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ENERGY UTILIZATION IN NORMAL AND DIABETIC RATS

THE UNIVERSITY OF ARIZONA  M.S. 1984
ENERGY UTILIZATION IN NORMAL AND DIABETIC RATS

by

Csilla Veronica Dudás

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In Partial Fulfillment of the Requirements
for the Degree of
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1984
STATEMENT BY AUTHOR

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Már Sokszor Enekeltem . . .

Már sokszor énekeltem rólatok,
Még többször is énekek, szép csillagok.
En úgy szeretlek titeket!
Egy szebb világgal hiteget
Sugárotok;
S ti egyre mosolygotok,
S oly jól esik nekem,
Oda tekintenem,
Hol egy kis vidámság van
E szomorú világban.

Petőfi, 1846
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ABSTRACT

Energy utilization was determined in normal and diabetic, Sprague-Dawley rats, using a comparative technique. Maintenance DE requirements were considerably higher in diabetic rats at 314.9 kcal/PBW/d in comparison with 139.0 for normals. Net efficiency values of converting DE to NE were 63.76 and 7.49% in normal and diabetic rats, respectively. Fat deposition efficiencies were 35.8 and 2.3%, while efficiency of lean deposition was 61.1 and 21.6% efficient in normal and diabetic rats, respectively.

DE costs of fat and lean gains were 1.14 and 5.5 kcal DE/kcal fat deposited and 2.55 and 10.6 kcal DE/kcal lean deposited, respectively, with efficiencies of fat and lean gain determined as 87.8 and 18.2% and 39.15 and 9.44% in normal and diabetic rats, respectively. Estimated gross energy of lean tissue was similar in both groups, 5.46 and 5.14 kcal/g, while fat was 10.10 kcal/g and 8.76 kcal/g in normal and diabetic rats.

Diabetic rats were significantly less efficient by all measures in energy utilization.
CHAPTER I

INTRODUCTION

Diabetes mellitus is a chronic systemic disease affecting most aspects of metabolism (primarily carbohydrate, but also fat and protein) and the vascular system due to deficiency of insulin or ineffectiveness of insulin present in the system (Harper, 1975; Ganong, 1975). There are two types of diabetes in humans: juvenile-onset (Type I) and mature-onset (Type II) diabetes. Type I is due to absolute deficiency of insulin from destruction of pancreatic B-cells by some means and Type II is due to ineffective use of insulin available because of obesity (changes in insulin receptors) or insulin resistance (Eli Lilly, 1980).

The alterations in metabolism associated with uncontrolled diabetes mellitus in experimental animals provides an opportunity for study of energy utilization and energetic efficiency in relation to insulin.

Energy balance is important in nutrition and economics since dietary energy and the interconversions which occur within the body are the driving forces for all metabolic reactions. Deficiencies in energy intake are not as visible as those associated with vitamins or minerals in
animals or human beings but long term effects of energy insufficiency are equally debilitating. In depth knowledge of bioenergetics aids in the understanding and in the treatment of metabolic diseases that occur in our society.

Bioenergetics is concerned with the conversion of various forms of energy into a form that is biologically useful to the organism and involves the application of the laws of thermodynamics, such as the law of energy conservation, and the law of initial and final states, to the living system. The basis of bioenergetics, as defined by the laws of thermodynamics, can be stated by the following formula (National Research Council (NRC), 1981):

\[
IE \ (Energy \ Intake) = Fecal \ E + Gaseous \ E + Urinary \ E \\
+ \ Surface \ E + Heat \ E + Net \ or \ Recovered \ E
\]

Energetic efficiency, a basic concept in bioenergetics, is the efficiency with which energy is utilized to deposit or gain new tissue or recovered as a product, such as eggs, milk, wool, etc., and can be defined as a ratio of the energy deposited to the metabolizable energy (ME) intake corrected for maintenance costs. This concept developed from the second law of thermodynamics (Rechcigl, 1979).

Energy utilization can be determined by direct or indirect measurements of heat production or by evaluation of carcass deposition of protein and fat. Heat production
measurements involve direct or indirect calorimetry, where heat produced by the animal is measured directly in a calorimeter (thermal exchange) by the first method, and $O_2$ consumed and $CO_2$ produced (respiratory exchange) is measured by the latter method. Measurements of carcass composition involve determination of the heats of combustion of carcass components, feed and feces (Blaxter, 1971; NRC, 1981).

The subject of energy balance, energy utilization, and related topics is very extensive. Even with all the information available, a search of the literature produced little work dealing specifically with energy utilization and energetic efficiency in diabetic animals. The experimental use of diabetic animals seems to have been generally restricted to a means of controlling insulin levels in order to develop and expand hypotheses dealing with feed intake regulatory mechanisms. Very little work has been done to investigate how energy utilization is affected by this disease. The fact that little research has been carried out in this area was the reason for proceeding with the experiment discussed in this thesis.
Normal Energy Utilization

Many studies have been conducted to evaluate energy utilization using experimental animals such as mice and rats, and most researchers have found rather similar rates of energetic efficiencies based on various modified experimental designs. It is difficult to compare and evaluate many of the individual studies because of differing methods and parameters used in the research.

Canolty and Koong (1976) used two lines of mice, one selected for rapid postweaning growth rate and the other selected randomly from a contemporarily mated line (control group), in an experiment to study energy utilization. The data, evaluated by multiple regression, showed that the maintenance energy requirement was the same for the two lines of mice at 176 kcal/physiological body weight (PBW; kg $W^{0.75}$). Efficiency of energy utilization, on the other hand, was significantly different and the rapid growth line had an energetic efficiency of 50% while the controls had an efficiency of 23%. Of the metabolizable energy available above maintenance, 79% was deposited as fat and 21% as lean in the rapid growth line, while the control line deposited
58% as fat and 42% as lean. Regression analyses of the change in lean energy/PBW on metabolizable energy intake (MEI)/PBW resulted in lean energy deposition coefficients of 10.4% for the rapid growth line and 9.6% for the control line. When the change in fat energy/PBW was regressed on MEI/PBW, the fat energy deposition coefficients were 39.3 and 13.4% for the rapid growth and control lines, respectively. From these data, the authors suggested that the two lines of mice used the ME available above maintenance in different proportions or at different efficiencies or both. It was concluded that mice from the rapid growth line used energy more efficiently for fat gain than did the control mice and that the maintenance energy requirement was the same for the two lines when expressed in relation to PBW. Comparison of lean energy deposition coefficients showed no difference between the two lines, but there was a significant difference in fat energy deposition coefficients indicating that the rapid growth line mice deposited more of the ME available as fat than did the controls.

Koong (1977) recalculated the above data using a Michaelis-Menten type equation to partition the ME available (MEA) for growth into fat and lean tissue synthesis (Figure 1). The efficiencies of fat synthesis were 57.5% for the rapid growth line, and 35.6% for the control mice.
Figure 1. Schematic for use of MEA for fat and lean gain

This results in the equation, $P = \frac{\text{MEA}}{K + \text{MEA}}$. $K$ is the level at which 50% of the MEA is utilized for fat synthesis. Rearranging the equation gives $\text{FG} = \frac{\text{MEA}}{(K+\text{MEA})E_f*\text{MEA}}$ and $\text{LG} = \frac{K}{(K+\text{MEA})E_1*\text{MEA}}$, where $K$, $E_f$ and $E_1$ were unknown and MEA, FG and LG were the parameters measured.

