BLOOD GLUCOSE AND PLASMA LIPIDS OF ZUCKER FATTY AND LEAN RATS FED DIETS CONTAINING CORNSTARCH AND SUCROSE

by

Patricia M. Sheehan

Dissertation submitted to the Graduate Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Human Nutrition and Foods

APPROVED:

S. J. Ritchey, Chairman

F. E. Bowen  
L. Ferreri

C. E. Polan  
F. W. Thye

May, 1981
Blacksburg, Virginia
ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to S. J. Ritchey, major professor, for his advice, encouragement and support throughout my graduate program, especially during the completion of this project. Appreciation is also extended to: Dr. F. W. Thye for assistance in planning of the project; Dr. C. E. Polan for his recommendations and advice in the planning and completion of this research; and Dr. P. E. Bowen for her helpful ideas concerning the interpretation of the results of this research. Special thanks and appreciation are extended to Dr. L. Ferreri for his invaluable assistance with the lipoprotein analysis and enthusiastic support.

I also thank for her laboratory assistance and expertise and for her valuable counsel throughout the completion of this research.

Finally, I especially thank my husband, for his encouragement and understanding throughout my entire graduate program.
## CONTENTS

### ACKNOWLEDGEMENTS

#### Chapter

<table>
<thead>
<tr>
<th>Chapter</th>
<th>page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>Human Obesity</td>
<td>3</td>
</tr>
<tr>
<td>The Zucker Fatty Rat</td>
<td>10</td>
</tr>
<tr>
<td>Metabolic Consequences of Dietary Carbohydrate Consumption in Humans</td>
<td>21</td>
</tr>
<tr>
<td>Metabolic Consequences of Dietary Carbohydrate Consumption in Rats</td>
<td>29</td>
</tr>
<tr>
<td>Metabolic Consequences of Dietary Carbohydrate Consumption in Obesity</td>
<td>36</td>
</tr>
<tr>
<td>III. MATERIALS AND METHODS</td>
<td>44</td>
</tr>
<tr>
<td>Animals and Diets</td>
<td>44</td>
</tr>
<tr>
<td>Experimental Design</td>
<td>47</td>
</tr>
<tr>
<td>Glucose Tolerance Tests</td>
<td>49</td>
</tr>
<tr>
<td>Sacrifice</td>
<td>50</td>
</tr>
<tr>
<td>Very Low Density Lipoprotein Isolation</td>
<td>51</td>
</tr>
<tr>
<td>Analysis of Individual VLDL and Plasma Samples</td>
<td>53</td>
</tr>
<tr>
<td>Gel Filtration Chromatography</td>
<td>54</td>
</tr>
<tr>
<td>Calculations and Statistical Analysis</td>
<td>58</td>
</tr>
<tr>
<td>IV. RESULTS AND DISCUSSION</td>
<td>59</td>
</tr>
<tr>
<td>Body Weights and Feed Intake</td>
<td>59</td>
</tr>
<tr>
<td>Glucose Tolerance</td>
<td>65</td>
</tr>
<tr>
<td>Plasma Lipids</td>
<td>77</td>
</tr>
<tr>
<td>VLDL Size Distribution</td>
<td>82</td>
</tr>
<tr>
<td>V. SUMMARY AND CONCLUSIONS</td>
<td>88</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>92</td>
</tr>
<tr>
<td>VITA</td>
<td>103</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Composition of Diets</td>
</tr>
<tr>
<td>2.</td>
<td>Body Weights of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets</td>
</tr>
<tr>
<td>3.</td>
<td>Daily Feed Intake of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets</td>
</tr>
<tr>
<td>4.</td>
<td>Glucose Tolerance Values of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets</td>
</tr>
<tr>
<td>5.</td>
<td>Areas Under Glucose Tolerance Curves of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets</td>
</tr>
<tr>
<td>6.</td>
<td>Plasma Lipid Values of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Experimental Design</td>
<td>48</td>
</tr>
<tr>
<td>2.</td>
<td>Glucose Tolerance Curves of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets</td>
<td>69</td>
</tr>
<tr>
<td>3.</td>
<td>VLDL Size Distribution of Zucker Fatty Rats Fed Sucrose or Cornstarch</td>
<td>83</td>
</tr>
<tr>
<td>4.</td>
<td>VLDL Size Distribution of Zucker Lean Rats Fed Sucrose or Cornstarch</td>
<td>84</td>
</tr>
<tr>
<td>5.</td>
<td>VLDL Size Distribution of Sprague-Dawley Rats fed Sucrose or Cornstarch</td>
<td>85</td>
</tr>
</tbody>
</table>
Chapter I
INTRODUCTION

Obesity is a major health problem in Western societies. Common characteristics of obesity are impaired glucose tolerance, hyperinsulinemia, and insulin insensitivity. Another abnormality often associated with obesity is hypertriglyceridemia (Gries et al., 1979). Consumption of high carbohydrate diets has been reported to cause further rises in serum insulin and triglyceride concentrations in obese subjects (Grey and Kipnis, 1971; Fiser and Bray, 1974). In contrast, Rodger et al. (1971) reported no change in fasting plasma insulin levels or plasma triglyceride concentration following consumption of diets containing primarily simple or complex carbohydrates by obese men.

The effects of various dietary carbohydrates have been extensively studied in normal weight humans and rats. Studies concerned with sucrose ingestion of normal weight humans have yielded conflicting results, but recent reports indicate that sucrose consumption results in increased fasting blood glucose levels (Reiser et al., 1979a), and increased plasma triglyceride levels (Reiser et al., 1979b). Glucose tolerance does not appear to be impaired after sucrose consumption, however (Dunnigan et al., 1970; Reiser et
al., 1979a). Similar results have been reported in rats, except that impaired glucose tolerance is also reported (Cohen and Teitelbaum, 1964; Blazquez and Quijada, 1969; Cohen et al., 1972; Bruckdorfer, 1974; Naismith and Rana, 1974; Vrana et al., 1974a; Laube et al., 1976; Hallfrisch et al., 1979).

The response of obese mammals to the consumption of various sources of dietary carbohydrate has not been elucidated. Research indicates that the response may not be the same as that of lean mammals. This problem was investigated using the Zucker fatty rat. This study was undertaken to test the hypothesis that the response of obese Zucker rats to diets containing sucrose or cornstarch is different from that of lean Zucker rats and normal Sprague-Dawley rats. To accomplish this, the following measures were made: fasting blood glucose concentration, glucose tolerance, fasting plasma triglyceride and cholesterol levels, fasting very low density lipoprotein triglyceride levels and very low density lipoprotein size distribution.
2.1 HUMAN OBESITY

Obesity is a disorder which is frequently characterized by hypertriglyceridemia and abnormal glucose tolerance (Gries et al., 1979). Berchtold et al. (1977) have reported that of 500 overweight male and female patients, 41% were glucose intolerant and 31% were hypertriglyceridemic. Only 12% of their subjects possessed no cardiovascular risk factors. In a study of 552 women, mild glucose intolerance was reported to be associated with the higher plasma triglyceride concentrations observed (Danowski et al., 1971).

Waxler and Craig (1964) studied 81 obese women and reported a tendency for high serum triglycerides in these women compared to normal weight controls. No statistical analyses were completed, however. Albrink and Meigs (1965) investigated serum triglycerides in relation to acquired and inherent obesity in men. Acquired obesity was generally obesity which occurred in adulthood, whereas innate obesity was present since childhood. Forearm skinfold fatness was used as an index of innate obesity and scapular skinfold thickness was used as a measure of acquired obesity. Hyper-
triglyceridemia was found to be associated with acquired obesity rather than inherent obesity. This difference may account for the observation that all obese persons are not hypertriglyceridemic.

A positive correlation between relative body weight (actual body weight/ideal body weight) and plasma triglyceride concentration has been reported (Sailer et al., 1966; Ford et al., 1968). Sailer et al. (1966) reported this relationship in normal subjects, as well as tolbutamide treated diabetics. In their normal subjects, 10 kg of excess weight resulted in 8 mg/100ml increase in triglycerides, whereas in tolbutamide treated patients, 10 kg of excess weight resulted in 28 mg/100 ml increase in triglycerides. This observation indicates a relationship between glucose intolerance and hypertriglyceridemia in obese humans.

The occurrence of hypertriglyceridemia in obesity may be the result of an increased rate of hepatic production of triglycerides or a decreased rate of removal of triglycerides from the plasma. Olesfsky and his coworkers (1974) have proposed an hypothesis to explain the origin of endogenous hypertriglyceridemia in humans. In this scheme, insulin resistance and hyperinsulinemia result in increased plasma triglyceride levels. It is considered that the basic underlying abnormality is cellular insulin resistance. With
this condition, blood glucose uptake by the peripheral tissues is decreased causing hyperglycemia, which results in increased secretion of insulin by the pancreas. The hyperinsulinemia that results stimulates hepatic triglyceride synthesis and secretion. This, according to the hypothesis, results in elevated plasma triglyceride levels. There is much experimental evidence in support of this hypothesis. It is possible that insulin resistance may not be the primary lesion, however (Nestel and Goldrick, 1976). The cause and effect relationship of hyperinsulinemia and insulin resistance has not been clearly established. Hyperinsulinism may be a compensatory result of tissue insulin resistance, as suggested by Olefsky et al. (1974) or hypersecretion of insulin by the pancreas may be the basic abnormality and overexposure to insulin may cause the peripheral tissues to have reduced response to insulin (Nestel and Goldrick, 1976). Regardless of whether hyperinsulinemia or cellular insulin resistance occurs first, these conditions do seem to be related to hypertriglyceridemia which is often observed in obesity.

