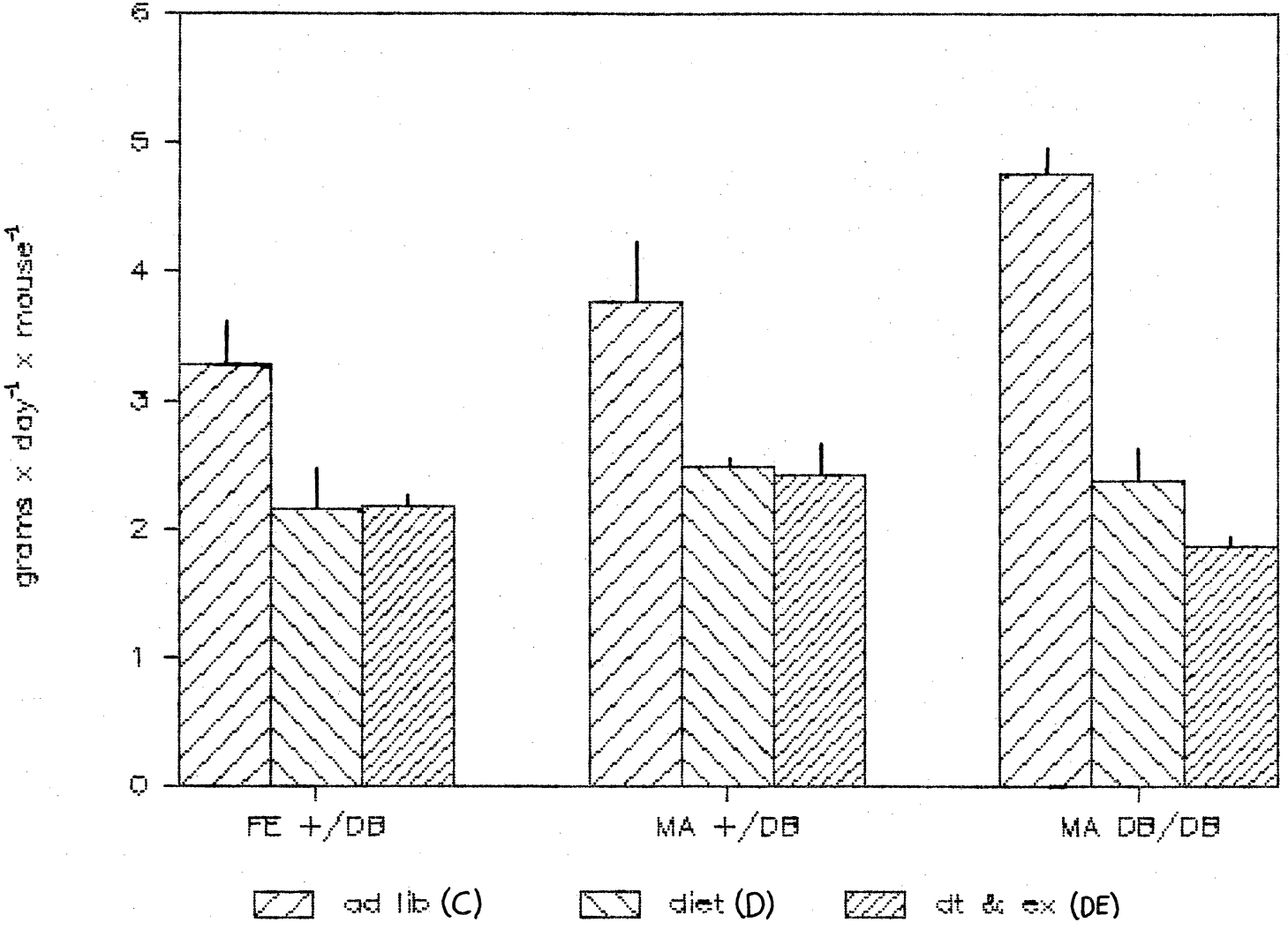
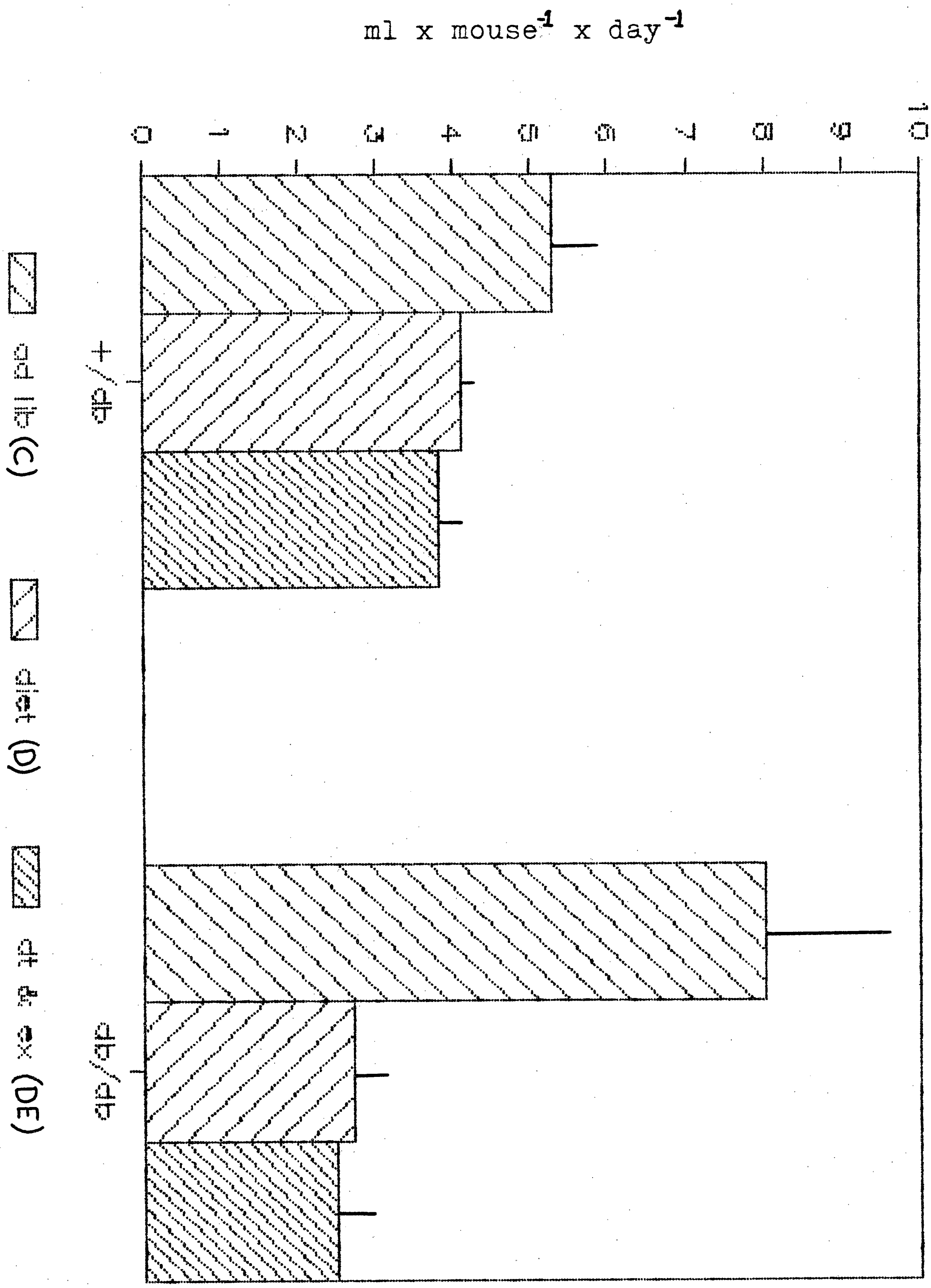