Efficiencies of lean tissue synthesis were 39.2 and 17.4% for the rapid growth line and controls, respectively. This evaluation indicated that the rapid growth line mice not only used a larger proportion of the MEA for fat and lean gain but also used it at a higher efficiency. By application of the Michaelis-Menten equation to the data, Koong further clarified the results obtained by Canolty and Koong (1976). The new method for estimating energetic efficiencies was thought to give better results and less error than standard multiple regression techniques.

In a similar study with congenitally lean and obese Zucker rats (littermates bred for leanness or obesity), Deb, Martin and Hershberger (1976) found results comparable to those of Canolty and Koong (1976). Obese rats had a greater
energetic efficiency for gain above the maintenance requirement than did lean rats (51.4 vs. 21.4%) but protein gain was significantly lower in the obese rats.

The maintenance requirement was not different for either lean or obese rats (130.5 and 122.0 kcal ME/PBW/d, respectively). Deb et al. concluded that obese rats had a higher energetic efficiency which was not related to a lower maintenance requirement.

A rat study by Schoenborne and Canolty (1980) examined the effects of meal frequency on body composition and energy utilization. They found that meal frequency, whether the animals were fed once or four times/day, had no effect on either body composition or energy utilization. The lean energy deposition coefficient was 12% and the fat energy deposition coefficients were 22% (one meal/day) and 30% (four meals/day). Gross energetic efficiencies were 34% and 42% for the two groups (one meal/d and four meals/d), respectively.

McNiven, Wiktorsson and Eriksson (1981) found that the maintenance energy requirement of rats was proportional to PBW using restricted and overfed rats. Results showed a maintenance requirement of 94.36 kcal/PBW, which was felt to agree with the interspecies equation for basal metabolic rate of 70 kg W^{-75} (Kleiber, 1947). No differences in digestible energy (DE) or ME were observed across all treatments and digestion efficiencies were the same
regardless of feed intake. They concluded that protein and fat gains were nonlinear with energy intake and that rate of fat deposition increased while protein decreased.

Similarly, Pullar and Webster (1977) employed genetically lean and obese Zucker rats to determine the energetic cost of fat and protein deposition. Costs of fat and protein deposition are usually defined as the portion of food energy (usually in terms of ME) required to deposit an amount of body fat or body protein. They found, by regression analyses, that the ME required to deposit one gram of protein and one gram of fat was approximately the same, 12.6 and 12.8 kcal, respectively. Assuming that the energy content of protein is 5.6 kcal/g and that of fat is 9.4 kcal/g, these correspond to energetic efficiencies of 44.4% for protein gain and 73.4% for fat gain.

Feed Intake Regulation

According to Anand (1967), energy balance in an animal is maintained by an equilibrium of several factors: (1) feed intake, (2) physical activity, (3) heat production, and (4) energy storage in the body. Although there are many theories regarding control of feed intake, there is much controversy as to the exact mechanism which regulates feed intake.

Presently, the glucostatic (or glucosensitive) theory is considered to be a short-term regulatory
mechanism. The hormones which are thought to be involved in this mechanism are the pancreatic hormones, insulin and glucagon. Both have been shown to alter feed intake since insulin and glucagon control glucose availability and utilization in the body. Mayer and Bates (1952) theorized that as glucose availability or utilization decreased there is a concurrent increase in feed intake. He demonstrated that glucoreceptors exist in the hypothalamus which are sensitive to plasma glucose levels. Glucose is thought to be a key compound because it is the major source of energy for the brain (Harper, 1975; Ganong, 1975). Anand (1967) reported that electrical activity in the ventro-medial hypothalamus (VMH) measured by electroencephalograph, changed in proportion to blood glucose changes during hunger and satiety. Glucose administered intravenously caused increased electrical activity in the satiety (VMH) center while hypoglycemia, induced by insulin injections, produced decreased electrical activity.

Panksepp and Nance (1972) have also indicated that the VMH was involved in control of feed intake. In observing normal and diabetic rats with and without VMH lesioning, it was found that the VMH affected feed intake through slow metabolic integration of nutrients, not by sensory stimulus or directly by the rapid breakdown of incoming nutrients. The metabolic state of the VMH cells determined feeding behavior in diabetic rats, because
lesioning of the medial hypothalamus of diabetic animals lessened their response to feed restriction. Glucose injections into the VMH decreased daily feed intake, but not meal size immediately after the injection, suggesting long-term regulation by the VMH rather than short-term satiety. It also was observed that feed intake did not increase as significantly after daily treatment with insulin in VMH lesioned rats as in non-lesioned animals. This indicated that the VMH contained cells which control feeding behavior (increased intake) during chronic hyperinsulinemia.

de Castro, Paullin and DeLugas (1978) manipulated insulin, glucagon, and somatostatin levels in several ways to determine the effects on feed intake: (1) insulin injections to increase insulin levels, (2) glucagon injections to increase blood glucose, (3) somatostatin injections to decrease both insulin and glucagon levels, and (4) induction of diabetes to decrease insulin levels and increase glucagon levels. They established that insulin decreased glucose supply and increased its utilization (also predicted by the glucostatic theory). This decreased blood sugar resulted in increased feed intake and ultimately increased body weight. Glucagon, being an antagonist of insulin, had the opposite effect by increasing glucose supply and decreasing glucose utilization. Consequently, both feed intake and body weight decreased. Somatostatin, which is antagonistic to both insulin and glucagon, had no
effect on either feed intake or body weight. Similar results were observed in both normal and diabetic rats.

De Castro et al. (1978) found that the insulin/glucagon ratio rather than the level of either hormone alone was a major determinant of feed intake and body weight set point. Body weight increased when insulin increased relative to glucagon and it decreased when glucagon increased relative to insulin. Neither insulin nor glucagon was changed by administering somatostatin, and as expected, there was no change in feed intake or body weight. In diabetic animals, glucagon levels were high relative to insulin and resulted in decreased body weight. Somatostatin administered to diabetic animals to decrease glucagon levels resulted in an expected increase in body weight.

Brandes (1977) rejected the glucostatic theory that insulin caused a change (increase) in feeding behavior by producing nonbehavioral neuroendocrine responses due to a decrease in glucose utilization in the cells and activating a receptor mechanism for feeding by the resulting hypoglycemia. Brandes proposed that insulin-induced overeating (IIO) was due to a neurological dysfunction in CNS metabolism resulting from insulin-induced hypoglycemia. He found that hypoglycemia existed without overeating in rats and that overeating did occur in insulin-treated rats with mild neurological dysfunction. Overeating in these hypoglycemic animals was not due to a feeling of hunger.
which was rarely present during the acute, severe hypoglycemia. High dose, short-term IIO caused increases in meal size and frequency which suggested that hunger was not the major reason for overeating. Therefore, the CNS pathophysiology caused by hypoglycemia was suggested as the cause of overeating and not behavioral glucostat.

Much of the research dealing with food intake regulation has involved plasma insulin and glucagon levels. It has been determined (as mentioned by Anand, 1967) that glucoreceptors exist in the brain in areas related to feed intake, so the glucostatic theory has a role, even though it may be small, in feed intake regulation. Plasma insulin may be a short-term regulator, while insulin present in cerebro-spinal fluid (CSF) may be the long-term control of feeding and body weight. Woods et al. (1979) theorized that CSF insulin concentration may be the signal for the feedback system regulating body fat stores. There were several reasons for this postulate: (1) insulin is present in the CSF; (2) plasma insulin concentration (short-term fluctuations) does not affect CSF insulin concentration; (3) lean individuals have lower CSF insulin levels than obese individuals and CSF insulin levels fall in both types of individuals during a fast; and (4) insulin receptors exist in areas of the brain and spinal cord. In order to test this hypothesis, insulin was infused into the CSF of baboons at a level equal to 25% of plasma insulin concentration.
There was a dose-related decrease in feed intake and a decrease in body weight during insulin infusion. Glucagon infusion, on the other hand, had no effect on feed intake compared to baseline levels. Feed intake returned to control level at the end of the experimental period. Insulin infusion did not affect plasma insulin or glucose levels, indicating no movement of insulin from the CSF into the plasma.