The conditions of hyperinsulinemia and insulin resistance almost always occur together in obesity. Rabinowitz and Zierler (1962) were among the first investigators to report the presence of insulin resistance and endogenous hy-
perinsulinemia in obesity. Upon infusion of insulin into the cannulated forearm of obese subjects, glucose uptake was less than in control subjects. It was also reported that insulin infusion inhibited free fatty acid release from the adipose tissue of the forearm of both lean and obese subjects, but that this inhibition occurred to a lesser degree in the obese subjects. Arterial plasma insulin concentrations were reported to be higher for obese subjects compared to normal weight subjects. In addition, every obese subject had a plasma insulin concentration that exceeded those of the control subjects.

Other researchers have also reported hyperinsulinemia in obese subjects (Bagdade et al., 1967; Drenick et al., 1973; Pykalisto et al., 1975; Schade and Eaton, 1974; Taskinan and Nikkila, 1977; Misbin et al., 1979). Bagdade et al. (1967) studying obese and nonobese adult males, observed that fasting serum insulin levels were significantly higher in obese subjects compared to nonobese subjects. These investigators also studied the insulin response to an oral glucose tolerance test. Obese subjects had a greater insulin response to the 100 grams of glucose administered. This increased response of insulin to the glucose load could be indicative of insulin resistance in the obese subjects.
In a study of 80 obese patients, a positive correlation was reported between triglyceride production rate and plasma triglyceride concentration \((r = 0.78)\) (Reaven and Bernstein, 1978). Grundy et al. (1979) also observed this relationship in many of their obese subjects. In others however, transport rates of very low density lipoprotein (VLDL) triglyceride were increased without hypertriglyceridemia. This suggests that these subjects had an increased ability to clear triglycerides from the blood. Possibly then both overproduction of VLDL triglyceride and insufficient clearance of VLDL triglyceride contribute to the development of hypertriglyceridemia of obesity.

If insufficient clearance of triglycerides were a contributing factor in hypertriglyceridemia, a difference in activity of lipoprotein lipase (LPL) might be expected in obese and nonobese subjects, since to enter the peripheral tissues the VLDL triglycerides are hydrolyzed by LPL (Mayes, 1979). Insulin plays an important role in regulation of adipose tissue LPL activity in humans (Pykalisto et al., 1975). Hyperinsulinemia and insulin resistance would be expected to have an effect on LPL activity in obesity. It has been demonstrated repeatedly that in human obesity, adipose tissue LPL activity is increased when expressed per adipocyte. This increase is not evident when activity is ex-
pressed per gram of adipose tissue, however (Campbell et al., 1974; Taskinan and Nikkila, 1977; Lithell and Boberg, 1978). Therefore, on a weight basis, there is no difference in adipose tissue LPL activity between obese and nonobese subjects.

Pykalisto et al. (1975) reported that adipose tissue LPL activity per $10^6$ fat cells was positively correlated with obesity and with hyperinsulinemia which accompanies obesity. Obese subjects studied with basal hyperinsulinemia had significantly higher adipose tissue LPL activity. The authors suggest therefore that the large adipocytes of their obese subjects were not resistant to the action of insulin in relation to LPL activity. It would appear that obese hyperinsulinemic individuals may have an increased capacity for removal of plasma triglycerides since LPL activity is increased per adipocyte and adipose tissue mass is greater in obese subjects. Therefore, in most obese subjects hypertriglyceridemia is not caused by lack of LPL activity. Increased hepatic triglyceride production coupled with increased removal however, may provide an explanation for the mild elevation of triglyceride levels frequently observed in obesity (Pykalisto et al., 1975).

Obesity in adults is often linked with hyperglycemia and glucose intolerance (Berger et al., 1978). It is re-
ported that approximately 50% of obese adults exhibit glucose intolerance (Duncan et al., 1968; Berchtold et al., 1977). Hyperglycemia and mild glucose intolerance are most likely related to insulin resistance and hyperinsulinemia that are often present in obesity (Berger et al., 1978). The relationship of hyperinsulinemia, insulin resistance, and glucose tolerance in human obesity was investigated by Drenick et al. (1973). Obese subjects with normal basal insulin levels had a normal response to a glucose load, whereas in obese subjects with hyperinsulinemia and insulin resistance, impaired glucose tolerance was also observed. Therefore, a situation exists of impaired glucose tolerance in the presence of high serum insulin levels in many obese humans.

In summary, obesity in adults is often associated with hypertriglyceridemia, hyperglycemia, and abnormal glucose tolerance. Although the precise nature of the relationship is unclear, it is thought that insulin resistance, related to excess adiposity, and hyperinsulinemia may be at the root of the problem.
2.2 THE ZUCKER FATTY RAT

The Zucker fatty is a genetically obese strain of rat that has been suggested as an appropriate model for the study of human obesity (Malewiak et al., 1971; York, 1975). This strain, in which obesity is transmitted as a Mendelian recessive trait, was first reported by L. M. Zucker and T. F. Zucker in 1961 (Zucker and Zucker, 1961). The obese Zucker rat is generally characterized by hyperphagia, polydipsia, polyuria, adipose tissue hypertrophy and hyperplasia, smaller skeletal mass, increased efficiency of energy utilization, normal or slightly elevated blood glucose concentration, hypertriglyceridemia, hypercholesterolemia, and insulin resistance. Decreased reproductive function, hypothyroidism, hyperinsulinemia and hypoglucaagonemia, as well as other endocrine abnormalities, are also reported in the Zucker fatty rat (Bray, 1977).

It has been suggested that the primary defect of the Zucker fatty rat involves an abnormality of the hypothalamus. Several observations point to the involvement of the hypothalamus in the cause of obesity in the Zucker rat. Smaller pituitaries have been reported in fatties compared to lean controls (Bray et al, 1973). Female fatty rats have delayed vaginal opening, prolonged estrous cycles, decreased uterine and ovary weights, and are infertile (Saiduddin et
al., 1973; Bray et al., 1973). In addition, uptake and release of radiolabelled iodine was reported to be lower in obese compared to lean Zucker rats, indicating thyroid hypofunction (Bray and York, 1971).

Although these observations indicate hypothalamic involvement, several investigations have yielded evidence which indicates that the obesity of the Zucker fatty rat is fundamentally different from the rat with a ventromedial hypothalamic (VMH) lesion. Zucker fatty rats respond differently than VMH lesioned rats in several instances. In the VMH lesioned rat, supradiaphragmatic vagotomy results in normalization of body weight, whereas the obesity of fatty rats does not respond to vagal transection (Opsahl and Powley, 1974). In addition, obese Zucker rats develop obesity at an earlier age than do VMH lesioned rats (Powley and Morton, 1976).

It is possible that the primary lesion of the Zucker fatty rat is not located in the hypothalamus at all. Powley and Morton (1976) investigated the effects of hypophysectomy on the obesity of the Zucker fatty. Hypophysectomy caused no further development of obesity although previously acquired obesity was maintained. Atrophy of the adrenals and gonads also resulted from complete hypophysectomy. These researchers concluded that since obesity acquired prior to
hypophysectomy was maintained after surgery, the genetic lesion of the Zucker fatty rat was not focused in the hypothalamo-hypophyseal control mechanisms. For this reason also, it was concluded that the obesity is not due to hypersecretion of pituitary, adrenal, gonadal or thyroid hormones. Therefore, it was suggested that the obesity of the Zucker rat was not due to hypothalamo-hypophyseal mechanisms or to any of the major target endocrine organs controlled by the pituitary. One of the endocrine systems controlled by the hypothalamus or pituitary may have a permissive role in supporting the development of obesity in the Zucker rat, however (Powley and Morton, 1976). It can be concluded from the preceding discussion that the primary lesion of the Zucker fatty rat has not been elucidated. Although hypothalamic involvement has been suggested, the lesion may or may not be focused in this organ.

Obese Zucker rats utilize energy intake more efficiently than lean littermates (Bray et al., 1973; Pullar and Webster, 1974; Zucker, 1975; Deb et al., 1976; Jenkins and Hershberger, 1978; Young et al., 1980), but this efficient energy utilization is in favor of fat synthesis rather than protein synthesis. Bray et al. (1973) reported that with caloric intake equal, obese rats stored 50% more calories than lean rats. In addition, 90% of the ingested calories
of obese Zucker rats was stored as fat whereas, only 60% of the stored calories of lean Zucker rats was stored as fat. Several investigators have reported decreased efficiency of protein utilization of obese rats fed similar amounts of protein as lean rats (Bray et al., 1973; Pullar and Webster, 1974; Young et al., 1980; Dunn and Hartsook, 1980) or more protein than lean rats (Bray et al., 1973; Deb et al., 1976). Therefore, less lean body mass and smaller skeletons are observed in obese Zucker rats compared to lean controls (Zucker and Zucker, 1963; Dunn and Hartsook, 1980; Young et al., 1980). It has been suggested that the decreased protein synthesis observed in the Zucker fatty rat is due to a deficiency of growth hormone (Zucker, 1975).