grams x day-1 x mouse-1
0
1
2
3
4
5
6
FE +/DB
MA +/DB
MA DB/DB
ad lib (C)
diet (D)
dt & ex (DE)

FIGURE 6. Water consumption of normal mice, +/db (sexes combined), and male diabetics (db/db) in milliliters per day per mouse. The different treatment groups for each mouse phenotype are grouped together for comparison. Values represent means ± standard deviation over the three week period.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(C) ad lib
(D) diet
(DE) diet & ex

Water consumption for animals in each group is shown in Figure 6. Polydipsia occurred in diabetic animals compared to normal animals (sexes combined) in group C (8.0 ±1.7 vs. 5.3 ±0.7 ml · d^{-1} · m^{-1}, respectively). Normal D and DE mice groups reduced water intake compared to group C about 1.0-1.5 ml with no difference between groups D and DE. Among diabetic mice the water consumption was lowered in groups D and DE by about 5 ml from group C; again there was no difference between groups D and DE.

Metabolic Efficiency

Metabolic efficiency (Table 3) is the change in body weight divided by the food consumed per mouse over the three week experimental period. Normal male mice demonstrated a decreased metabolic efficiency ($p<0.05$) from groups C to D and DE (35 ±26, 13 ±16, 16 ±29, respectively), showing no difference between values for groups D and DE. Diabetic animals showed a significant decline in metabolic efficiency ($p<0.05$) from groups C to D to DE (119 ±4, 51 ±23, 30 ±37, respectively).

Comparisons between normal male and diabetic mice show greater metabolic efficiency among the diabetic mice in groups C and D; $p<0.01$, $p<0.05$, respectively. DE groups were not statistically different, and the

TABLE 3. Metabolic efficiency[a] over the three week program. Values[b] are given for normal (+/db) female and male mice and diabetic (db/db) mice fed *ad libitum* (group C), subjected to diet-restriction (group D) and those subjected to diet-restriction and exercise (group DE).

	Treatment			
Group	C	D	E	p values
+/db female	43 ±21 [c]	25 ±25	-9 ±20	NSD
+/db male	35 ±26 [dx]	13 ±16 [ex]	16 ±29 [x]	x: $p < 0.05$
db/db male	119 ±4 [cdy]	51 ±23 [ey]	30 ±37 [y]	y: $p < 0.05$
p values	c,d: $p < 0.01$	e: $p < 0.05$	NSD	

a metabolic efficiency is derived from the increased change in body weight (g) per food consumed (g) $\times 10^3$.

b Values represent means ± standard deviation (n=3).

c,d,e p values of comparisons of various groups with the same treatment.

x,y p values of comparisons of treatments within the same phenotype group.

NSD (no significant difference)

DE diabetic mice group normalized their metabolic efficiency to that of normal male mice fed *ad libitum* (30 ±37, 35 ±26, respectively).

Fasting Serum Glucose Levels

Normal female mice had lower levels of serum glucose ($p<0.05$) following diet-restriction (group D) and diet-restriction plus exercise (group DE) in comparison to group C levels (84 ±13 mg/dl, 98 ±8 mg/dl, 192 ±40 mg/dl, respectively, in Table 4). However, no significant differences in fasting glucose levels were observed between normal male and diabetic mice in any of the treatment groups. There was, however, an apparent gradual decline from groups C to D to DE in normal male mice (157 ±45 mg/dl, 111 ±42 mg/dl, 84 ±31 mg/dl, respectively). Diabetic mice showed a decrease in serum glucose from group C to both groups D and DE (276 ±102 mg/dl, 215 ±35 mg/dl, 215 ±43 mg/dl, respectively).

Diabetic mice had an approximate two-fold increase in fasting glucose levels from normal male mice in all treatment groups. Groups D and DE were significantly elevated ($p<0.01$) while group C was not, possibly due to the large variances observed in the diabetic mice group C.

TABLE 4. Fasting serum glucose (mg/dl) of normal (+/db) female and male mice and diabetic (db/db) mice at 8 weeks of age fed *ad libitum* (group C), subjected to diet-restriction (group D), and those subjected to diet-restriction and exercise (group DE).[a]

	Treatment			
Groups	C	D	DE	p values
+/db female	192 ±40 [x]	84 ±13 [x]	98 ±8 [x]	x: $p < 0.05$
+/db male	157 ±45	111 ±42 [b]	84 ±31 [c]	NSD
db/db male	276 ±102	215 ±35 [b]	215 ±43 [c]	NSD
p value	NSD	b: $p < 0.01$	c: $p < 0.01$[d]	

[a] Values represent means ± standard deviation (n=3).
[b,c] p values of comparisons of various groups with the same treatment.
[x] p values of comparisons of treatments within the same phenotype group.
[d] significantly different by Student's t-test.
NSD (no significant difference)

Fasting Serum Insulin

No significant differences were found in any group comparisons of fasting serum insulin levels (Table 5). However, control diabetic animals had an apparent two to three-fold elevation of serum insulin levels compared to group C normal mice. In group D diabetic animals there was a 33% higher insulin concentration compared to normal male animals (98 ±46 vs. 65 ±76 µU/ml, respectively). Diabetic animals in group DE showed greater serum insulin concentrations compared to group DE normal mice (85 ±51 vs. 33 ±32 µU/ml, respectively).