It seems that many factors are involved in control of feed intake. None of the theories in existence is individually the accounts for the regulation of feeding. The answer probably lies in a combination of all these hypotheses.

**Diabetes**

Diabetes mellitus is a chronic systemic disease affecting most aspects of metabolism (primarily carbohydrate, but also fat and protein) and the vascular system due to insulin deficiency or ineffectiveness of insulin present in the system (Harper, 1975; Ganong, 1975). The two types of diabetes occurring in human beings are juvenile-onset (Type I) and mature-onset (Type II). Type I is due to absolute deficiency of insulin from destruction of pancreatic β-cells by some means and Type II is due to ineffective use of insulin available because of obesity.
(changes in insulin receptors) or insulin resistance (Eli Lilly, 1980).

The abnormalities seen in diabetes can be explained by the varied functions of the anabolic hormone, insulin. In general, diabetes is primarily a malfunction in carbohydrate metabolism. Lack of insulin from the $\beta$-cells causes a decrease in glucose uptake by peripheral tissues giving rise to hyperglycemia, the major symptom of uncontrolled diabetes. Protein catabolism increases due to glucose deficiency in the peripheral cells thus glucogenic amino acids are provided to the system for gluconeogenesis in the liver and kidneys (protein catabolism also increases urinary nitrogen loss). This consequent increase in glucose production, little of which enters target cells, causes severe hyperglycemia. The high blood glucose exceeds the renal glucose threshold and glycosuria results. Glucose is osmotically active and the hyperglycemia and glycosuria cause osmotic diuresis (polyuria), which leads to dehydration. Dehydration in turn causes polydipsia (increased water intake). Insulin deficiency also causes malfunctions in lipid metabolism. Lipolysis increases in the absence of insulin therapy and this increases plasma free fatty acid (FFA) concentrations which result in increased production of ketone bodies and cause ketosis. Ketosis also increases the dehydration already present and may lead to acidosis.
This state of excess glucose in the blood and a state of starvation within cells of the peripheral tissues, informs the body that more glucose is needed. This may be the signal for increased release of glucagon. Glucagon is a catabolic hormone, which mobilizes glucose from liver storage and fatty acids from adipose tissue. It is a direct antagonist of insulin. Since insulin and glucagon are two of the most important metabolic hormones, the insulin/glucagon (I/G) molar ratio is a determinant of the body's metabolic state at any point in time (Ganong, 1975). When the body requires energy, as in starvation or fasting, the I/G ratio is low, favoring glucagon release in order to increase glycogen breakdown and gluconeogenesis. In the fed state, when energy requirements are low, the I/G ratio is high favoring nutrient deposition as glycogen, protein and fat.

Type I diabetes is often characterized by hyperglucagonemia, which only increases the hyperglycemia already existing in uncontrolled diabetes.

**Alloxan Diabetes**

Alloxan, the ureide of mesoxalic acid, was first reported to be a diabetogenic compound in 1943 by several groups of researchers. Dunn, Sheehan and McLetchie (1943) and Goldner and Gomori (1943) found that alloxan caused necrosis of the islets of Langerhans. Jacob (1937) observed
the following triphasic action by alloxan: (1) an immediate hyperglycemia, which peaks within 2-3 hours after alloxan administration; (2) a severe and often fatal hypoglycemia; and (3) chronic, final hyperglycemia (if the animal survives the second phase). Goldner and Gomori (1944) reported that the first phase hyperglycemia can be prevented by administering insulin simultaneously with alloxan, but the ensuing two phases still occurred. The first hyperglycemic phase was caused by increased glycogenolysis. The second phase (hypoglycemia) was not the cause of islet cell necrosis, but was only a sign of it, according to these researchers. Ridout, Ham and Wrenshall (1944), along with other groups, investigated the histological process of alloxan administration. Hypoglycemia occurred after β-cell degeneration apparently due to the tremendous increase in insulin levels as insulin was leached out of dying and dead islet cells. β-cell necrosis could be observed as soon as one hour after alloxan injection. Hypoglycemia occurred only 4-5 hours later. Complete necrosis was seen within 12 hours of alloxan injection.

Data presented by Ishibashi et al. (1981) in several papers, by Malaisse (1982) and by Sener, Malaisse-Lagae and Malaisse (1982) indicated that alloxan acted within the β-cell by (1) initially stimulating the pentose-monophosphate shunt with the formation of H2O2; and (2) subsequently inhibiting both the
pentose-monophosphate shunt and the tricarboxylic acid cycle pathways through the formation of $\text{OH}^*$ from $\text{O}_2^-$ and $\text{H}_2\text{O}_2$.

Malaisse (1982) found that islet cells accumulate alloxan very rapidly, within 2-5 minutes after alloxan treatment. Among several tissues examined, islet cells were the most sensitive to peroxide compounds. These two factors, rapid accumulation and sensitivity, may explain the diabetogenic action of alloxan.

Alloxan causes effects which do or do not necessarily occur in spontaneous diabetes mellitus in humans. George and Augusti (1978) reported that the metabolic defects in alloxan diabetes vs. Type II (human) diabetes mellitus may be different because in alloxan diabetes the defect is a lack of endogenous insulin while in maturity-onset diabetes the endogenous insulin is inactive. They found serum component differences between alloxan diabetic rabbits and diabetic (Type II) men. Serum pyruvate and phospholipids were considerably higher in alloxan diabetes than in adult-onset diabetes. Duffy (1945) found that ketonuria did not occur in alloxan diabetes with ordinary diets, but could be induced by excess glucose. Ketonuria, in humans, is typical of Type I, but not Type II diabetes which alloxan-diabetes resembles.
Feed Intake in Diabetes

Feed intake has been studied extensively for a variety of reasons. There is a normal variation in daily intake in animal species. The major reason for studying this area is to determine what factors (hormonal, neural, environmental) control overall feed intake and energy balance in an animal species.

Kumaresan and Turner (1965) studied the effects of alloxan on feed consumption in rats to evaluate the role of insulin in feed intake regulation. They reported that after an initial 67% decrease in feed intake (compared to control period) following a single alloxan injection, intake increased up to 56% above the control period. These results agreed with most of the work on alloxan-treated rats.

Early work regarding the effect of insulin on feed intake regulation was done by Mayer and Bates (1952). In an experiment alloxan-diabetic rats became hyperphagic and this was thought to be caused by decreased glucose utilization in the feed regulating areas of the brain. The hyperphagia was considered to be a "defense" mechanism against glucoprivation. Administering insulin to these animals alleviated the high rate of feed intake.

In order to evaluate the role of insulin in energy balance regulation, and consequently in feed intake regulation, Thomas, Scharrer and Mayer (1976) employed alloxan-diabetic rats. An increase in feed intake was
observed which resulted from an increase in meal size rather than meal frequency. Feed intake increased 2-3 times over the control period after an initial decrease in feeding behavior immediately after alloxan injection. Thomas et al. (1976) and Steffens (1969) reported that in normal rats given insulin injections the reverse was true, meal frequency increased and not meal size. Diabetic rats had a higher rate of energy intake than VMH lesioned rats due to their inability to store fat for future utilization. Therefore, diabetic animals required each meal to supply the immediate energy necessary for daily activity.