Obese Zucker rats increase in percent body fat beginning at 2 weeks and continuing until 14 weeks of age. After this point, total body fat increases, but percent body fat plateaus due to continued body growth (Zucker and Antoniades, 1972). Gruen et al. (1978) reported that at as young as 5 weeks old, obese rats weighed more than lean rats. Increased fat pad weight was also noted at this age. This pattern continued at 10, 13, and 20 weeks of age. Fat cell number was not significantly greater in the obese rat until 20 weeks of age. In contrast, fat cell size was greater in obese rats compared to lean rats at all ages.
studied. Similar results were reported by York and Bray (1973). This pattern of hyperplasia and hypertrophy of the adipose tissue of Zucker fatty rats is in contrast to that of VMH lesioned rats which exhibit only hypertrophy (Johnson et al., 1971). Restriction of feed intake causes less increase in weight of Zucker fatty rats, but the animals are still fatter than lean controls (Zucker and Zucker, 1962; Bray et al., 1973).

The Zucker fatty rat has been studied extensively in an attempt to characterize the metabolic abnormalities present and to try to understand the causes of the various abnormalities. Hyperinsulinemia in the Zucker fatty rat has been reported by many investigators (Lemonnier, 1971; Stern et al., 1972; York et al., 1972; Schonfeld et al., 1974; Laburthe et al., 1975; Thenen and Mayer, 1975; Steele et al., 1979; Bach et al., 1980). Serum insulin levels of obese rats rise until about 15 weeks of age after which there is a decline. At all ages however, levels are elevated above control concentrations (Zucker and Antoniades, 1972).

Shino et al. (1973) have reported hypertrophy of the pancreatic islet cells up to 24 weeks of age. Hypertrophy was accompanied by degranulation of the B cells. At 52 weeks of age, when it was noted that plasma insulin and tri-
glyceride levels declined, proliferation of fibrous tissue and granulation of the islet cells were observed.

Decreased insulin sensitivity has also been reported in the Zucker fatty rat. York et al. (1972) reported a smaller hypoglycemic response in obese rats injected with exogenous insulin compared to controls. Insulin also had less effect on free fatty acid levels in obese rats compared to lean controls. Using in vitro techniques, Stern et al. (1972) reported less stimulation of glucose catabolism to CO$_2$ in adipose tissue of obese Zucker rats compared to lean Zucker rats. The same effect was noted in diaphragm tissue in which glucose conversion to glycogen was studied. Therefore the Zucker fatty rat appears to exhibit insulin resistance in muscle, as well as, adipose tissue.

Zucker fatty rats tend to have normal or slightly elevated fasting blood glucose concentrations (Zucker and Zucker, 1962; Eaton et al., 1976; Bach et al., 1980; Wade, 1980). Zucker and Antoniades (1972) reported no difference in blood glucose levels of fasting lean and obese rats at 6 or 50 weeks of age. At 15 weeks of age however, obese Zucker rats had blood glucose concentrations which exceeded those of lean Zucker rats. Although obese Zucker rats had increased blood glucose concentrations compared to lean rats, the values were still within normal limits. At 6 and
50 weeks of age, glucose tolerance curves for lean and obese Zucker rats were reported to be virtually identical. At 20 weeks however, obese Zucker rats exhibited a slower rate of removal of glucose from the blood (Zucker and Antoniades, 1972). This age of 20 weeks approximately corresponds to the age during which slightly elevated blood glucose levels were reported by these researchers. Slightly elevated blood glucose levels were also noted in obese Zucker rats at 2 to 3 months of age and 6 to 8 months of age by Triscari et al. (1979). Glucose disappearance rates for lean and obese Zucker rats at 2 and 8 months were similar, however. Expressed on a per organ basis, hepatic and kidney PEP carboxykinase activities were increased in obese Zucker rats compared to lean Zucker rats. Obese animals also had increased kidney glucose production rates and increased hepatic glycogen synthesis compared to lean rats. It was concluded therefore, that elevated blood glucose concentrations in obese Zucker rats were due to increased synthesis as opposed to impaired disappearance of glucose (Triscari et al., 1979).

Lactescent plasma, due to hyperlipemia, is a distinguishing characteristic of Zucker fatty rats (Zucker and Zucker, 1962). These rats have been reported to have 2 to 10 times the amount of lipid in their blood compared to lean
Zucker rats (Zucker and Zucker, 1962; Schonfeld and Pflegar, 1971). Plasma triglycerides have been reported to be as much as 377% higher in obese compared to lean rats (Bach et al., 1977). Plasma cholesterol concentrations in the same study were only 48% higher than lean controls. This pattern of extreme elevation of triglyceride concentration with moderate elevation in cholesterol concentration has been observed by other investigators as well (Zucker, 1972; Schonfeld et al., 1974; Bach et al., 1980). It was thought that the elevated blood lipids observed in obese Zucker rats might be due to the increased feed intake of these rats. Fatty rats pairfed to lean littermates retained increased plasma triglycerides and cholesterol concentrations, however (Zucker and Zucker, 1962; Schonfeld and Pflegar, 1971).

Plasma lipoproteins of Zucker rats have been studied by Schonfeld and his coworkers (Schonfeld et al., 1974; Witztum and Schonfeld, 1979). Plasma VLDL concentration was found to be increased 7 times and particles were enriched with triglyceride in obese Zucker rats compared to lean controls. In addition, low density lipoprotein (LDL) and high density lipoprotein (HDL) concentrations were doubled with lipid compositions similar to lean rats (Schonfeld et al., 1974).

Witztum and Schonfeld (1979) reported the apoprotein composition of VLDL from Zucker fatty and lean rats. The
major apoproteins present in VLDL are ApoB, ApoC, and ApoE. ApoB is the primary structural protein of the VLDL particles, constituting about 40% of the VLDL apoproteins. The C apoproteins consist of 3 main classes, ApoC-I, ApoC-II, and ApoC-III (Morisett et al., 1975). These apoproteins are reported to comprise 40% to 80% of VLDL apoproteins (Schaefer et al., 1978). ApoC-II is the major activator of adipose tissue LPL and it is thought that ApoC-III is an inhibitor of adipose tissue LPL (Kashyap et al., 1977). Witztum and Schonfeld (1979) reported that VLDL protein of fatty rats was 3.7 mg/dl higher compared to controls. Most of this increase was due to ApoC rather than ApoE or ApoB. The apoprotein content of VLDL particles varies with density, but for any given density fraction, VLDL of fatty rats had less ApoB and ApoE and more ApoC than did VLDL of lean rats. In addition, of the ApoC proteins, there was more ApoC-II and less ApoC-III present in VLDL of obese rats. These alterations in ApoC content may be important in clearance of VLDL triglyceride from the blood since ApoC-II is the major activator of LPL and ApoC-III is a possible inhibitor of LPL (Kashyap et al., 1977).

Several studies have indicated that there is increased hepatic fatty acid synthesis and triglyceride secretion in the Zucker fatty rat. Martin (1974) reported that in vivo hepatic lipogenesis was elevated in the Zucker fatty rat.
Hepatic enzymes related to lipogenesis that were found to be elevated include glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, ATP citrate lyase and malic enzyme. Taketomi et al. (1975) reported increased activities of glucose 6-phosphate dehydrogenase, acetyl CoA carboxylase, ATP citrate lyase and malic enzyme in livers of obese rats compared to lean controls. Monodesaturase activity was also reported to be increased in liver tissue of obese Zucker rats compared to lean controls (Wahle, 1974).

Using a liver perfusion technique, Schonfeld and Pflegar (1971) reported a 2.5 fold increase in hepatic triglyceride secretion rate in Zucker fatty rats compared to lean controls. An average of 94% of the triglyceride in the perfusate was identified as VLDL triglyceride upon ultracentrifugation. Using a similar perfusion technique, Witztum and Schonfeld (1979) reported that livers of obese Zucker rats secreted larger, triglyceride enriched, less dense VLDL compared to livers of lean Zucker rats.

Adipose tissue of adult obese Zucker rats has been reported to have an increased lipogenic capacity (Bray et al., 1970; York and Bray, 1973). This phenomenon was not observed by Martin (1974) however, who reported similar in vivo adipose tissue lipogenic rates in adult lean and obese rats. Activities of glucose 6-phosphate dehydrogenase,
6-phosphogluconate dehydrogenase, and glycerol phosphate dehydrogenase were found to be similar in lean and obese rats. These differences may partially be due to the age of animals used and differences in methodology.

Considering adipose tissue lipogenesis developmentally, Martin and Lamprey (1975) reported that only at 5 weeks of age was lipogenic capability greater in obese compared to lean rats. In fact at 5 weeks of age, lipogenesis was 4 times higher in adipose tissue of obese compared to lean rats. At 7, 10, and 14 weeks of age however, lean and obese rats had similar adipose tissue lipogenic capacities. Therefore, these researchers concluded that the increase in adipose tissue mass of the Zucker fatty rat is due to increased hepatic lipogenesis and VLDL secretion rather than increased adipose tissue lipogenesis.

Zucker fatty rats have increased adipose tissue LPL activity when expressed per fat cell (Stern et al., 1972; DeGasquet et al., 1973; Gruen et al., 1978). However, when expressed per gram of tissue, there is no difference in LPL activity in lean and obese rats. This is similar to the situation described for human obesity. In the obese Zucker rat, adipocyte size does not appear to be the cause of increased LPL activity, since it has been reported that when studying cells of similar size, cells of obese rats have
more LPL activity than cells of lean rats (Gruen et al., 1978).