Glucose/Insulin Ratio

The Glucose/Insulin ratio was calculated to compare the relationship between serum glucose and insulin levels (Table 6). There was no difference within each phenotype. However, it was noted that within each phenotype the C and DE groups had similar ratios while the ratios decreased in the D groups. Serum glucose and insulin levels were lower in the DE groups than in the C groups in all mice phenotypes (Table 4 and 5) but the relative proportions of each are the same for both groups. Group D serum insulin levels are elevated in all mice groups causing decreases in the Glu./Ins. ratio compared to the DE groups.

TABLE 5. Fasting serum insulin[a] (μU/ml) of normal (+/db) female and male mice and diabetic (db/db) mice at 8 weeks of age fed *ad libitum* (group C), subjected diet-restriction (group D), and those to diet-restriction and exercise (group DE).

	Treatment		
Groups	C	D	DE
+/db female	32 ±2	56 ±23	19 ±11
+/db male	54 ±57	65 ±76	33 ±32
db/db male	111 ±88	98 ±46	85 ±51

[a] Values represent means ± standard deviation which show no significant difference by all comparisons (n=3).

TABLE 6. Ratio of serum glucose/serum insulin of each mouse[a] to determine glucose-insulin relationships between normal (+/db) female and male mice and diabetic (db/db) mice fed *ad libitum* (group C), subjected to diet-restriction (group D), or subjected to diet-restriction plus exercise (group DE).

	Treatment			
Group	C	D	DE	p values
+/db female	6.1 ±1.7 [bx]	1.7 ±0.8 [xy]	6.4 ±3.4 [y]	x : p<0.05 y : p<0.05
+/db male	4.9 ±2.8 [b]	3.6 ±2.5	4.8 ±3.7	NSD
db/db male	3.1 ±1.2 [b]	2.6 ±1.6	3.3 ±2.0	NSD
p value	b: p < 0.05	NSD	NSD	

[a] Values represent means ± standard deviation (n=3).
[b] p values of comparisons of various groups with the same treatment.
[x,y] p values of comparisons or treatments within the same phenotype group.
NSD (no significant difference)

TABLE 7. Percent body fat[a] of normal (+/db) female and male mice, and diabetic (db/db) mice fed *ad libitum* (group C), subjected to diet-restriction (group D), and those subjected to diet-restriction and exercise (group DE).

	Treatment			
Groups	C	D	DE	p values[e]
+/db female	20.1 ±4.6	12.9 ±2.7	10.3 ±5.1	NSD
+/db male	19.0 ±4.5 [b]	13.4 ±2.3 [c]	11.0 ±3.8 [d]	NSD
db/db male	47.2 ±5.0 [b]	37.2 ±3.7 [c]	40.35 ±2.1 [d]	NSD
p values	b: $p < 0.01$	c: $p < 0.01$	d: $p < 0.05$	

a Values represent means ± standard deviation(n=3).
b,c,d p values of comparisons of various groups with the same treatment.
e p values of comparisons of treatments within the same phenotype group.
NSD (no significant difference)

Diabetic mice had lower Glucose/Insulin ratios in each treatment group compared to normal male mice with a significant reduction in group C ($p<0.05$) but not in groups D and DE. The decreased ratios are due to the increased serum insulin levels of diabetic mice compared to normal mice.

Percent Body Fat

There was an apparent gradual decline in percent body fat from groups C to D to DE in all mice phenotypes except group DE of diabetic mice had a slightly greater amount than group D (Table 7). Diabetic mice retained elevated amounts of body fat over normal male mice in each group (C: 47.2 ±5.0% vs. 19.0 ±4.5%, $p<0.01$: D: 37.2 ±3.7% vs. 13.4 ±2.3%, $p<0.01$: DE: 40.35 ±2.1% vs. 11.0 ±3.8%, $p<0.05$, respectively).

Liver Histology

Livers from normal female and male mice showed no difference in their histology so only male tissues are compared against the tissues of male diabetic mice. Normal control male mice group C show expanded hepatocytes (Figure 7a) with densely filled cytoplasm and enlarged cell nuclei (10.4 ±2.1 µm). The nuclear contents are dispersed signifying cellular activity. Fat deposits are small and dispersed throughout the tissue. There is a moderate presence of lacunae (black arrows) which

appear as irregular elongated cross-sections of vesicles. Group C diabetic mice have enlarged hepatocytes (Figure 7b) with large fat deposits (black arrow) and condensed nuclei (7.6 ±0.9 µm). Lacunae are contracted due to the enlarged cells. Diet-restricted normal male mice (group D) have thinned hepatocytes with slightly enlarged nuclei (8.0 ±2.1 µm) (Figure 8a). Lacunae are of moderate size and hepatic cytoplasm is fairly dense. There is no apparent fat deposits seen in their hepatocytes. The diabetic mice group D still show densely filled hepatocytes (Figure 8b) with dense cytoplasm and small fat deposits. Lacunae are sparse and small and nuclei are condensed (7.4 ±0.9 µm).

Diet-restricted and exercised normal male mice (group DE) exhibit further reduction in the diameter of hepatocytes (Figure 9a) and enlargement of lacunae. No apparent fat deposits were observed. The nuclei are slightly condensed (8.2 ±0.1 µm), similar to group D normal male mice. Diabetic exercised mice group DE also show thinning of hepatocytes with no apparent fat deposits (Figure 9b). Nuclei are further condensed, 6.0 ±1.7 µm, and lacunae are enlarged due to hepatocyte shrinkage.

PAS stained glycogen deposits appear as dark granular dots located

within hepatocyte cytoplasm. Normal male mice group C have fairly dense glycogen deposits (black arrows) dispersed throughout the liver section (Figure 10a). The hepatocytes of diabetic mice group C contain large glycogen reserves (black arrows) associated with large fat deposits (Figure 10b).