The hyperphagia observed in diabetic animals can be explained by the various metabolic aberrations of diabetes mellitus such as decreased glucose utilization, low liver glycogen and decreased body fat stores. An exception was found with the feeding of high-fat diets to diabetic animals. Excessive overeating did not occur in this case according to Friedman (1972). The effect of diet composition on hyperphagia in alloxan diabetes and whether metabolic abnormalities continued in these animals was studied by Friedman (1978). He found that diabetic rats adjusted feed intake only when the caloric density of the diet was changed by altering dietary fat levels, while normal rats adjusted intake when either dietary fat or carbohydrate content was altered. The basal diet contained 23% fat, 52% cornstarch and 20% casein by weight. Intake
was approximately the same for both groups fed the basal diet. Normal rats increased intake by the same amount when either fat or carbohydrate was diluted with non-nutritive filler. As fat content was diluted, diabetic rats became hyperphagic, and with carbohydrate dilution diabetics ate approximately the same amount as they had eaten of the basal diet. If the basal diet was diluted with glucose (no change in caloric density) normal rats decreased intake to basal diet levels, while diabetic rats became hyperphagic.

The next step in this study was to determine if feeding a high fat diet alleviated or reversed any of the metabolic impairments in alloxan diabetes. Blood glucose, insulin activity, liver glycogen and body fat analyses were conducted. Groups of diabetic rats were fed either a low-fat or a high-fat diet. As in the previous experiment, diabetic animals on the low-fat diet were hyperphagic and those on high-fat diets ate normal amounts. Both groups weighed less than normal controls. Blood glucose was high in both groups, although the high-fat group had slightly lower values compared to the low-fat diet group. Insulin levels were 20% lower in both dietary groups compared to normal controls. Plasma free fatty acids (FFA) were twice as high in the high-fat group as compared to normals. Only the high-fat diet group showed ketonuria. Both groups lost comparable amounts of weight. Liver glycogen was one-half
of normal and carcass fat was low in both groups, especially the low-fat group. All diabetic animals had glycosuria.

Friedman found that diabetic rats utilized fats more readily than carbohydrates for daily energy needs after body-fat stores were depleted. Hyperphagia in diabetic rats was a closely regulated, compensatory mechanism to replace the loss of utilisable fuels as seen in the relationship between dietary fat content and feed intake. The observation that intake increased as dietary fat was diluted with filler, in a manner similar to normal rats, indicates that alloxan-diabetic rats used fat as the major utilisable fuel.

Friedman's results also suggested that hunger in diabetic rats was due to reduced levels of circulating fats, since the difference in dietary fat intake was reflected in differences in plasma FFA levels. They demonstrated a direct relationship between dietary fat and plasma FFA. It should be noted that diabetic rats, given a choice between low-fat and high-fat diets, had a preference for high fat diets.

Several of the theories explaining hunger and hyperphagia in diabetic rats were questioned (or disproved) by the results of the study above. Mayer and Bates (1952) and Smith and Epstein (1969) suggested that hyperphagia was caused by decreased utilization of glucose. This was contradicted since diabetic rats in Friedman's study
continued to have glycosuria, elevated blood glucose and low plasma insulin despite normal intakes of the high-fat diet. Blood glucose was slightly lower, probably because of decreased carbohydrate intake.

Another theory promoted by Panksepp and Ritter (1975) stated that overeating in diabetic animals was due to decreased body nutrient stores. Normal intake was maintained in the present experiment on the high-fat diet despite severe weight loss and considerable loss in carcass fat. Friedman concluded that because stored fuels were reduced, the immediate availability of oxidizable fuels was the determinant of feed intake.

Diabetic rats lose considerable amounts of energy in urine with glycosuria and ketonuria, therefore this had been suggested as a cause of overeating by Panksepp and Nance (1972). However, glycosuria and ketonuria continued to be present in rats eating normal amounts of high-fat diets.

Liver glycogen was half the normal concentration in diabetic rats fed the high-fat diet (Friedman) and this also had been thought to be a reason for hyperphagia by Russek (1976). Although results of Friedman's study were inconsistent with the hypothesis that liver glycogen levels control feeding, his results did not eliminate the possibility that hunger may have occurred when the supply of utilizable fuels in the liver was low. Thus, high levels of
FFA in the liver could have been a signal to the diabetic rat to eat less, because of the availability of FFA as a utilizable fuel.

**Summary**

The subject of energy balance, energy utilization and related topics is very extensive. Even with all this information available, it is difficult to find work dealing with diabetic animals specifically concerning diabetic energy utilization and energetic efficiency. The use of diabetic animals seems to be only as a means of controlling insulin levels in order to develop and expand hypotheses dealing with feed intake regulatory mechanisms. Very little work has been done to investigate how energy utilization is affected by this disease. The fact that little research has been carried out in this area was the reason for proceeding with the experiment discussed in this thesis.
CHAPTER III

EXPERIMENTAL PROCEDURE

Sixty-five male, Sprague-Dawley rats were used in this investigation. Rats were kept on a 12-hour light-dark cycle at 23°C +/- 1°C and 50% relative humidity. Rats were fed Ralston-Purina rat chow ad libitum until the beginning of the experimental phase, at which time all rats were weighed and then fasted for 24 hours. Initial body weights ranged from 105 to 177 g. The rats were divided into two groups, such that their total average body weights were the same.

One group of 32 rats was injected intraperitoneally with a single dose of alloxan monohydrate (Sigma), 150 mg/ml .85% NaCl at a level of 150 mg/kg body weight (Turlapaty, 1980; Kennedy and Lukens, 1944). A single intraperitoneal dose of saline (.85% NaCl, 1 ml/kg) was administered to a second group of 33 rats. Alloxan-injected rats were tested for the presence of diabetes by the appearance of glucose in the urine 5 days after alloxan treatment using Tes-Tape (Eli Lilly Co.). All rats were fed a pelleted experimental diet (Table 1) ad libitum for 9 days prior to the start of the experiment.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>12.77</td>
</tr>
<tr>
<td>Ground Corn</td>
<td>72.30</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>.18</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>.25</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.00</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>5.00</td>
</tr>
<tr>
<td>AIN-76 Minerals$^1$</td>
<td>3.50</td>
</tr>
<tr>
<td>AIN-76 Vitamins$^2$</td>
<td>1.00</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>.20</td>
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**Calculated Nutrient Composition**

<table>
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<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, %</td>
<td>17.50</td>
</tr>
<tr>
<td>DE, kcal/g$^3$</td>
<td>3.79</td>
</tr>
<tr>
<td>Fat, %</td>
<td>7.85</td>
</tr>
<tr>
<td>Fiber, %</td>
<td>6.62</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>.61</td>
</tr>
<tr>
<td>Total Phosphorus, %</td>
<td>.73</td>
</tr>
</tbody>
</table>

$^1$Supplied the following mg/kg of diet: 5200 Ca, 4000 P, 1020 Na, 3600 K, 500 Mg, 54 Mn, 35 Fe, 6 Cu, 30 Zn, .2 I, .1 Se, 2 Cr, 1560 Cl, and 1000 SO$_4$.