The Zucker fatty rat has been presented as a useful model for the study of human obesity. Complications of obesity present in this genetically obese strain of rat include hyperinsulinemia, insulin resistance, and hypertriglyceridemia. Generally, blood glucose levels are normal or slightly elevated and glucose tolerance is not abnormal. This animal therefore may provide a useful model for the study of hypertriglyceridemia associated with obesity.

2.3 METABOLIC CONSEQUENCES OF DIETARY CARBOHYDRATE CONSUMPTION IN HUMANS

The effect of feeding various carbohydrates to normal weight animals and humans on glucose tolerance and plasma triglyceride concentration has been extensively investigated. Dietary carbohydrates studied include fructose, glucose, galactose, maltose, sucrose, lactose and starch from various sources. In addition, acute and chronic ingestion of carbohydrate have been investigated. This review will concentrate primarily on studies in which sucrose and starch were compared and in which dietary treatment can be considered longterm.
Ambiguous results have been reported by investigators studying the effects of sucrose consumption on fasting glucose concentrations and glucose tolerance in normal weight humans. Anderson et al. (1973) compared liquid diets of 40% or 80% of calories as sucrose with a control diet of 40% of calories as unspecified carbohydrate. Neither experimental diet resulted in fasting blood glucose levels that differed from control values. An improvement in glucose tolerance was noted for both diets compared to the control diet, however. The control and experimental diets differed in ways other than carbohydrate source, however. For instance, the experimental diets were liquid whereas the control diet consisted of solid foods. In addition, the source of carbohydrate in the control diet was not indicated.

Dunnigan et al. (1970) reported that sucrose consumption resulted in elevated fasting blood glucose concentrations in subjects fed a diet containing 70% of total dietary carbohydrate as sucrose compared to subjects fed 15% of dietary carbohydrate as sucrose. The total amount of carbohydrate in the test diets was 45%. Glucose tolerance tests conducted indicated insignificantly higher blood glucose concentrations in subjects consuming sucrose compared to those consuming starch.
Reiser et al. (1979a) also reported elevated fasting blood glucose levels in subjects fed 30% of calories as sucrose compared to those fed 30% of calories as wheat starch. In addition, these researchers observed normal glucose tolerance curves after an oral sucrose load in subjects consuming diets containing sucrose compared to those consuming starch. A unique feature of this study that may have influenced the results is that a gorging pattern of food intake was used. At breakfast, 10% of the diet was consumed and at dinner the remaining 90% was consumed. This regime was employed in order to accelerate any dietary effects that may occur with longer feeding periods. In both of these studies that indicated elevation of fasting blood glucose levels with sucrose feeding (Dunnigan et al., 1970; Reiser et al., 1979a), foods normally consumed rather than formula diets were used.

No alteration in glucose tolerance pattern was reported by MacDonald and Braithwaite (1964) who fed diets containing 500 grams of sucrose or raw maize starch. In this study, carbohydrate was fed in equal portions 6 times each day.

A comparison of the effects of consumption of sucrose or bread on fasting blood glucose levels and glucose tolerance curves indicated no significant effect of diet on fasting blood glucose concentration (Cohen et al., 1966). Glu-
cose tolerance curves of subjects consuming the sucrose diet were significantly higher than when the same subjects consumed the bread diet, however. The diets contained 65% and 69% of calories as bread and sucrose, respectively.

Thompson et al. (1978) studied glucose response to diets containing 45% or 65% of calories as either sucrose or corn syrup. The diets were fed in 3 meals and 1 snack daily for 10 days. No effect of diet on fasting plasma glucose concentration or response to an oral glucose load was reported. Using a portable constant withdrawal pump, plasma glucose concentrations were monitored for a 24 hour period in each subject. There was no change in 24 hour integrated glucose concentration due to diet. Fluctuations of glucose concentration were greater when subjects were consuming the corn syrup diet compared to the sucrose diet, however. These researchers question the validity of oral glucose tolerance tests. They suggest that glucose response to an oral glucose tolerance test may not accurately reflect glucose homeostasis during the ingestion of an experimental diet.

Fasting plasma insulin concentrations and insulin response to a glucose tolerance test have been investigated after consumption of diets containing sucrose or starch in an effort to ascertain the presence of an effect of the source of dietary carbohydrate on hyperinsulinemia and insu-
lin sensitivity. Reports in this area are inconclusive, however. Decreased (Anderson et al., 1973) and increased (Reiser et al., 1979a) fasting serum insulin concentrations, as well as, no change in fasting serum insulin concentrations (Dunnigan et al., 1970; Thompson et al., 1978) have been reported following consumption of diets containing sucrose compared to those containing starch. Generally, in studies in which fasting blood glucose levels were not altered by dietary treatment, fasting insulin levels were also not altered (Dunnigan et al., 1970; Thompson et al., 1978). Most often no change in insulin response to a glucose load has been reported (Dunnigan et al., 1970; Anderson et al., 1973; Thompson et al., 1978). Reiser et al. (1979a) observed increased insulin response to a sucrose load, however and concluded that consumption of a diet containing sucrose as the source of carbohydrate resulted in decreased insulin sensitivity in their subjects. In contrast, Anderson et al. (1973), who noted improvement in glucose tolerance with sucrose consumption and no change in insulin response to a glucose load, concluded that insulin sensitivity was improved with sucrose consumption. Although there were differences in experimental design in these studies, as noted previously, variability of individual response to the dietary treatments also probably plays an important role in the
differences in insulin response observed. Therefore, no conclusion as to the effect of source of dietary carbohydrate on insulin sensitivity and hyperinsulinemia in normal weight humans can presently be reached.

Consumption of diets containing sucrose as the source of carbohydrate is most frequently associated with increased fasting plasma triglyceride levels (MacDonald and Braithwaite, 1964; Nestel et al., 1970; Naismith et al., 1974; Reiser et al., 1979b). Feeding a diet of 30% carbohydrate as sucrose resulted in an increase of fasting plasma triglycerides in normal weight healthy subjects compared to the same subjects consuming a diet of 30% wheat starch (Reiser et al., 1979a). The same pattern was observed in normal subjects consuming a diet which contained 49% total carbohydrate, 300 grams of which was sucrose (Naismith et al., 1974). In this study, fasting plasma triglycerides were increased from 129 mg/dl on a self-selected diet, to 147 mg/dl on the diet containing sucrose.

Hayford et al. (1979) reported no difference in fasting plasma triglyceride concentration in subjects fed diets of 45% of calories as sucrose or 45% of calories as corn syrup. There was also no difference when subjects were fed diets of 65% of calories as sucrose or corn syrup. In this study, plasma triglyceride concentrations throughout a 24 hour per-
iod were also measured using a portable constant withdrawal pump. Triglyceride integrated concentrations were significantly higher with sucrose consumption at the 45% or 65% levels compared to corn syrup consumption. These researchers therefore, questioned the usefulness of fasting plasma triglyceride measurements for indication of the status of overall plasma triglyceride values.

Other researchers have also reported no effect of sucrose feeding on fasting plasma triglyceride concentrations (Dunnigan et al., 1970; Mann and Truswell, 1972). In both of these studies sucrose provided only part of the dietary carbohydrate fed. This may have obscured an effect of sucrose on plasma triglyceride values, but this type of feeding regime has been suggested to more closely estimate actual consumption patterns (Mann and Truswell, 1972). In addition, as was evident in the discussion of the effect of sucrose consumption on plasma glucose concentrations and glucose tolerance, there is considerable individual variation in response of plasma triglyceride concentration to dietary carbohydrate treatments.

Most reports indicate no effect of sucrose consumption on plasma cholesterol concentration (MacDonald and Braithwaite, 1964; Dunnigan et al., 1970; Mann and Truswell, 1972; Hayford et al., 1979). Some investigators however,
have reported increases in blood cholesterol levels due to sucrose consumption (Naismith et al., 1974; Reiser et al., 1979b). The increase observed by Reiser et al. (1979b) may have been due to the gorging pattern of feeding employed.

Nestel et al. (1979) investigated VLDL changes due to feeding a sucrose enriched diet to normal weight men. Diets fed contained 45% of calories as unspecified carbohydrate or 70% of calories as carbohydrate (55% of calories from sucrose). After 10 days of feeding, an increase in VLDL triglyceride concentration was observed. A trend for increased VLDL-B apoprotein concentration was also reported. However, since VLDL triglyceride to ApoB ratios were increased with sucrose feeding, VLDL particles became enriched with triglyceride. Similar findings were reported by Schonfeld et al. (1976) feeding an 80% carbohydrate diet to men and women in good health for 4 to 5 days. In addition, the amount of ApoC-II increased relative to the amount of ApoC-III in VLDL after consumption of the high carbohydrate diet.
2.4 METABOLIC CONSEQUENCES OF DIETARY CARBOHYDRATE CONSUMPTION IN RATS

Numerous studies have been conducted to investigate the metabolic consequences of feeding various types of dietary carbohydrate to rats. Fasting blood glucose concentrations, response to a glucose load, fasting blood lipid concentrations, and hepatic and adipose tissue lipogenic enzyme activities have been extensively studied.