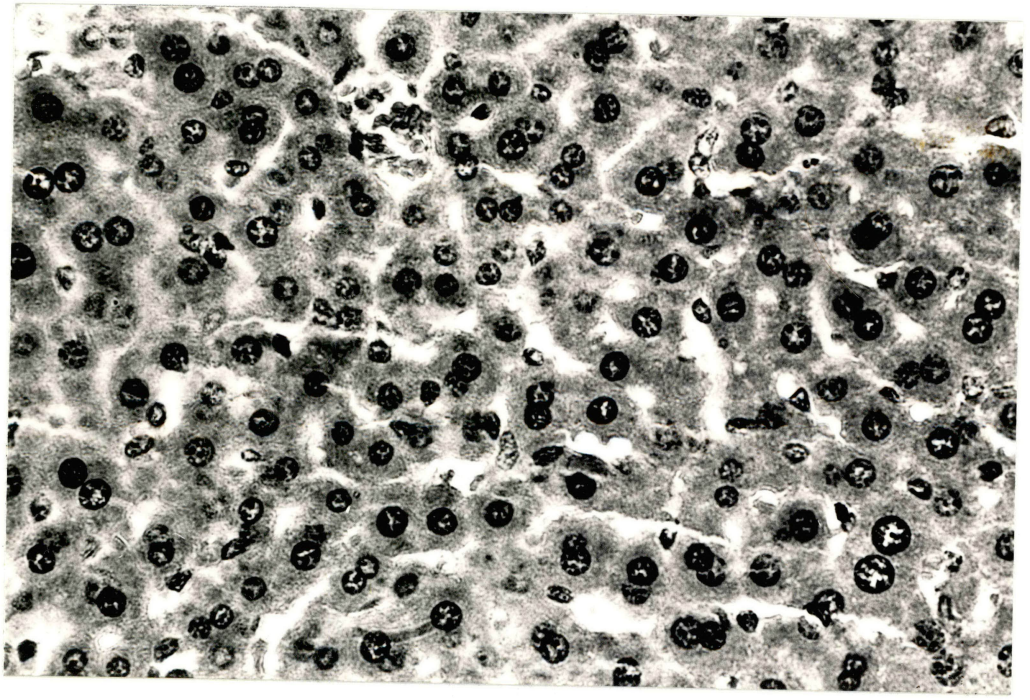
In hepatocytes of normal male mice group D glycogen deposits were scarce (black arrows) and evenly dispersed (Figure 11a) while diabetic mice group D hepatocytes have moderate deposits (black arrow) in cells associated with hepatic veins and arteries (Figure 11b). Hepatocytes from DE groups show very little glycogen deposits in both normal and diabetic male mice (Figure 12a, 12b, respectively), however, there are more small massed deposits in cells of the diabetic mice (black arrows in Figure 12b).

FIGURE 7a. Liver section of a normal male (+/db) mouse fed *ad libitum* (group C) showing compact hepatocytes with enlarged nuclei. Lacunae are small (black arrows). H&E stain, X400.

FIGURE 7b. Liver section of a diabetic male (db/db) mouse fed *ad libitum* (group C) showing huge fat deposits with expanded hepatocytes. Lacunae are very small. H&E stain, X400.

FIGURE 8a. Liver section of a diet-restricted normal (+/db) male mouse (group D). Thinned hepatocytes, contracted small nuclei, and no apparent fat deposits are visible. Lacunae are of moderate size due to hepatocyte cell thinning. H&E stain, X400.

FIGURE 8b. Liver section of a diet-restricted diabetic (db/db) mouse (group D). Compact hepatocytes, have small fat deposits and small contracted nuclei. H&E stain, X400.



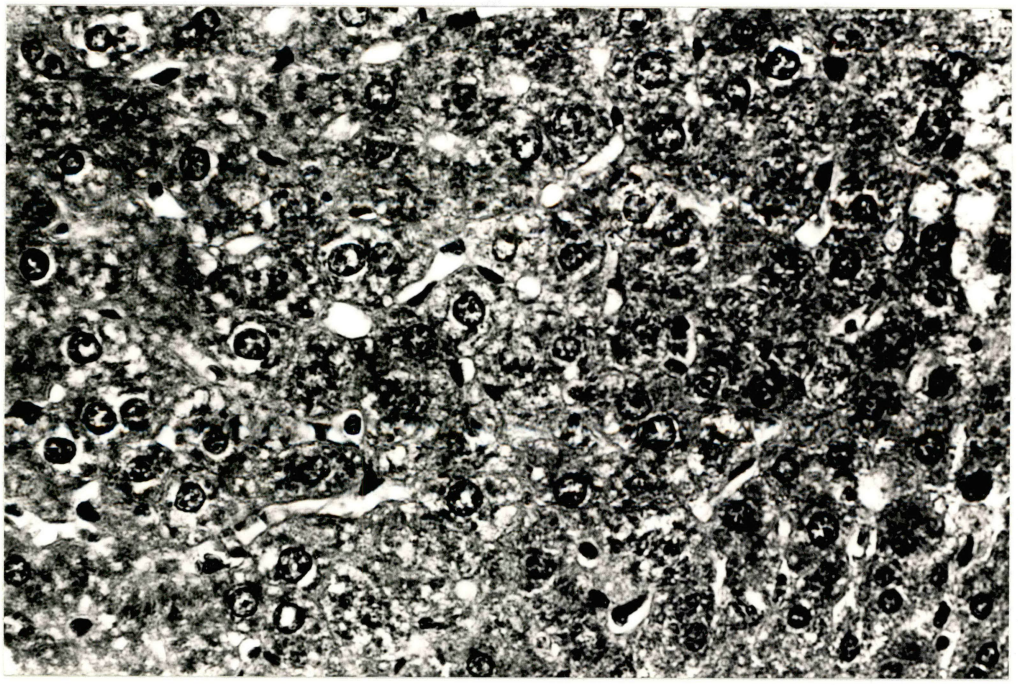
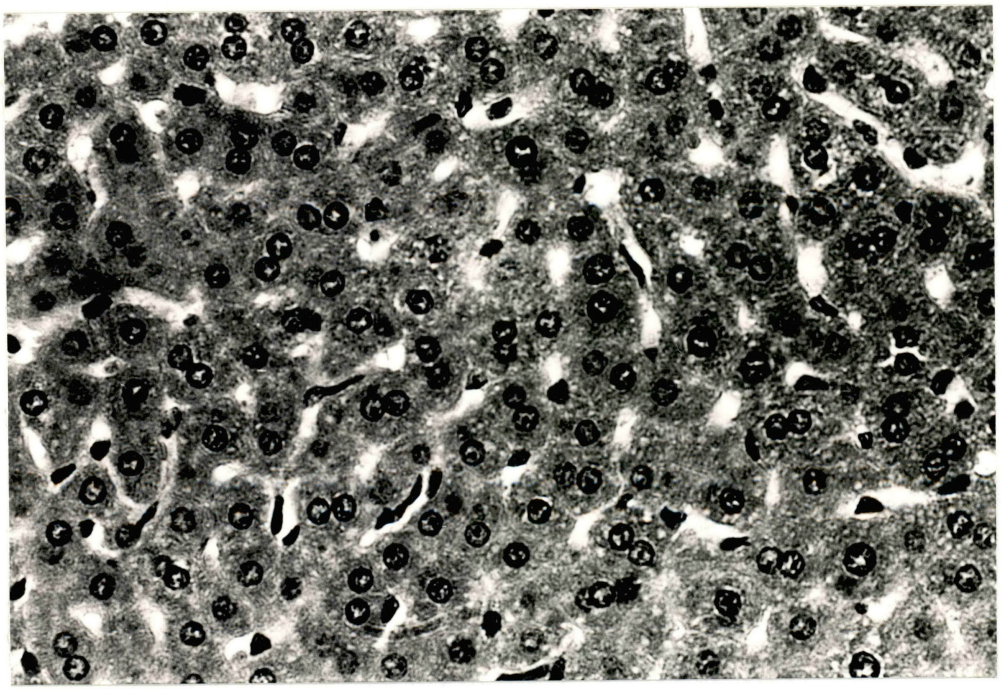


FIGURE 9a. Liver section of a normal mouse (+/db) on diet-restriction and exercise (group DE). Notice even thinner hepatocytes and slightly smaller, less dense nuclei than a diet-restricted normal mouse (group D mouse in Figure 8a). Lacunae are large due to cell size shrinkage. H&E stain, X400.