$^2$Supplied the following per kg of diet: 6 mg thiamin HCl, 6 mg riboflavin, 7 mg pyridoxine HCl, 30 mg nicotinic acid, 16 mg calcium pantothenate, 2 mg folic acid, .2 mg biotin, 10 µg cyanocobalamin, 4,000 IU vitamin A, 1,000 IU vitamin D, 50 IU vitamin E, and 50 µg vitamin K.

$^3$Determined
Seven rats, 4 normal and 3 diabetic, were chosen randomly, fasted 24 hours, sacrificed by anesthetization with CO₂, and the carcasses stored frozen until the end of the experiment. These rats formed the control group from which initial body composition was estimated.

The remaining normal and diabetic rats were each subdivided into 3 groups such that the total average body weights were the same. One normal and one diabetic group were fed ad libitum and the other four groups were fed at restricted levels calculated as a percentage of the ad libitum intake of normal or diabetic rats (Table 2). All rats were weighed every other day and feed intake was measured daily.

Water intake of all rats was measured for seven days during the second week of the experimental period (Table 3). Spilled food was collected, dried and weighed, and corrections made to feed intake daily for the duration of the experiment. Total fecal collection was done for 13 days, from the second through the fourth week of the experiment for all rats (Table 3).

At the end of 4 weeks, all rats were fasted for 24 hours and sacrificed by anesthetization with ether. All carcasses were frozen prior to analysis.

Carcasses were prepared for analysis by grinding, followed by lyophilization for 4 days and regrinding in a Wiley mill. Carcass moisture content was determined by net
Table 2. Experimental design and feeding schedule

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Feeding Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal</td>
<td>9</td>
<td>Ad libitum</td>
</tr>
<tr>
<td>2. Normal</td>
<td>9</td>
<td>60%</td>
</tr>
<tr>
<td>3. Normal</td>
<td>9</td>
<td>40%</td>
</tr>
<tr>
<td>4. Diabetic¹</td>
<td>5</td>
<td>Ad libitum</td>
</tr>
<tr>
<td>5. Diabetic¹</td>
<td>5</td>
<td>80%</td>
</tr>
<tr>
<td>6. Diabetic¹</td>
<td>5</td>
<td>60%</td>
</tr>
</tbody>
</table>

¹Alloxan treated
Table 3. Water intakes and total fecal weights of normal and diabetic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water Intake (ml/d)</th>
<th>Total Fecal Output (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad lib</td>
<td>33.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>60%</td>
<td>23.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>25.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>40%</td>
<td>19.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad lib</td>
<td>288.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>80%</td>
<td>209.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60%</td>
<td>154.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>42.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-e</sup>Means not having common letter superscripts are significantly different (P<.05)
weight difference before and after lyophilization. Fat was extracted from the carcasses with chloroform:methanol (9:1, v/v), filtered through Whatman #5 paper into round bottom flasks and dried by evaporation under vacuum with a Büchi Rotovapor-R apparatus. A Parr adiabatic bomb calorimeter was used to determine gross energy (GE) of feed, feces and carcasses. Some carcass samples were pelleted using a hand press for ease of determining GE. GE of extracted fat samples was determined by combining the fat (liquid) with reagent grade benzoic acid (Mallinckrodt) and pelleting.

Fecal samples were oven-dried for one week at 71°C, singed to remove hair and ground to a fine powder in a Wiley mill before GE determinations were carried out. The diet was also ground and analyzed for GE.

In this study, digestible energy (DE) was used in expressing dietary energy intakes. In equation form, DE is defined as DE = IE - FE, while metabolizable energy (ME) is ME = IE - (FE + UE + GE) where GE of feed, feces, urine and gaseous excretion are represented by IE, FE, UE and GE, respectively.

Data were analyzed by linear and multiple regression and by the simplex method of Koong (1977).
CHAPTER IV

RESULTS

Diabetic rats displayed typical symptoms of alloxan diabetes, hyperphagia, polydipsia, polyuria and glycosuria. All rats injected with alloxan, with the exception of one, tested positive for urine glucose with Tes-Tape (>2%). None of the diabetic rats seemed to show symptoms of ketosis or ketonuria, which was not tested.

Dry matter digestibility and digestible energy (DE) were not significantly different between the normal and diabetic groups and averaged 85.3% and 3.8 kcal/g, respectively (Table 4). Total feed intakes for the 4-week experimental period, were significantly (P<.05) different between ad libitum-fed normal and diabetic rats, with the diabetic animals eating nearly twice the amount of feed as normal rats (1043.8 vs. 632.0 g/rat). All diabetic rats lost weight after alloxan injection and before the experimental period began. All restricted-fed rats, both normal and diabetic, lost weight during the 4-week experimental period. Body weight changes were -11.59 and -30.87 g/rat for the two restricted normal groups and -.60 and -1.61 g/rat for the two restricted diabetic groups. Normal and diabetic rats fed ad libitum were the only
groups to gain in relation to their initial weights. The change in body weight was 88.0 g/rat for normal rats and 29.0 g/rat for diabetic rats (Table 4).

Carcass fat content decreased with increasing feed restriction among normal rats from 30.6% (dry-weight basis) for the ad libitum group to 21.4% with 40% of ad libitum intake (Table 4). Diabetic rats maintained approximately 12% body fat even when feed intake was restricted, however the highest degree of restriction still provided energy intakes which were comparable to those of the full-fed normal rats. Body fat contents of the diabetic rats were significantly (P<.05) lower than those of the normal rats. Linear regression of dry carcass fat (%) on carcass moisture (%) content gave a significant negative correlation, indicating that as fat content decreased water increased in the body. This was observed in both normal and diabetic rats. Slope values were -.964 and -.766 for the normal and diabetic rats, respectively (Table 5).

Restricted feeding caused a loss in lean body mass in all feed-restricted rats. Changes in lean body mass in normal rats were -8.2 and -16.6 g/rat for the two restricted groups, while the two restricted groups of diabetic rats lost -4.3 and -6.2 g/rat. Diabetic ad libitum-fed rats maintained lean weight at 35% less than ad libitum-fed normal rats. Lean body weight changes were 8.6 and 24.4 g/rat in diabetic and normal rats, respectively (Table 4).
Table 4. Effect of alloxan diabetes on growth, feed consumption, and carcass composition of rats

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad lib</td>
<td>60%</td>
</tr>
<tr>
<td>Number of rats</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Dry matter digestibility, %</td>
<td>85.9</td>
<td>86.3</td>
</tr>
<tr>
<td>DE consumption, kcal/d</td>
<td>95.8c</td>
<td>67.4d</td>
</tr>
<tr>
<td>Final body weight, g</td>
<td>203.1a</td>
<td>149.1b</td>
</tr>
<tr>
<td>Carcass moisture, %</td>
<td>58.9c</td>
<td>62.2b</td>
</tr>
<tr>
<td>Carcass fat(^1), %</td>
<td>30.6a</td>
<td>25.9b</td>
</tr>
<tr>
<td>Body weight change, g/rat</td>
<td>88.0a</td>
<td>-20.1c</td>
</tr>
<tr>
<td>Lean Change, g/rat</td>
<td>24.4a</td>
<td>-8.2c</td>
</tr>
<tr>
<td>Fat Change, g/rat</td>
<td>21.7a</td>
<td>-3.4bc</td>
</tr>
<tr>
<td>Carcass energy change, kcal/PBW(^2)/d</td>
<td>23.9a</td>
<td>-7.8c</td>
</tr>
<tr>
<td>Lean energy change, kcal/PBW/d</td>
<td>10.1a</td>
<td>-5.3c</td>
</tr>
<tr>
<td>Fat energy change, kcal/PBW/d</td>
<td>13.7a</td>
<td>-2.5c</td>
</tr>
</tbody>
</table>