Fasting blood glucose levels have been reported to be increased in rats with longterm feeding of diets containing a high percentage of sucrose (Blazquec and Quijada, 1969; Hallfrisch et al., 1979). Hallfrisch et al. (1979) fed diets of 54% sucrose or cooked cornstarch to weanling rats for 11 to 13 weeks and observed significantly increased serum glucose concentrations in sucrose fed rats compared to starch fed rats. In addition, at 1/2 hour and 2 hours after an intraperitoneal glucose injection, rats fed sucrose had significantly higher serum glucose levels than rats fed cornstarch. Other researchers, also feeding high levels of carbohydrate, have reported no effect of sucrose intake on fasting plasma glucose levels (Cohen and Teitelbaum, 1964; Bruckdorfer et al., 1974; Waterman et al., 1975). Feeding diets of 67% sucrose or starch for 40 days, Cohen and Teitelbaum (1964) noted no differences in fasting blood glu-
cose in their rats due to diet. These authors did observe impaired glucose tolerance in rats fed sucrose compared to starch, however. Altered glucose tolerance was also noted in rats fed diets of 40% starch and 27% sucrose or 34% starch and 33% sucrose. Abnormal response to an oral or intraperitoneal glucose load has also been reported in rats fed a 70% sucrose diet for 6 to 10 weeks compared to rats fed a 70% wheat starch diet (Vrana et al., 1974a). In sucrose fed rats, blood glucose levels rose higher and remained elevated for a longer period of time compared to starch fed rats. A similar response of blood glucose concentrations during a glucose tolerance test has been observed in rats fed a 70% fructose diet for 4 weeks compared to those fed a 70% glucose diet for 4 weeks (Vrana et al., 1978). At 30, 60, and 120 minutes after an oral glucose load, fructose fed rats had higher blood glucose levels than glucose fed rats. This could lend support for implication of the fructose moiety of sucrose as causing the metabolic abnormalities that result after sucrose feeding. Although no conclusion can be reached concerning the effect of sucrose feeding on fasting blood glucose levels in rats, it does appear that prolonged sucrose feeding leads to altered glucose tolerance in rats.
Basal serum insulin concentrations and insulin response to a glucose load are not affected in a consistent manner by sucrose feeding. Hyperinsulinemia has been reported in sucrose fed rats when compared to starch fed controls (Gardner et al., 1977; Hallfrisch et al., 1979). Others have reported no effect of longterm ingestion of sucrose on fasting serum insulin concentrations (Cohen and Teitelbaum, 1964; Blazquez and Quijada, 1969; Vrana et al., 1974a).

During glucose tolerance tests of sucrose fed rats, insulin levels are not consistently different from insulin levels of starch fed rats. Hallfrisch et al. (1979) reported no significant difference in insulin response to a glucose load between sucrose and starch fed rats. This response coupled with the abnormal glucose tolerance curve of sucrose fed rats of this study may be indicative of insulin resistance due to sucrose feeding. Another pattern which could also be indicative of insulin resistance was observed by Vrana et al. (1974a). In these sucrose fed rats, insulin levels increased to a greater extent during a glucose tolerance test compared to starch fed rats. Since blood glucose levels also increased to a greater extent compared to starch fed rats in this study, insulin resistance of the peripheral tissues may be indicated.
Insulin sensitivity of adipose tissue of rats fed sucrose is decreased compared to rats fed starch (Blazquec and Quijada, 1969; Vrana and Kazdova, 1970; Vrana et al., 1971; Vrana et al., 1974a; Bruckdorfer et al., 1974). In these studies, in vitro techniques were used in which the incorporation of radioactivity of labelled glucose into lipid of adipocytes incubated in the presence or absence of insulin was determined. In contrast, Vrana and Kazdova (1970) reported no effect of diet on insulin sensitivity of rat diaphragm tissue, as measured by the incorporation of labelled glucose into glycogen. Using similar methodology, Blazquec and Quijada (1969) reported decreased sensitivity of diaphragm tissue to insulin in sucrose fed rats compared to starch fed rats. In this study however, the starch containing diet was 49.5% carbohydrate and the sucrose diet contained 68% sucrose. This discrepancy may in part be due to the differences in response observed in sucrose fed rats in these two studies.

In vivo insulin insensitivity of sucrose fed rats has been observed by Vrana et al. (1974a) who studied disposition of a glucose load with simultaneously injected insulin. At 30 minutes after the injection, sucrose fed rats had 72.9% of the initial glucose load remaining in the blood and starch fed rats had 56.2% of the initial load remaining.
Similar results were reported by Hallfrisch et al. (1979). These researchers found that when insulin was injected with glucose in an intraperitoneal glucose tolerance test, serum glucose levels remained higher in sucrose fed rats compared to starch fed rats.

Sucrose feeding has been repeatedly reported to cause elevated plasma triglyceride levels in rats compared to starch feeding (Hallfrisch et al., 1979; Bruckdorfer, 1974; Laube et al., 1976; Cohen et al., 1972; Naismith and Rana, 1974). It has been suggested that the hyperlipidemic effect of sucrose is due to the more rapid digestion and absorption of disaccharides compared to polysaccharides (Naismith, 1971; Naismith and Rana, 1974). Naismith and Rana (1974) have indicated that monosaccharides and disaccharides are digested and absorbed more rapidly than starch. Therefore, sucrose ingestion would result in greater concentrations of monosaccharides reaching the liver and this, in turn, would result in increased hepatic lipogenesis. Since glucose and maltose ingestion can also result in hyperlipidemia, Naismith and Rana (1974) concluded that the rate of sucrose absorption is more important in the production of hypertriglyceridemia than is the presence of the fructose moiety in the sucrose molecule. Cohen et al. (1972) have implicated the fructose moiety of the sucrose molecule, however. Fruc-
Tose is thought to be a more efficient precursor for hepatic lipid synthesis since it enters the lipogenic pathway beyond two of the control points, glucokinase and phosphofructokinase. In addition, fructose is metabolized primarily by the liver rather than by the peripheral tissues. This would also lead to an increase in hepatic lipogenesis since the liver would be subjected to a larger hexose load with fructose compared to glucose (Cohen et al., 1972).

Witztum and Schonfeld (1978) studied changes in VLDL composition and structure in Sprague-Dawley rats fed lab chow plus fructose (60:40) compared to those fed only lab chow. VLDL were enriched with triglyceride after carbohydrate feeding. The proportion of VLDL protein composed of ApoB and ApoE fell, whereas the proportion of ApoC rose in carbohydrate fed rats. In addition, these researchers reported that perfused livers of carbohydrate fed rats secreted larger, triglyceride enriched VLDL. This indicates that hepatic VLDL synthesis may contribute to hypertriglyceridemia observed with sucrose feeding.

Since endogenous triglycerides are synthesized primarily by the liver, the effect of dietary carbohydrate on the activities of several lipogenic enzymes has been investigated. Naismith and Rana (1974) have reported increased activities of hepatic pyruvate kinase and glucose 6-phosphate
dehydrogenase in rats fed a 68.2% sucrose diet compared to rats fed a 68.2% starch diet. Cohen et al. (1972) also reported increased activities of hepatic pyruvate kinase and glucose 6-phosphate dehydrogenase with feeding of a 72% sucrose diet compared to feeding a 72% starch diet. In addition acetyl CoA carboxylase was studied and a five fold increase in the activity of this enzyme was seen in sucrose fed rats. These changes in enzyme activity with sucrose feeding suggest an increase in hepatic triglyceride secretion would therefore lead to the hypertriglyceridemia observed.

LPL activity of adipose tissue, heart and diaphragm has been studied in relation to dietary carbohydrate (Vrana et al., 1974b). Rats fed a 70% sucrose diet for 2 months had significantly higher LPL activity in heart and diaphragm tissue compared to rats fed a 70% starch diet. There was no significant difference in LPL activity of adipose tissue of rats fed sucrose or starch, however. A mechanism for these differences was not suggested. These data do however support the hypothesis that hypertriglyceridemia observed in sucrose feeding is caused by increased hepatic triglyceride synthesis rather than by decreased triglyceride removal.

It appears that feeding diets which contain sucrose as the source of carbohydrate to rats may result in elevated
blood glucose concentrations and impaired glucose tolerance. Sucrose feeding also results in peripheral insulin resistance in rats although fasting insulin levels are probably not affected. Elevated plasma triglyceride levels occur in rats fed sucrose containing diets and this increase has been attributed to increased hepatic de novo triglyceride synthesis as opposed to decreased removal of circulating triglycerides.

2.5 METABOLIC CONSEQUENCES OF DIETARY CARBOHYDRATE CONSUMPTION IN OBESITY

Most studies dealing with dietary carbohydrate in obesity in humans are actually concerned with hyperlipidemic subjects, some of whom are also obese. In addition, emphasis has been placed on quantity of carbohydrate rather than quality of carbohydrate.

The effect of feeding high and low carbohydrate diets to obese humans has been studied by Grey and Kipnis (1971). Obese female subjects consumed diets containing either 25% of calories as carbohydrate or 62% of calories as carbohydrate for 3 weeks. The type of carbohydrate consumed was not indicated. These researchers reported that blood glucose levels were not altered due to dietary treatment. Prior to treatment, the obese subjects were hyperinsulinemic.
The low carbohydrate diet caused a decrease in plasma insulin concentrations, whereas the high carbohydrate diet resulted in increased plasma insulin levels. Similar results were reported by Fiser and Bray (1974) who studied 4 obese females. In addition, it was found that feeding of a high carbohydrate diet caused elevated plasma triglyceride levels.