FIGURE 9b. Liver section of a diabetic (db/db) mouse on diet-restriction and exercise (group DE). Hepatocytes are thinned with no visible fat deposits. H&E stain, X400.



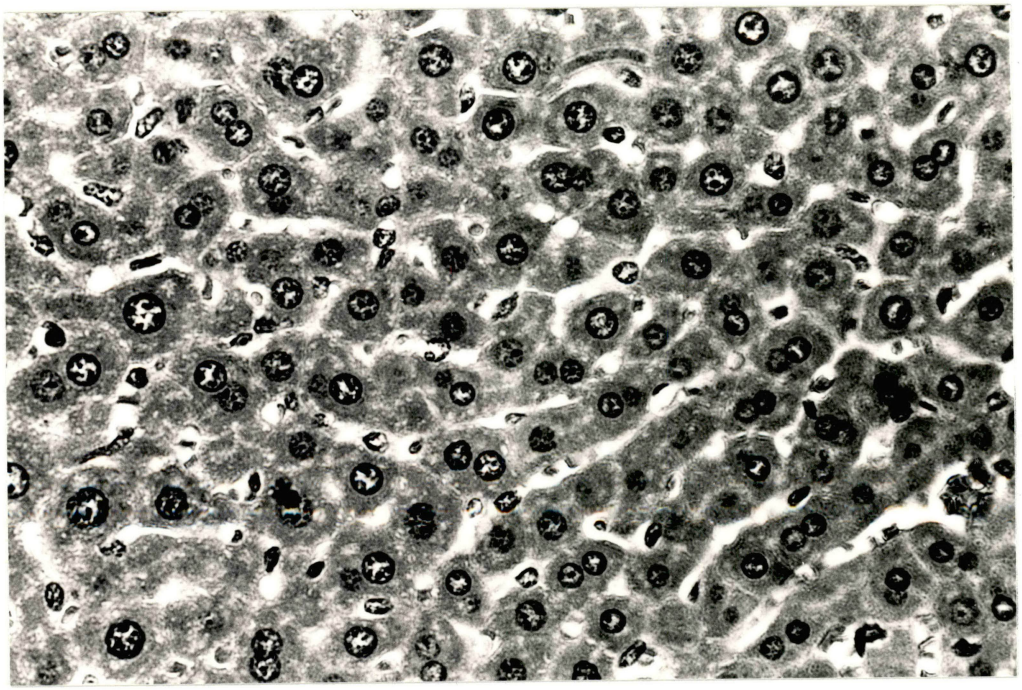
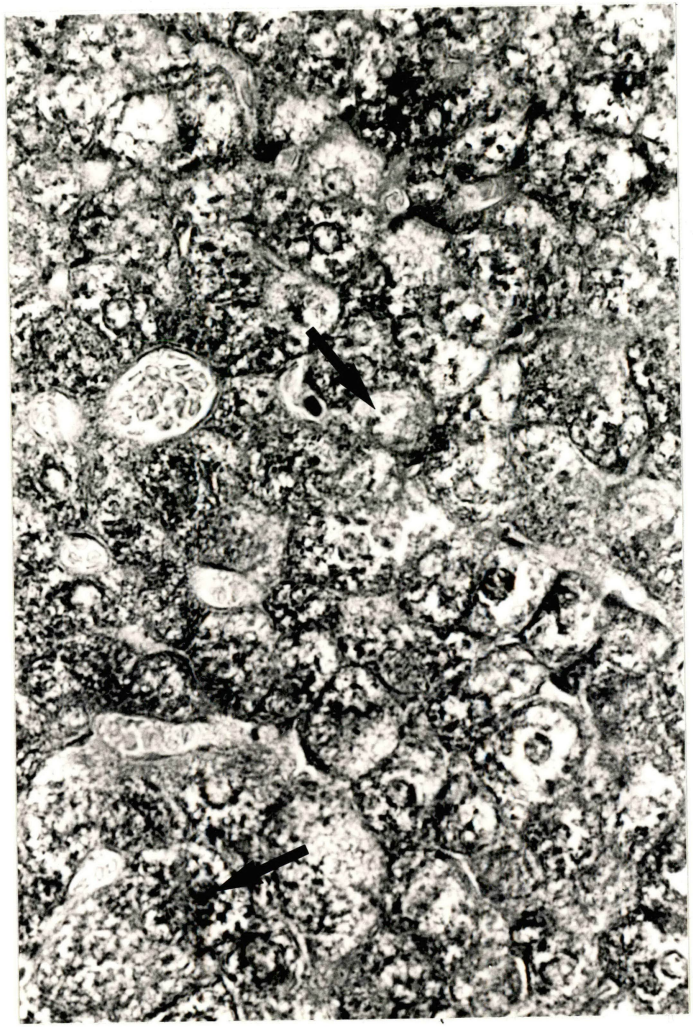


FIGURE 10a. PAS stain for hepatic glycogen on a normal male mouse fed *ad libitum* (group C) showing fairly dense glycogen deposits (black arrows). Methylene blue counterstain, X400.

FIGURE 10b. PAS stain for hepatic glycogen on a diabetic male mouse fed *ad libitum*, (group C) with large glycogen deposits associated with large fat deposits (black arrow). Methylene blue counterstain, X400.