\(^a\)-\(^e\)Means not having common letter superscripts are significantly different (P<.05)

\(^1\)Dry-weight basis

\(^2\)PBW = Physiological body weight = kg BW\(^{0.75}\)
Table 5. Linear regression equations developed to estimate energetic efficiencies

<table>
<thead>
<tr>
<th>x = DE Intake (kcal/PBW(^1)/d)</th>
<th>r</th>
<th>Slope</th>
<th>y-Intercept</th>
<th>x-Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y = Carcass Energy Change (kcal/PBW/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>.938</td>
<td>.637</td>
<td>88.84</td>
<td>139.1</td>
</tr>
<tr>
<td>Diabetic</td>
<td>.702</td>
<td>.075</td>
<td>23.18</td>
<td>314.9</td>
</tr>
<tr>
<td>y = Carcass Fat Change (g/PBW/d)</td>
<td></td>
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<td></td>
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<tr>
<td>Normal</td>
<td>.884</td>
<td>.040</td>
<td>-5.41</td>
<td>136.5</td>
</tr>
<tr>
<td>Diabetic</td>
<td>.619</td>
<td>.003</td>
<td>-.59</td>
<td>281.7</td>
</tr>
<tr>
<td>y = Carcass Lean Change (g/PBW/d)</td>
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<tr>
<td>Normal</td>
<td>.906</td>
<td>.112</td>
<td>-15.32</td>
<td>137.3</td>
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<tr>
<td>Diabetic</td>
<td>.778</td>
<td>.042</td>
<td>-15.54</td>
<td>347.9</td>
</tr>
<tr>
<td>y = Carcass Fat Energy Change (kcal/PBW/d)</td>
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<td>.358</td>
<td>-48.83</td>
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<td>.023</td>
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<td>y = Carcass Lean Energy Change (kcal/PBW/d)</td>
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<td></td>
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<tr>
<td>Normal</td>
<td>.906</td>
<td>.611</td>
<td>83.63</td>
<td>137.3</td>
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<tr>
<td>Diabetic</td>
<td>.778</td>
<td>.216</td>
<td>79.80</td>
<td>347.9</td>
</tr>
<tr>
<td>y = Carcass Fat (%) x = Carcass Water (%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
<td>Diabetic</td>
<td>.710</td>
<td>-.766</td>
<td>55.65</td>
<td>70.1</td>
</tr>
</tbody>
</table>

\(^1\)PBW = Physiological body weight (kg BW\(^{.75}\))
Linear regression analysis of daily DE intake (DEI)/kg physiological body weight (PBW, kg$^{.75}$) regressed on change in carcass energy (CEC)/PBW/d (Table 5) showed that maintenance DEI for normal rats was 139.1 kcal/PBW (x-intercept). The y-intercept (DEI = 0) estimated fasting heat production (FHP) as 88.8 kcal/PBW/d and energetic efficiency (slope of regression line) was 63.8% (Figure 2a). Diabetic rats had a maintenance DEI of 314.9 kcal/PBW/d, a FHP of 23.2 kcal/PBW/d and an energetic efficiency of only 7.5% (Figure 2b).

The amount of feed necessary for maintenance was calculated by regressing feed intake (g/d) on CEC (kcal/d), the x-intercept gives the amount of feed in g/d; this was 18.77 g feed/d for normal rats and 32.09 g feed/d for diabetic rats. The slope of this regression line predicted dietary NE as 1.97 and .25 kcal/g feed for normal and diabetic rats, respectively.

Linear regressions of carcass fat or carcass lean changes (g/PBW/d) on DEI/PBW/d were used to predict the amount of feed energy required to deposit 1 g of either fat or lean tissue and were calculated as the reciprocal of the slope. DEI requirements for carcass fat gain were 25.2 kcal DE/g fat deposited for normal rats and 370.4 kcal DE/g fat deposited by diabetic rats (Figures 3a and 3b). Carcass lean gain in normal rats required 8.97 kcal DE/g gain and
NORMAL RATS

Figure 2a. Plot of energy balance (kcal/PBW/d) vs. DE intake for normal rats. Energetic efficiency estimated as 63.8 (slope).

$Y = 0.638X - 88.85 \ (r = 0.938)$
Figure 2b. Plot of energy balance (kcal/PBW/d) vs. DE intake for diabetic rats. Energetic efficiency estimated as 7.5% (slope).
NORMAL RATS

\[ Y = 0.0397 \times - 5.41 \quad (r = 0.884) \]

DE INTAKE (kcal/PBW)

Figure 3a. Plot of fat change (g/PBW/d) vs. DE intake (kcal/PBW). Slope estimates 0.0397 g fat stored/kcal DE or 25.2 kcal DE needed/g fat fat stored in normal rats.
Figure 3b. Plot of fat change (g/PBW/d) vs. DE intake (kcal/PBW). Slope estimates .0027 g fat stored/kcal DE or 370.4 kcal DE needed/g fat stored in diabetic rats.
23.6 kcal DE/g lean gain was required by diabetic rats (Table 5 and Figures 4a and 4b).

Similar regressions of carcass fat energy or lean energy gains (kcal/PBW/d) on DEI/PBW/d estimated energetic efficiencies or energy deposition coefficients of fat energy gains in normal rats of 35.8% in comparison with 2.3% for diabetic rats. Energetic efficiencies were 61.1% for normal animals and 21.6% for diabetics for the conversion of DE to NE in lean body mass (Table 5).

The cost of deposition of fat or lean tissue was determined by multiple regression in the form:

\[ y = a x_1 (\text{Lean Change/PBW}) + b x_2 (\text{Fat Change/PBW}) + c \]

where \( y \) is the dependent variable and \( x_1 \) and \( x_2 \) are the independent variables (Table 6). The heat of combustion (kcal/g) of either body fat or lean can be determined in this way. Thus, regressing total carcass energy (kcal/g) as the dependent variable on final carcass lean (g) and final carcass fat (g) estimated heats of combustion of 5.46 and 5.14 kcal/g protein in normal and diabetic rats, respectively. Fat values were 10.10 kcal/g in normal and 8.76 kcal/g in diabetic rats. By bomb calorimetry, GE of carcass fat in normal and diabetic rats were 9.01 and 8.73 kcal/g, respectively.
NORMAL RATS

\[ Y = 0.112 \times - 15.32 \ (r = 0.906) \]

Figure 4a. Plot of lean change (g/PBW/d) vs. DE intake (kcal/PBW). Slope estimates .112 g lean gain/kcal DE or 8.92 kcal DE needed/g lean gain in normal rats.
Figure 4b. Plot of lean change (g/PBW/d) vs. DE intake (kcal/PBW). Slope estimates .042 g lean gain/kcal DE or 23.8 kcal DE needed/g lean gain in diabetic rats.
Table 6. Multiple regression equations developed to evaluate energy utilization