Feeding a high sucrose or glucose diet (70 to 80% of calories) to hyperlipemic patients has been found to result in elevated plasma triglyceride levels compared to feeding a high starch diet (Kaufman et al., 1966). Kuo et al. (1967) reported increased hepatic and adipose tissue lipogenic activities in hypertriglyceridemic patients fed diets containing large amounts of sucrose. The body weights of the subjects were not given in either of these studies.

Rodger et al. (1971) compared the effects of a diet based primarily on simple carbohydrate to that of a diet based primarily on complex carbohydrate in 5 overweight male patients. The patients had normal fasting blood glucose concentrations and fasting hypertriglyceridemia. The diets fed contained 40% to 50% of calories as carbohydrate. In one diet 75% of the carbohydrate was derived from simple carbohydrates and in the other diet 75% of the carbohydrate was derived from complex carbohydrates (starch and pectins).
Oral glucose tolerance tests were improved after both experimental diets and glucose tolerance tests were similar following both diets. Mean fasting insulin levels were not affected by either dietary treatment. Insulin response to an oral glucose load was greater after the complex carbohydrate diet compared to either the pretreatment period or the simple carbohydrate diet treatment. Plasma triglyceride and cholesterol concentrations were not affected by the dietary manipulations of this study. The influence of individual carbohydrates cannot be assessed in this study since diets did not contain a single source of dietary carbohydrate. In addition, each diet contained both simple and complex carbohydrates. This study does provide some indication of the response of the obese individual to alterations in dietary carbohydrate, however.

There have been several studies conducted dealing with the effects of feeding high and low carbohydrate diets to Zucker obese rats. Bryce et al. (1977) reported no difference in plasma glucose concentrations in obese Zucker rats fed diets with 62% of calories as cornstarch or 11% of calories as cornstarch. Using the same diet, Stern et al. (1975) reported a decrease in plasma glucose levels in obese rats fed the high carbohydrate diet. Diet had no effect on lean Zucker rats in either study. Using diets containing
69% of calories as carbohydrate or 16% of calories as carbohydrate, Lemonnier et al. (1974) reported no effect of diet on serum glucose levels in lean or obese Zucker rats. Plasma insulin concentrations have been reported to be unaffected by feeding diets high or low in carbohydrate to lean or obese Zucker rats (Malewiak et al., 1977; Bryce et al., 1977; Stern et al., 1975; Lemonnier et al., 1974). Adipocytes of obese rats have been found to be less sensitive to insulin compared to those of lean rats, regardless of dietary treatment (Stern et al., 1975). Adipocytes of lean Zucker rats were found to be insensitive to insulin when rats were fed the low carbohydrate diet compared to the high carbohydrate diet. These researchers (Stern et al., 1975) suggest the possibility of a different response to the level of dietary carbohydrate in lean and obese Zucker rats. Malewiak et al. (1977) observed no change in serum VLDL triglyceride levels in lean or obese Zucker rats fed diets containing 52% or 7% of carbohydrate as wheat flour.

Thenen and Mayer (1976) studied the effects of diets containing 64.3% carbohydrate as fructose, glucose, sucrose, or cornstarch on lean and obese (ob/ob) mice. No significant differences were observed in plasma insulin or cholesterol levels in lean or obese mice due to diet. The only differences seen in plasma triglyceride levels in any group
were in lean mice fed fructose and cornstarch. Mice fed fructose had significantly lower plasma triglyceride concentrations than lean mice fed cornstarch. Although some differences were observed in plasma glucose concentrations of mice fed the various carbohydrates, levels were not different in lean or obese mice fed sucrose or cornstarch. Therefore, feeding sucrose compared to cornstarch in this genetically obese strain of mouse had no effect on plasma glucose, insulin, triglyceride or cholesterol levels. The response of plasma triglycerides to dietary carbohydrate in lean and obese mice does not correspond to that observed in normal weight humans or rats. The authors suggest that this difference may be due to the use of mice as the experimental animal. Another reason why this animal may not be a good model to study the effects of dietary carbohydrate in human obesity and hypertriglyceridemia is that the hyperlipidemia observed in these mice is due to elevated plasma cholesterol levels rather than elevated triglyceride levels.

Two recent studies have indicated that genetically obese rats respond to dietary carbohydrate in a similar manner to lean rats. Michaelis et al. (1980) fed lean and obese Zucker rats diets containing 54% sucrose, invert sugar, or cooked cornstarch for 4 weeks. Sucrose feeding caused increased activities of hepatic lipogenic enzymes.
In both lean and obese rats, activities of hepatic glucose 6-phosphate dehydrogenase, malic enzyme and fatty acid synthetase were significantly increased with sucrose compared to starch diets. Since both lean and obese Zucker rats responded to sucrose feeding in a similar manner, it was concluded that Zucker fatty and lean rats are a useful model for the study of the metabolic effects of dietary carbohydrate.

The effects of feeding various dietary carbohydrates has also been studied in a new strain of genetically obese rat, LA-cp (Michaelis et al., 1981). These obese rats exhibit hypertriglyceridemia, hyperinsulinemia, hypercholesterolemia, and increased activities of hepatic glucose 6-phosphate dehydrogenase and malic enzyme, as do Zucker fatty rats. Lean and obese rats were fed diets containing 54% carbohydrate as sucrose or cooked starch. Obese and lean rats responded in a similar manner to the dietary treatment. Liver glucose 6-phosphate dehydrogenase and malic enzyme activities were increased and fasting plasma triglycerides and insulin levels were increased in both lean and obese rats fed sucrose diets compared to those fed starch diets.

The effects of consumption of various carbohydrates by obese humans or animals has not been adequately determined.
The human obese population is a heterogeneous group which makes investigation difficult. Studies with obese mice have not yielded much information applicable to human obesity, however a recent study of Zucker fatty rats indicates that this animal responds in a similar manner as lean Zucker rats to dietary carbohydrate. The primary objective of this study is to determine the effects of feeding diets containing sucrose and cornstarch as the source of carbohydrate on several parameters of carbohydrate and lipid metabolism in lean and obese Zucker rats. This strain of rat has been chosen as a model of human obesity accompanied by hypertriglyceridemia, hyperinsulinemia, and insulin resistance. Since the Zucker fatty rat innately exhibits many of the characteristics induced by sucrose feeding in lean rats, it was thought that obese Zucker rats may respond to a lesser extent to the dietary treatment compared to lean Zucker rats. The presence or absence of a differential effect of the type of dietary carbohydrate on glucose and lipid metabolism of lean and obese rats was determined. An intraperitoneal glucose tolerance test was administered and fasting blood glucose levels were determined to assess the effect of dietary carbohydrate on glucose metabolism of the Zucker fatty and lean rats. In addition, fasting plasma triglyceride and cholesterol levels and VLDL size distribution and
triglyceride concentrations were measured as an indication of the effect of the dietary treatment on lipid metabolism in the lean and obese rats.
Chapter III
MATERIALS AND METHODS

3.1 ANIMALS AND DIETS

Lean and obese Zucker rats (Fab-Labs, New City, NY) and Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were used for this experiment. All rats were females, 8 to 10 months of age. Rats were allowed at least 4 days for acclimatization to the laboratory and were fed lab chow (Rodent Chow 5001, Ralston Purina Co., St. Louis, MO) during this period.

During the experiment, rats were housed in suspended wire mesh cages. Animals were housed in a room with a 12 hour light-dark cycle and controlled temperature. A small humidifier was used intermittently in an effort to maintain the humidity of the room at at least 45%, since the Zucker rats had dry skin. This was aggravated by low humidity causing scratching and skin sores. Rats were weighed each Tuesday and Friday and were fed on alternating days. Tap water and food were allowed ad libitum throughout the experiment.

Two diets were fed during the experiment. The composition of each diet is presented in table 1. The diets were
similar except that by weight one contained 60% sucrose and the other contained 60% cornstarch. Based on values in Agriculture Handbook No. 456 (1975), sucrose and cornstarch provided 57% and 56% of dietary calories, respectively. Furthermore, the diets supplied 4.0 and 3.9 calories/gram, respectively.
## TABLE 1
Composition of Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Cornstarch</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient</td>
<td>g/100 g diet</td>
<td></td>
</tr>
<tr>
<td>Cornstarch(^1)</td>
<td>60.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Sucrose(^2)</td>
<td>0.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Casein, vitamin-free(^2)</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Corn Oil(^3)</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Alphacel(^2)</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>AIN Mineral Mixture(^2)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>AIN Vitamin Mixture(^2)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>L-methionine(^2)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Choline(^2)</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^1\) CPC International Inc., Englewood Cliffs, NJ.

\(^2\) ICN Pharmaceuticals, Cleveland, OH.

\(^3\) Mazola Corn Oil, Best Foods, Englewood, NJ.
3.2 EXPERIMENTAL DESIGN

Rats were divided into 6 groups in a 3 x 2 factorial design with strain or genotype and type of dietary carbohydrate as the 2 main factors (figure 1). Rats of each strain or genotype were assigned to a dietary treatment by controlled randomization to ensure similar distribution of body weights for each dietary treatment within strain or genotype. Each experimental group consisted of 13 rats. Since a limited number of glucose tolerance tests could be completed in one day, 3 to 4 rats per group were begun on the dietary treatment on each of 4 days. Diets were fed for 4 weeks, at which point a glucose tolerance test was administered. Feeding was then resumed for 2 more weeks before sacrifice to allow for recovery from any stress imposed by the glucose tolerance test.
Figure 1: Experimental Design
3.3 GLUCOSE TOLERANCE TESTS

After 27, 28 or 29 days of dietary treatment, an intra-peritoneal glucose tolerance test was administered to each rat. Each day, 1 rat from each of the 6 experimental groups was given the glucose tolerance test. Rats were fasted 12 hours prior to the test. Order of glucose injection was determined in a random manner.