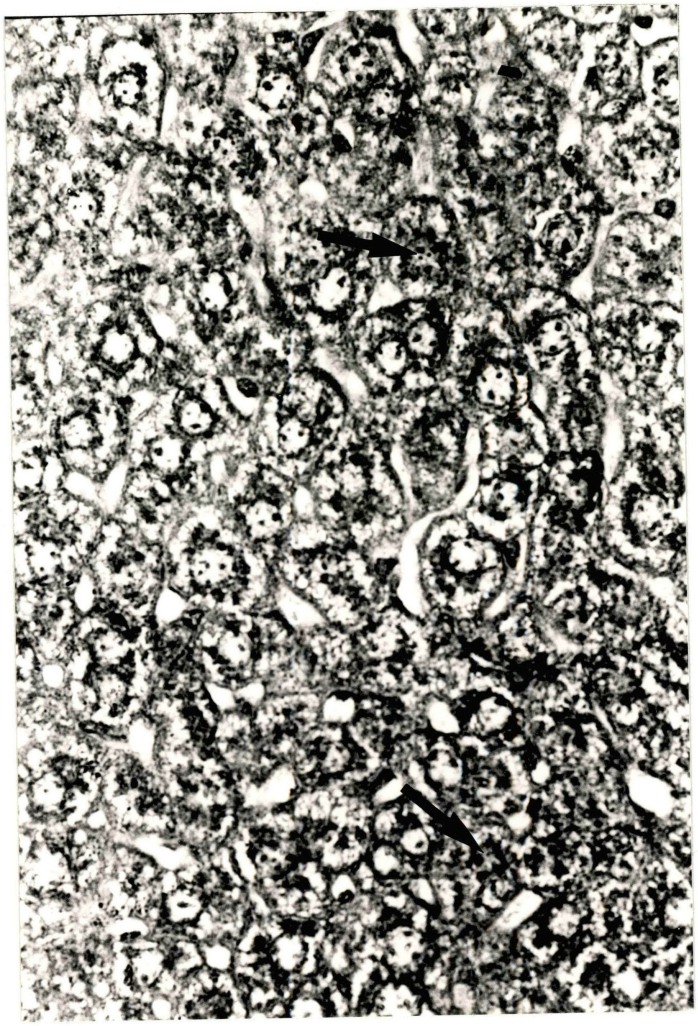
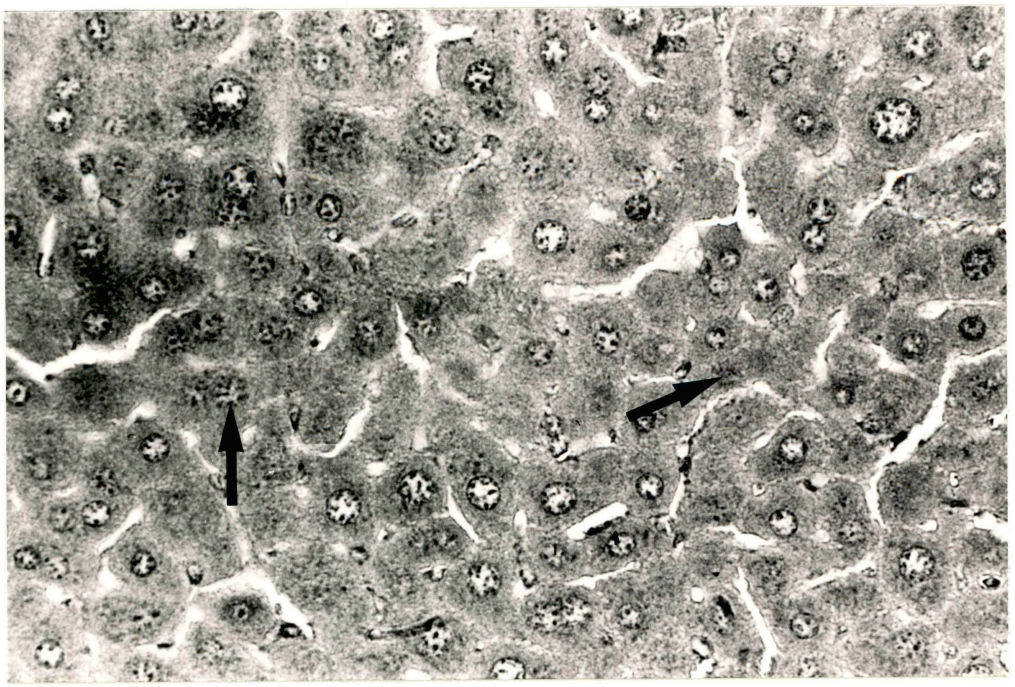


FIGURE 11a. PAS stain for hepatic glycogen on a normal male mouse on diet-restriction (group D). Small glycogen deposits (black arrows) are scarce. Methylene blue counterstain, X400.

FIGURE 11b. PAS stain for hepatic glycogen on a diabetic male mouse on diet-restriction (group D). Hepatocytes surrounding the hepatic arteries contain moderate glycogen stores. Methylene blue counterstain, X400.