<table>
<thead>
<tr>
<th></th>
<th>( r )</th>
<th>( a )</th>
<th>( b )</th>
<th>( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DE(^1)/PBW(^2)/d = a LC(^3)/PBW/d + b FC(^4)/PBW/d + c</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>.96</td>
<td>12.77</td>
<td>14.35</td>
<td>-136.26</td>
</tr>
<tr>
<td>Diabetic</td>
<td>.82</td>
<td>84.56</td>
<td>-114.95</td>
<td>416.37</td>
</tr>
<tr>
<td><strong>DE/d = a LEC(^5)/d + b FEC(^6)/d + c</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>.94</td>
<td>2.55</td>
<td>1.14</td>
<td>72.04</td>
</tr>
<tr>
<td>Diabetic</td>
<td>.76</td>
<td>10.60</td>
<td>5.50</td>
<td>125.82</td>
</tr>
<tr>
<td><strong>CE(^7) = a L(^8) + b F(^9) + c</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>.99</td>
<td>5.46</td>
<td>10.10</td>
<td>-91.46</td>
</tr>
<tr>
<td>Diabetic</td>
<td>.99</td>
<td>5.14</td>
<td>8.76</td>
<td>-34.79</td>
</tr>
</tbody>
</table>

\(^1\)DE = Digestible energy consumed (kcal)
\(^2\)PBW = Physiological body weight (kg BW\(^{75}\))
\(^3\)LC = Carcass lean change (g)
\(^4\)FC = Carcass fat change (g)
\(^5\)LEC = Carcass lean energy change (kcal)
\(^6\)FEC = Carcass fat energy change (kcal)
\(^7\)CE = Total carcass energy (kcal)
\(^8\)L = Carcass lean (g)
\(^9\)F = Carcass fat (g)
DEI/d regressed on lean energy change/d and fat energy change/d resulted in the equations:

\[ y = 2.55 x_1 + 1.14 x_2 + 72.04 \text{ (normal)} \]
\[ y = 10.56 x_1 + 5.50 x_2 + 125.82 \text{ (diabetic)} \]

Reciprocals of \( x_1 \) and \( x_2 \) estimated lean energy deposition efficiencies of 39.2 and 9.4% for normal and diabetic rats, respectively. Efficiencies of fat energy deposition, calculated in the same way, were 87.8 and 18.2% in normal and diabetic rats, respectively. The results, for both fat and lean deposition, were much higher than those calculated by linear regression.

The cost of deposition of lean or fat mass also was determined by regressing DEI/d on lean energy change and fat energy change. Thus, 2.55 kcal DE were required to deposit 1 kcal protein in normal rats and 10.6 kcal DE were required/kcal protein deposited in diabetic rats. The predicted values for fat deposition were 1.14 and 5.5 kcal DE/kcal fat deposited in normal and diabetic rats, respectively.

An additional method of analysis, using a Michaelis-Menten type equation as discussed in Chapter 1, was used to analyze these data. \( K \) values of 44.47 and 37.66 kcal were obtained for normal and diabetic rats, \( E_f \) equaled 28.51 and 5.78% and \( E_l \) was 7.71 and 14.22%, respectively (Table 7). These results suggested that normal rats utilized energy more efficiently for fat storage, while
Table 7. Simplex analysis\(^1\) of DE utilization for fat and lean gains in normal and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K^2)</td>
<td>44.5</td>
<td>37.7</td>
</tr>
<tr>
<td>(E_f^3)</td>
<td>.285</td>
<td>.058</td>
</tr>
<tr>
<td>(E_l^4)</td>
<td>.077</td>
<td>.142</td>
</tr>
</tbody>
</table>

\(^1\) (Koong, 1977)

\(^2\) \(K =\) DE intakes at 50% energy deposition as fat

\(^3\) \(E_f =\) Efficiency of DE used for fat gain

\(^4\) \(E_l =\) Efficiency of DE used for lean gain
diabetics were more efficient with lean storage. The K values indicated that both groups of rats showed approximately the same point at which the DE available above maintenance was directed to equal amounts of fat and lean deposition.
CHAPTER V

DISCUSSION

The differences in body fat content between normal and diabetic rats were considerable, with the fat content of normal rats ranging from 21.4 - 30.6% and that of diabetic rats 12.1%. Diabetic rats lost considerable body fat within a few days after alloxan treatment as has been observed by Friedman (1978). Body fat content was negatively correlated with body water content in both normal and diabetic rats. This same relationship has been reported by other investigators (Reid, Maiorino and Parker, 1980; Pratt and Putney, 1959; Schoenborne and Canolty, 1980). Pratt and Putney (1959) observed a decrease in percent body water in fatter and also in older, heavier animals.

Some of the results, such as hyperphagia and polydipsia, in diabetic rats were expected since other workers had found increased feed intakes in diabetic as compared with normal rats. Duffy (1945), Kumaresan and Turner (1965), Thomas et al. (1976) and Friedman (1978) listed hyperphagia as a symptom of alloxan diabetes. Kumaresan and Turner (1965) found a 56% increase in feed intake compared to control animals two weeks after an injection of alloxan. In the present study, diabetic rats
ate approximately 61% more than normal rats. It was interesting to note that with increased feed intake in diabetic animals, fecal volume likewise increased. The fecal pellets of these rats were much larger and seemed to be less dense than normal (Table 3). The GE values of the feces were not different between the groups when expressed on a unit weight basis. Dry matter digestibilities for the rats averaged 85.3% and were not significantly different between normal and diabetic animals. Burlacu et al. (1973) observed similar digestibilities (76.7 - 81.5%) in normal weaned pigs, as did Chan, Koong and Stern in 1982 (83%) for both normal and diabetic rats.

Likewise, the apparent dietary DE values were similar for all groups and were not apparently affected by either feeding level or alloxan treatments. Deb et al. (1976) and McNiven et al. (1981) have reported no differences in dietary DE between ad libitum and restricted rats.

DE intakes of the diabetic rats were quite high in relation to the normal animals, but marked differences occurred in the utilization of that energy at the metabolic level. In view of the metabolic alterations associated with diabetes, it was not surprising to find a high maintenance requirement of 314.89 kcal DE/PBW/d in diabetic rats; more than two times that of the normal maintenance requirement determined as 139.05 kcal DE/PBW/d. Deb et al. (1976)
calculated a maintenance requirement of 130.5 kcal ME/PBW/d in lean rats, while Schoenborne and Canolty (1980) and McNiven et al. (1981) observed lower maintenance requirements of 99.7 kcal GE/PBW/d and 94.36 kcal ME/PBW/d, respectively, in rats. A maintenance requirement of 143.6 kcal ME/PBW/d was reported for weaned pigs by Burlacu et al. (1973). A higher maintenance requirement was suggested by Canolty and Koong (1976) at 176 kcal ME/PBW/d in both rapid growth line and control line mice. A direct comparison of the literature values is not possible due to the fact that most values have been calculated on the basis of ME while the results of the present experiment were calculated as DE.

Diabetic rats undoubtedly lost considerable amounts of energy (glucose) in urine because of the defect in glucose utilization. Lack of insulin also appeared to affect metabolic pathways involving fat and protein degradation and biosynthesis. Duffy (1945) and Friedman (1978) did not note ketosis or ketonuria in their studies with alloxan treated rats nor were these symptoms detected in the diabetic rats in this study. Energetic efficiencies of conversion of dietary DE to net energy (NE) of maintenance and gain in the diabetic rats were only 12% of those obtained with the normal animals. Energy intakes were more than adequate in the diabetic animals, but it was not effectively utilized in metabolism.
Reported energetic efficiencies, the conversion of DE or ME to NE, are quite variable and highly dependent on dietary composition and the energy expression values used, as well as the age and physiological state of the experimental animals employed. Reid et al. (1980) suggested a 45.8% efficiency of DE utilization for rabbits. Schoenborne and Canolty (1980) found an efficiency of 34% for the conversion of GE to NE in rats. Efficiencies of ME utilization in a rapid growth line and control mice were 50 and 23%, respectively, in the study by Canolty (1976). Deb et al. (1976) observed similar efficiencies of ME utilization above maintenance in obese and lean rats, 51.4 and 21.4%, respectively. In weaned pigs, Burlacu et al. (1973) calculated a 78% efficiency of converting ME to NE, which is higher than the 63.76% observed in normal rats in this experiment.