Blood samples were obtained from the tail of each rat. During blood collection, the rat was held with her head and most of her body in a sleeve to help calm her. The tail was warmed in approximately 50°C water and blotted dry. A clean horizontal cut was made close to the tip of the tail with a razor blade and the tail was stroked to stimulate bleeding. The first drop of blood was removed from the tail and discarded. Blood was collected into a sample cup containing a small amount of sodium heparin and sodium fluoride. After collection of approximately 200 ul of blood, it was stirred and clots were removed. The blood sample was then vortexed and placed on ice until assayed for glucose.

Blood samples were collected in this way immediately prior to glucose injection and 15, 30, 90, 150 and 180 minutes after injection. Immediately after the first blood sample was obtained, each rat was intraperitoneally injected with 7.5 mg glucose/g metabolic body weight ($BW^{3/4}$). The in-
Injection solution was 100 mg glucose/ml 0.9% saline. During the glucose tolerance test, rats were kept in plastic cages and allowed free access to water.

As quickly as possible after blood samples were collected, they were assayed for glucose using the Technicon Auto Analyzer II method (Technicon Instruments Corporation, Tarrytown, NY). Standards of 25, 50, 100, 150, 200, 250 and 300 mg glucose/dl were used to generate a standard curve by linear regression each day.

Due to instrument failure, 1 rat from each treatment group did not undergo a glucose tolerance test. These rats were fasted for 12 hours however, to make them similar to the other animals in each group.

3.4 SACRIFICE

At the end of 6 weeks of dietary treatment, rats were sacrificed. Animals were fasted for at least 6 hours prior to sacrifice. Rats were anesthetized with sodium pentobarbital (5 mg/100 g body weight, intraperitoneally). At sacrifice, rats that partially awakened were further anesthetized by inhalation of methoxyflurane (Metofane, Pitman-Moore Inc., Washington Crossing, NJ).
Rats were killed by heart puncture using a 21 gauge, 1.5 inch needle and a Vacutainer tube containing disodium EDTA. After blood was drawn, each tube was inverted to thoroughly dissolve and mix the EDTA and was placed on ice until centrifugation. Blood was centrifuged at 2500 rpm for 20 minutes at 4°C for separation of plasma. Plasma from each rat was removed from packed cells and stored at 4°C until aliquots were taken for VLDL isolation. Remaining plasma was then frozen until analyzed.

3.5 VERY LOW DENSITY LIPOPROTEIN ISOLATION

Very low density lipoproteins (VLDL) were isolated from a plasma sample of each individual rat, as well as, from pooled samples within each group. Pooled samples consisted of plasma of 3 to 5 rats within a treatment group and were used for gel filtration chromatography. An equivalent amount of plasma from each rat within a pool was used.

VLDL were isolated from plasma by the method of Schonfeld et al. (1974) within 3 days after plasma collection. An aliquot of plasma was pipetted into the bottom of a cellulose nitrate centrifuge tube (2.5 in. x 0.5 in.; 6.5 ml). The aliquot size depended upon the amount of plasma obtained from each animal and the amount of plasma available
for the pooled samples. Samples were overlaid with cold EDTA-saline (0.16 M NaCl, 1 mM in disodium EDTA, pH 7.4). Saline was layered down the side of the tube with a Pasteur pipet to create a layer of saline above the plasma. Tubes were filled to leave a minimal air bubble when caps were in place. Caps were hand tightened onto the filled tubes using a wrench. Tubes were placed in a precooled Ti 50.3 rotor and centrifuged at 40,000 rpm for at least 20.5 hours at 10° C in a Beckman Model L2-65 preparative ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA).

After ultracentrifugation, VLDL were removed from each tube within 1 hour of the end of centrifugation. Tube caps were carefully removed and rinsed with a small amount of cold EDTA-saline into a 5 ml graduated cylinder. The top turbid layer of fluid in the centrifuge tube was then completely aspirated into a 5 ml syringe, removing with it the VLDL. This step was accomplished in as small a volume as possible. Due to the small amount of plasma centrifuged for some samples, the final VLDL volume sometimes exceeded the original plasma sample. This situation was avoided when possible. Individual and pooled VLDL samples were stored at 4°C until further analysis was completed.
3.6 ANALYSIS OF INDIVIDUAL VLDL AND PLASMA SAMPLES

VLDL and plasma samples from each individual rat were analyzed in duplicate for triglyceride concentration by the method of Giegel et al. (1975). The only modification made was that all reagent and sample volumes were half of that indicated by Giegel et al. (1975). In this method, triglycerides are extracted with nonane. Glycerol is cleaved from the triglyceride using sodium hydroxide and is then oxidized to formaldehyde with periodate. The formaldehyde formed reacts with acetylacetone to form the colored compound, 3,5-diacetyl-1,3-dihydrolutidine.

Triolein (Sigma Chemical Co., St. Louis, MO) was used as a standard for this determination. Concentrations of triolein standards were 25 mg/dl, 50 mg/dl, 100 mg/dl and 200 mg/dl. An error of 10% between duplicates was accepted for this assay.

Total cholesterol concentration was determined for each plasma and VLDL sample using an SR Direct Cholesterol Test Set (Stanbio Laboratory, Inc., San Antonio, TX). The only deviation from this method was that all reagent and sample volumes were half of that indicated in the instructions. This is a colorimetric procedure based on the Liebermann-Buchard reaction. Cholesterol standards (200 mg/dl and 400 mg/dl) provided in the test set were used to prepare stan-
dards of 50 mg/dl and 100 mg/dl in glacial acetic acid. 
Three standards (50, 100, and 200 mg/dl) were used to generate standard curves. An error of 5% between duplicate samples was accepted for this assay.

3.7 GEL FILTRATION CHROMATOGRAPHY

The size distribution of VLDL of pooled samples was investigated using gel filtration chromatography. Pooled VLDL samples were chromatographed on a Sepharose 2B column (Pharmacia Fine Chemicals, Piscataway, NJ). The Sepharose 2B was packed in a waterjacketed 1.6 cm x 100 cm column (Pharmacia Fine Chemicals, Piscataway, NJ). Gel bed height was 88 cm for each chromatographic run. Column buffer used was 0.16 M NaCl with 1mM disodium EDTA and 0.02% sodium azide (as a preservative) (pH 7.4). A peristaltic pump was used to maintain a constant flow rate of 10.8 ml/hour. This pump was placed between the column and the UV monitor. A second peristaltic pump was used to circulate water of ambient temperature (25°C) through the waterjacket of the column. The column was packed according to standard procedures for use with agarose gels (Bio-Rad Laboratories, 1975). After packing, the column was equilibrated with column buffer at a slow flow rate to achieve a stable bed height. This required 2 to 3 days.
Samples were applied to the column in a 2 to 4.5 ml volume using a sample cup. After the sample had entered the column, the sample cup was rinsed with column buffer. Peaks were monitored at 280 nm using a Buchler Fracto-Scan UV monitor (Searle Analytic Inc., Fort Lee, NJ), and 90 drop (approximately 3 ml) fractions were collected using a Buchler Fractomette Alpha 200 fraction collector (Searle Analytic Inc., Fort Lee, NJ). To determine the void volume of the column, isolated chylomicrons were applied to the column.

Blood, for chylomicron isolation, was obtained from the investigator who had eaten breakfast 2 hours prior to blood drawing. Blood was collected into 3, 15 ml Vacutainer tubes containing disodium EDTA. Immediately after collection, the blood was centrifuged at 2500 rpm for 20 minutes at 4°C. Plasma was removed from packed cells and immediately prepared for ultracentrifugation. Approximately 5 ml of plasma was pipetted into the bottom of a polyallomer centrifuge tube (3 3/4 in. x 9/16 in.; 14 ml). Plasma was overlaid with saline (0.15 m NaCl, 1mM in disodium EDTA, pH 7.4). Samples were placed in a SW40 Ti rotor (Beckman Instruments Inc., Palo Alto, Ca) and centrifuged at 22,500 rpm (90,000g) for 140 minutes at 10°C in a Beckman model L2-65 preparative ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). After centrifugation, turbid fluid, indicating the presence
of chylomicrons, was aspirated from the tube, as described for VLDL samples. Chylomicrons equivalent to 10 ml of plasma were immediately applied to the agarose column. The maximum point of the chylomicron peak was determined to be the void volume of the column. This point was tube 23, or approximately 69 ml. Bovine serum albumin, Fraction V was used to determine the elution volume of a small molecular weight compound from the agarose column. Bovine serum albumin eluted at tube 59 or approximately 177 ml. The total bed volume of the column was determined by filling the empty column with water to the level of the gel. The total bed volume was 182 ml.

Due to adsorption of sample material to the flow cell of the UV monitor, accurate absorbance readings at 280 nm for column fractions could not be obtained. Therefore, the absorbance of each column fraction at 280 nm was determined manually and chromatograms for each sample were plotted. Generally, 3 peaks were observed for each sample.