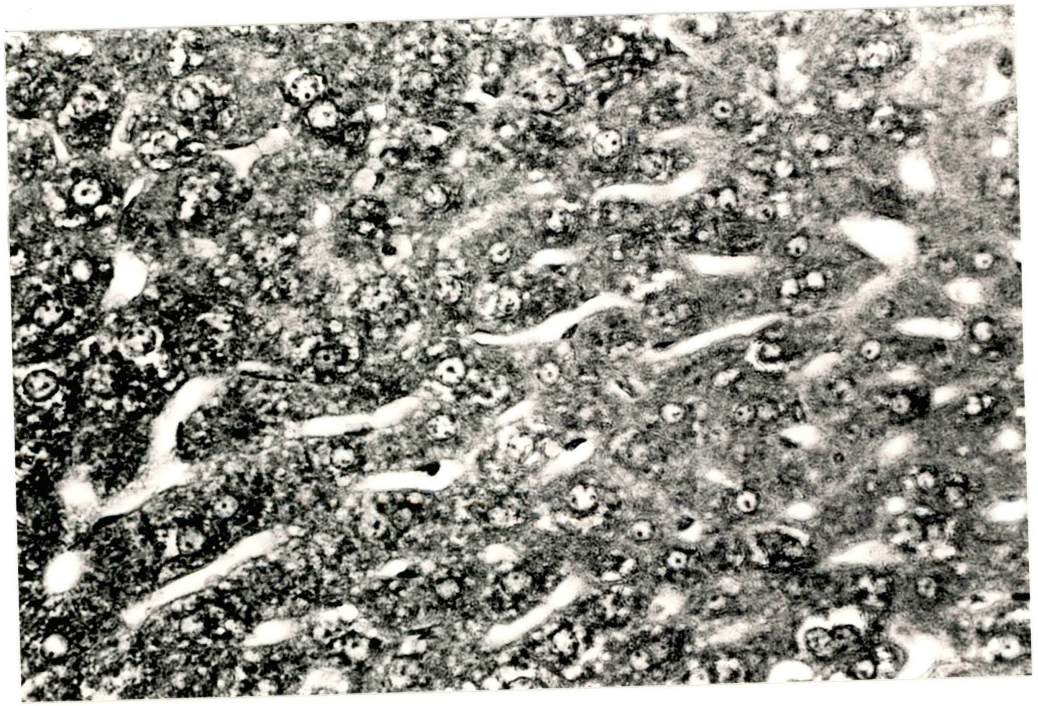
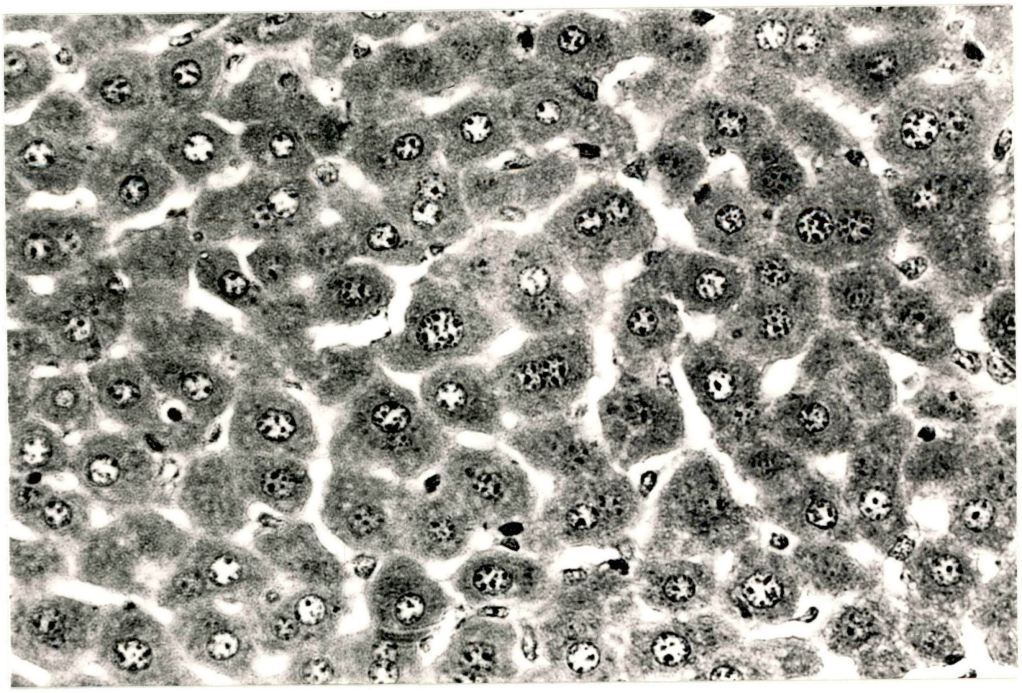
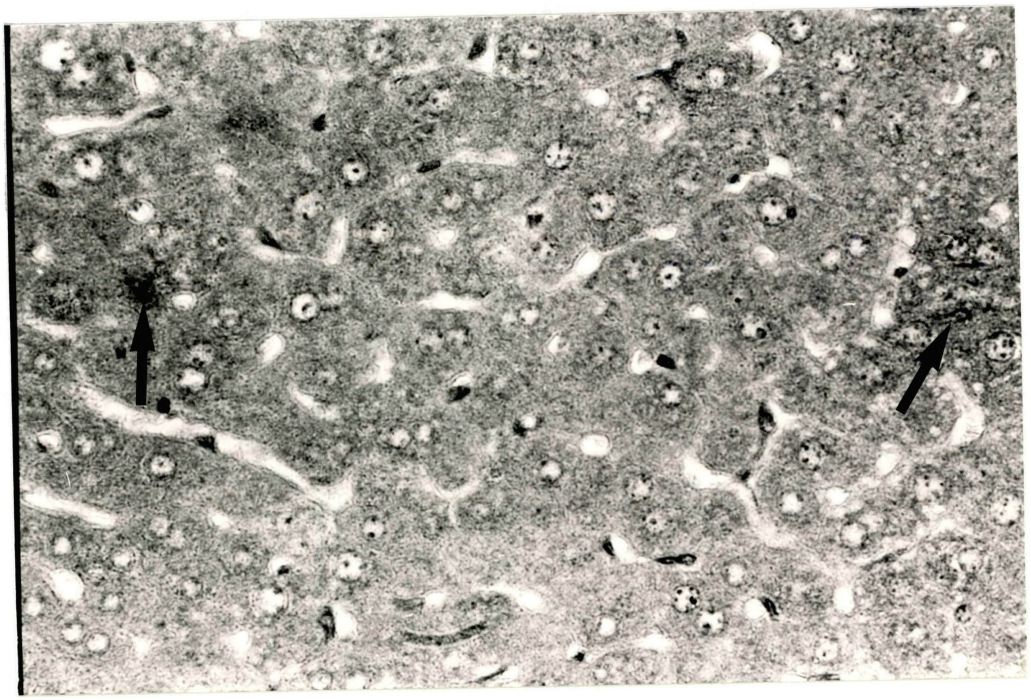


FIGURE 12a. PAS stain for hepatic glycogen on a normal male mouse on diet-restriction and exercise (group DE) illustrating very few glycogen deposits. Methylene blue counterstain, X400.

FIGURE 12b. PAS stain for hepatic glycogen of a diabetic male mouse on diet-restriction plus exercise (group DE). Only sparse glycogen deposits exist (black arrows). Methylene blue counterstain, X400.





DISCUSSION

Normal (+/db) female mice showed reductions ($p<0.05$) in body weight compared to normal males in all treatment groups throughout the three week study. All other physiological parameters measured in this study showed no significant differences between normal female and male mice. Normal female mice values were not used in statistical comparisons with diabetic male mice.

Body weights of normal male mice and diabetic mice (Figure 4) show no difference between D and DE groups at the end of the three week program. Human studies have also shown no absolute reduction in body weight by the addition of exercise (31,34,37). Thus weight may be controlled mostly by diet-restriction alone. Diabetic mice groups D and DE normalized their body weights to that of normal mice fed *ad libitum* as they displayed similar growth rates throughout the study. They maintained a 2-3g weight increase; however, diabetic mice had initial body weights greater ($p<0.05$) than the normal males by 3-4g which was not correctable by the diet and exercise treatment prescribed in this study. Starting mice at four weeks of age could compensate for this

phenomenon. Exercise in the diabetic mice lowered ($p<0.01$) the percent increase in body weight but this effect due to exercise was not observed in normal male mice. Exercise reduced the percent increase in body weight in diabetic mice more than that of normal males, thus suggesting that the weight gain in the diabetic mice is reduced whereas normal mice which ate normal amounts of food are not effected by the prescribed treatments. This is consistent with the same type of treatment performed on obese (ob) mice (30) indicating that exercise prevents the accumulation of nutrient stores by increasing the need for metabolic fuel in the body.