Fasting heat production (FHP) is the amount of heat produced by the body due to physical activity and ongoing body processes not directly related to feeding. The value of 88.8 kcal/PBW/d obtained with normal rats was similar to that observed by McCracken and McNiven (1983) in force-fed rats where their values ranged from 78.63 to 80.78 kcal/PBW/d. Forsum, Hillman and Nesheim (1981) calculated total heat production of 77.4 and 65.9 kcal/PBW/24 hours in ad libitum and restricted rats, respectively. Heat production, whether fasting or total, has been shown to
decrease during restricted feed intake, therefore, the low FHP in all diabetic rats was not surprising. Total heat production decreased to 78% of prefasting levels after a 2-day fast in a study conducted by Cumming and Morrison (1960).

FHP for diabetic rats in this study was only 26% of that determined for normal animals. This difference may be due to lower levels of nutrients moving into cells of diabetic animals for anabolic and catabolic processes, thus less heat is produced by metabolism.

The dietary NE predicted by regressing feed intake (g/d) on CEC (kcal/d) was found to be 1.97 kcal/g feed for the normal rats in this study. The diabetic rats had an NE of only .25 kcal/g feed, which was only 13% of the dietary NE predicted for the normal rats. These values also reflect the poor energetic efficiencies of the diabetic animals.

Energetic efficiencies or energy deposition coefficients, from linear regressions, for fat and lean tissue were lower in diabetic than in normal rats, 2.3 vs. 35.8% for DE conversion to fat tissue and 21.6 vs. 61.1% for lean tissue. This suggested that the diabetic rats were not as efficient as normal rats in utilizing dietary DE to deposit fat or lean tissue. This was corroborated by the efficiency of fat or lean deposition determined by multiple regression, 9.44 and 39.15% for lean and 18.2 and 87.8% for fat deposition in diabetic and normal rats, respectively.
Burlacu et al. (1973) calculated approximately the same efficiencies of protein and fat synthesis, 78.13 and 78.74%, respectively, in normal weaned pigs. Efficiency of fat deposition in normal rats in our study was lower than that observed by these researchers. Schoenborne and Canolt (1980) calculated 22% as the coefficient and Canolt and Koong (1976) observed 39.3 and 13.4% in rapid growth and control line mice, respectively. These values were similar to the results in the present study.

The lean energy deposition coefficients were lower than other reported values: 12% (Schoenborne and Canolt, 1980), 10% (Canolt and Koong, 1978) and 10.4% for rapid growth line and 9.4% for control mice, respectively, (Canolt and Koong, 1976). The resulting inefficiency was also observed when comparing the actual cost of fat or lean deposition. It cost the diabetic rat 10.60 kcal DE/kcal lean deposited, while the cost was only 2.55 kcal DE/kcal lean deposited for the normal rat. Fat deposition is a more efficient process than lean deposition, when the metabolic processes involved in protein degradation, transport and resynthesis for deposition are considered (Blaxter, 1971). Thus, normal rats required only 1.14 kcal DE/kcal fat deposited compared to 5.5 kcal DE/kcal fat deposited in diabetic rats. Diabetic rats utilized fat more efficiently (Friedman, 1978) when the amount of fat in the diet was high as compared to carbohydrate for daily energy needs and were
less likely to deposit fat in their tissues (Thomas et al., 1976). The results of our experiment seem to be consistent with previous research.
CHAPTER VI

CONCLUSIONS

Twenty-seven normal and 15 diabetic (by alloxan injection) Sprague-Dawley rats were fed the same experimental diet either ad libitum or at one of two restricted levels, ranging from 80 to 40% restriction based on a percentage of ad libitum intake, for 25 days. Feed and feces were collected and analyzed for gross energy (GE) and dietary digestible energy (DE) calculated. Rats were sacrificed and the carcasses were analyzed for GE, fat and moisture content and lean was calculated by difference. Raw data were analyzed by linear and multiple regression techniques in order to evaluate energy utilization and energetic efficiencies for both groups of rats.

The efficiency of converting DE to net energy (NE) in normal rats was 63.8%, while that of the diabetic animals was 7.49%. Fasting heat productions (FHP) were 88.8 and 23.18 kcal/physiological body weight (PBW; kg W\(^{-75}\)) per day in normal and diabetic rats, respectively. The predicted maintenance requirements in normal and diabetic rats were 139.05 and 314.89 kcal DE/PBW/d. Dietary NE values, determined by linear regression of feed intake/d on body energy change/d, were 1.97 kcal/g for normal rats and
only .25 kcal/g feed for diabetic rats fed the same diet. As expected, these data indicate very poor energy utilization of diabetic rats and reflect the loss of energy in urine.

Efficiencies of lean tissue deposition, determined from linear regressions, were 35.8% for the normal rats in comparison with 2.3% for the diabetic rats. Using multiple regression analyses, these values were 39.15% in normal and 9.44% in diabetic rats. The energy cost of depositing lean tissue in normal rats was 2.55 kcal DE/kcal lean deposited, while the value for diabetic rats was four times higher at 10.6 kcal DE/kcal lean gain.

Fat deposition efficiencies were 61.1 and 21.6%, respectively, when determined from linear regressions, and the values calculated using multiple regressions were 87.8 and 18.2% in normal and diabetic animals, respectively. The energy costs of fat deposition were less than lean deposition cost at 1.14 kcal DE/kcal fat deposited in normal rats and 5.5 kcal DE/kcal fat in diabetic rats (Table 8).

The above results suggested extreme energetic inefficiency in diabetic rats as compared to normal rats. This was not a surprising outcome when the overall metabolic defects of diabetes mellitus are considered. Lack of insulin causes primarily a decrease in glucose utilization and secondarily a reduction in both fat and protein metabolism.
Table 8. Energetic efficiencies calculated from linear and multiple regressions

<table>
<thead>
<tr>
<th>Regression Technique</th>
<th>Linear</th>
<th>Multiple</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance DE/PBW(^2) (kcal)</td>
<td>139.1</td>
<td>39.2</td>
</tr>
<tr>
<td>Efficiency of fat gain (%)</td>
<td>35.8</td>
<td>39.2</td>
</tr>
<tr>
<td>Efficiency of lean gain (%)</td>
<td>61.1</td>
<td>87.8</td>
</tr>
<tr>
<td>Gross energetic efficiency (%)</td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td><strong>Diabetic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance DE/PBW (kcal)</td>
<td>314.9</td>
<td></td>
</tr>
<tr>
<td>Efficiency of fat gain (%)</td>
<td>2.3</td>
<td>18.2</td>
</tr>
<tr>
<td>Efficiency of lean gain (%)</td>
<td>21.6</td>
<td>9.4</td>
</tr>
<tr>
<td>Gross energetic efficiency (%)</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)All efficiencies are based on DE

\(^2\)PBW = Physiological body weight (kg BW\(^{0.75}\))
This study would have been improved by determination of energy and nutrient loss not only in feces, but also in urine. The comparative slaughter technique employed in this experiment could be enhanced by respiration trials in which $O_2$ intake and $CO_2$ output are measured to provide direct estimates of FHP and diet induced thermogenesis.
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