The metabolic efficiency defined in this study as the percent increase in body weight per amount of food consumed was reduced ($p<0.05$) from treatment group C to D to DE of diabetic mice (Table 3). The diabetic DE mice group normalized its metabolic efficiency to that of normal male mice (NSD) while groups C and D were still elevated four-fold ($p<0.01$, $p<0.05$, respectively).

Water consumption was reduced in groups D and DE from group C in normal male and diabetic mice (Figure 6). Diabetic mice illustrated a greater decrease in water consumption, whereas the control diabetic animals displayed significant polydipsia. Water consumption is generally

considered an indicator of serum glucose levels with consumption increasing in direct proportion to increasing serum glucose levels (3). Thus, the reduced water consumption in diabetic D and DE animals would indicate a greater decrease in non-fasting serum glucose levels than in untreated diabetic animals. The therapy therefore seemed to be more beneficial among diabetic mice because of their greater reduction in water consumption than the controls.

Fasting plasma glucose levels were increased in diabetic mice compared to normal male mice in all treatment groups (D and DE, $p<0.01$; but C was NSD probably due to large standard deviations because of large individual variances commonly seen in diabetic mice). Glucose levels in *ad libitum* fed animals of both phenotypes were similar to those reported by Coleman (2). Exercise caused no decrease in serum glucose levels in diabetic mice and only slight decreases in normal male animals (NSD). Since the mice were fasted for eighteen hours, there seemed to be a reduction in serum glucose from fasting which could have masked the proposed additional reduction of exercise. In contrast, human studies showed reduced circulating concentrations of glucose by exercising because exercise stimulates glucose uptake and its oxidative rate by the

muscles (37,41).

The large standard deviations of fasting serum insulin levels reported in this study are common to this animal model. However, several observations may be interpreted as indicators of hyperinsulinemia in all of the treatment groups of diabetic mice compared to normal male mice. Exercise apparently further reduced the insulin levels from D to DE groups of normal male and diabetic mice illustrating that exercise was slightly more effective than diet-restriction alone in increasing insulin sensitivity or response and in lowering serum glucose levels in normal male mice.

Exercise seems to have increased insulin sensitivity as indicated by Glucose/Insulin ratios (Figure 6). Apparently, the DE groups had an increased amount of serum glucose to serum insulin levels compared to D groups. Similarly, a walking program on obese men improved the glucose/insulin ratio by 156% (34), and exercise decreased the insulin response curve over time by glucose challenge (43). Although the serum glucose levels were the same or slightly reduced in diabetic and normal male mice, respectively, the fall in serum insulin levels mostly contributed to the increased Glu/Ins. ratio. Again, since the diabetic mice

nuclei became smaller and more dense from groups C to D to DE (Figure 7-12). This may be an indicator that cellular activity is reduced as in the form of gluconeogenesis, lipogenesis, and glycogenesis.

In this study, calculations of percent body fat utilized an estimated residual volume (RV) of 0.5ml. This RV estimation conflicts with Crosfill's suggestion (61) that the RV of a mouse should be one-third of the "eupnoepic or true mouse tidal volume" which would be ≈0.06ml. However, calculation with this value in the Siri equation yields values in the 70-80% range for percent body fat which is clearly inaccurate. Crosfill also used aneasthesized mice which show depressed respiration and it is likely this resulted in lower values. In humans the RV is twice the tidal volume, and this is more in agreement with the proposed RV estimate of 0.5 ml for the mouse (tidal volume ≈1.8 ml). Thus, the calculations reported herein should more accurately reflect the true percentage of body fat in the mouse.

Lastly, the diabetic mice had large increases in percent body fat compared to normal males: C and D groups, $p<0.01$; DE group, $p<0.05$ (Table 7). Mice on exercise programs showed no difference in percent body fat when compared to diet-restriction alone in diabetic mice, which suggests

that elevated adipose tissue deposition is not reduced by the addition of exercise to diet-restriction. Normal males had a slight decrease (NSD) in percent body fat by exercising. This supports previous studies which report that lipolysis in adipose tissues secretes FFA into the blood stream in increased amounts in order to fuel the increased metabolic demand (27,36,38,59). This is apparently not so in the diabetic animal.

The present study has shown that diet-restriction plus exercise is more effective than diet-restriction alone at reducing percent increases in body weight, normalizing metabolic efficiency, and reducing hepatic glycogen deposits in diabetic mice. These results occur from the increased metabolic need for glucose and the resultant decrease in availability of substrates for fats. There is evidence of an increased insulin sensitivity or response due to the exercise regime as evidenced by the decreased serum insulin levels and increased Glucose/Insulin ratios. However, the improvement by exercise is limited because most of the above parameters are only slightly reduced and are still elevated above normal levels. Serum glucose, body weight, and percent body fat values were similar in both diet and diet plus exercise groups in all mice. The latter set of findings suggest no effect on peripheral insulin resistance by

addition of exercise. In a similar study using the obese mouse model, exercise and matched-pair feeding still resulted in a two to three-fold increase of fat deposition in the obese animals (29). The diabetic mice in this study apparently also reduced linear growth by depleting skeletal and lean body weight below normal mouse levels at the expense of maintaining their increased amount of fat deposition.

An improved treatment regimen which might further reduce the diabetic syndrome of the diabetic mouse would be to combine more intense exercise with a smaller time interval of food availability or to combine exercise with a high protein diet. Though these suggestions would likely improve the metabolic status of the diabetic mouse to a level more like the normal mouse, it is not likely to prevent the diabetic syndrome because of the limited success in this and in numerous other studies. Bogardus (31) states the significance of diet and exercise on NIDDM human subjects as follows:

> Moderate physical therapy with a sharply restricted diet, would have no added effects in clinical parameters, of body composition, fasting serum glucose and glucose response to a mixed meal, but glucose storage capabilities would go up. However, diet plus exercise turns diabetic subjects more towards the normal state.

Despite its limitations, diet and exercise proves to be the best practical treatment of NIDDM because of the partial reduction of the diabetic state to more effective utilization of metabolic fuels and to increase insulin sensitivity and response. Behaviorally, the diabetic mice were observed to be more alert and active, thus more closely resembling their normal littermates and suggesting a trend towards normalcy. Hopefully, this can be achieved in NIDDM patients.

It was noted in this study that large variances in individual physiological values are encountered with diabetic subjects. Therefore, NIDDM patients should have a customized diet-restriction and exercise program for their specific needs. Because of the limited success of this study in normalizing the diabetic animals by diet-restriction plus exercise, it can be implied that this type of treatment program may also be limited in NIDDM patients. The genetic predisposition of the diabetic syndrome is a powerful regulator of the abnormal physiological state of the mouse or human; however, it may be improved more towards the norm by treatment designed to reverse the diabetic pathology.

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