One representative sample from each group was further investigated. The fractions composing each peak were pooled. Pooled fractions were dialyzed against deionized water using dialysis bags made from dialysis tubing tied securely at each end. Tubing used was Spec/Por 1 Membrane Tubing, 6000-8000 Molecular Weight Cut Off (Spectrum Medical
Industries, Los Angeles, CA). The dialysis tubing was boiled in 5% sodium bicarbonate for 15 minutes to rid it of glycerol. The tubing was then rinsed well with deionized water and checked for leaks by filling with water. One end of the tubing was tied securely and string was also tied firmly at that end. The bag was then filled with sample using a Pasteur pipet. After filling, the bag was closed by tying, incorporating as little air as possible into the bag. This end was also tied with string. Dialysis bags were lowered into a 6 liter container filled with deionized water and were secured to its side. Dialysis was carried out at 4°C with constant stirring. Water was changed 3 to 4 times daily and samples were dialyzed for at least 3 days. Dialyzed samples were transferred to vials or beakers of appropriate size and were frozen. Frozen samples were lyophilized for 72 hours or until dry. Each sample was then reconstituted in 1 ml deionized water. No attempts to further analyze the samples were successful.
3.8 CALCULATIONS AND STATISTICAL ANALYSIS

The area under the glucose tolerance curve for each rat with a complete curve was estimated using the equation, \((A+F)/2+B+C+D+E\), where A is blood glucose concentration before glucose injection, F is blood glucose concentration at 180 minutes after glucose injection, and B, C, D and E are blood glucose concentrations at 15, 30, 90 and 150 minutes after glucose injection, respectively (Bailey and Matty, 1972).

All data were analyzed by 2 x 3 factorial analysis of variance, with dietary treatment and strain or genotype as the two main factors. The effect of dietary treatment within each strain or genotype was determined by 1 (strain or genotype) x 2 (diet) analysis of variance. The effect of strain or genotype within each dietary treatment was determined by 1 (diet) x 3 (strain or genotype) analysis of variance. Comparisons of treatment means were made by Duncan's multiple range test.
Chapter IV
RESULTS AND DISCUSSION

4.1 BODY WEIGHTS AND FEED INTAKE

Initial body weights of rats within a genotype or strain were similar for both dietary treatment groups (table 2). This was expected since rats of each strain or genotype were divided into dietary treatment groups based on body weight. As expected, Zucker fatty rats had higher initial and final body weights than Zucker lean rats and Sprague-Dawley rats. In addition, Sprague-Dawley rats had higher body weights at the beginning and end of the study than Zucker lean rats. Therefore, for the same age and sex, the Zucker lean rat is a smaller rat than the very often used experimental rat, the Sprague-Dawley.

There was no difference in final body weights of Zucker fatty rats due to dietary treatment (table 2). This was also observed for Zucker lean rats. Sprague-Dawley rats fed sucrose had significantly higher final body weights than Sprague-Dawley rats fed cornstarch, however. This was due to a weight gain of the Sprague-Dawley rats fed sucrose.

There was no significant weight gain throughout the experiment for any group fed cornstarch. For Zucker fatty
TABLE 2

Body Weights of Zucker and Sprague-Dalwey Rats Fed Sucrose and Cornstarch Diets

<table>
<thead>
<tr>
<th>Strain or Genotype</th>
<th>Dietary Treatment</th>
<th>N</th>
<th>Body Weights</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>g</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>Zucker Fatty</td>
<td>Cornstarch</td>
<td>12</td>
<td>528 ± 82^1</td>
<td>572 ± 69</td>
<td></td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Cornstarch</td>
<td>13</td>
<td>235 ± 14</td>
<td>239 ± 15</td>
<td></td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Cornstarch</td>
<td>13</td>
<td>368 ± 26</td>
<td>384 ± 40^a</td>
<td></td>
</tr>
<tr>
<td>Zucker Fatty</td>
<td>Sucrose</td>
<td>13</td>
<td>523 ± 90</td>
<td>560 ± 78</td>
<td></td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Sucrose</td>
<td>13</td>
<td>235 ± 15</td>
<td>251 ± 17</td>
<td></td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Sucrose</td>
<td>13</td>
<td>360 ± 23</td>
<td>414 ± 25^a</td>
<td></td>
</tr>
</tbody>
</table>

2 x 3 ANOVA

<table>
<thead>
<tr>
<th>Diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>NS</td>
</tr>
<tr>
<td>Strain or Genotype</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Strain or Genotype x Diet</td>
<td>NS</td>
</tr>
</tbody>
</table>

^1 Values are means ± SD. Diet effects within strain or genotype based on a 1 x 2 ANOVA are indicated as follows: means with common superscripts within a column are significantly different from each other (p < 0.05).
rats, the lack of significant weight gain may be due to
greater variability of body weights encountered. For fatty
rats fed cornstarch, initial body weights ranged from 421
grams to 668 grams and final body weights ranged from 485
grams to 707 grams. Therefore, although it appears that
weight gain may have occurred in this group, it was not a
significant gain. Lean Zucker rats fed sucrose had final
body weights greater than initial body weights (p < 0.03).
This pattern was also observed for Sprague-Dawley rats fed
sucrose (p < 0.0001), but not for Zucker fatty rats fed su-
crose. Michaelis et al. (1980) have reported greater weight
gain in lean Zucker rats fed a sucrose diet compared to
those fed a cornstarch diet. Weight gain of Zucker fatty
rats was similar on both diets. Rats used in this study
were younger (7 weeks old) than those used in the present
study, so weight gain was observed on all diets investigat-
ed.

Differences in initial and final body weights that were
observed may be due to differences in feed intake. Feed in-
take results are presented in table 3. Obese Zucker rats
consumed the same amount of food on both the cornstarch and
sucrose diets. Lean Zucker and Sprague-Dawley rats fed su-
crose consumed significantly more food than lean Zucker and
Sprague-Dawley rats fed cornstarch (p < 0.05). This differ-
ence was evident with feed intake expressed on a daily basis (g/day) and on a daily basis per gram of metabolic body weight (g/day/g B.W.; MBW). Looking at the mean daily feed intakes (or daily feed intake/g MBW) and standard deviations however, these feed intakes do not appear to be different. Therefore although the difference in feed intake is statistically significant, it was a small difference that may not be generalizable to future groups of rats.

Pairfeeding had originally been considered for this experiment so that rats in each group would have the same consumption of dietary carbohydrate, and differences in carbohydrate consumption would not be a confounding factor. Ad libitum feeding was decided upon however, so that no animals would lose weight or be placed upon restricted intake during the study. It was speculated that rats of the same genotype or strain would have similar intakes on both dietary treatments. Although statistically this is not the case, food intakes were remarkably similar considering ad libitum feeding.

Feed intake based on metabolic body weight was calculated to enable comparison of feed intake among rats of different strain or genotype. On both diets, Zucker fatty rats had lower daily feed consumption based on metabolic body weight than Sprague-Dawley or Zucker lean rats (p < 0.05).
TABLE 3

Daily Feed Intake of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets

<table>
<thead>
<tr>
<th>Strain or Genotype</th>
<th>Dietary Treatment</th>
<th>Daily Intake</th>
<th>Daily Intake per Gram MBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucker Fatty</td>
<td>Cornstarch</td>
<td>19 ± 3</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Cornstarch</td>
<td>12 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.20 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Cornstarch</td>
<td>17 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zucker Fatty</td>
<td>Sucrose</td>
<td>19 ± 2</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Sucrose</td>
<td>13 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Sucrose</td>
<td>19 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

2 x 3 ANOVA

<table>
<thead>
<tr>
<th></th>
<th>Diet</th>
<th>Strain or Genotype</th>
<th>Strain or Genotype x Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p &lt; 0.03</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>Diet</td>
<td>p &lt; 0.0005</td>
<td>p &lt; 0.0001</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SD. Diet effects within strain or genotype based on a 1 x 2 ANOVA are indicated as follows: means with common superscripts within a column are significantly different from each other (p < 0.05).
Sprague-Dawley and Zucker lean rats consumed similar amounts of food when expressed per gram MBW regardless of dietary treatment. Zucker fatty rats fed the cornstarch diet consumed 0.17 g food/day/g MBW whereas, Sprague-Dawley and Zucker lean rats consumed 0.20 g food/day/g MBW. Similar results were obtained for rats fed the sucrose diet.

There are several possible explanations for the lower food intake of the obese Zucker rats expressed as daily food intake per gram of MBW. Metabolic body weight is a manipulation of body weight which allows comparison of animals of varying size. It is an estimate of actively metabolizing tissue. Since excess weight of the Zucker fatty rat is adipose tissue rather than all body tissues, comparison of lean and obese Zucker rats using MBW is not strictly correct. It was determined to be an appropriate basis upon which to compare food intake among strain and genotype groups, however. Other researchers have also used MBW for expression of data of Zucker rats (Deb et al., 1976; Jenkins and Hershberger, 1978).

Another explanation of the lower feed intake per gram of MBW of the Zucker fatty rat compared to the Zucker lean and Sprague-Dawley rats is the smaller skeletal and lean tissue mass reported for the Zucker fatty rat (Zucker and Zucker, 1963; Dunn and Hartsook, 1980; Young et al., 1980).
Therefore, the Zucker fatty rat is a larger rat than the Sprague-Dawley or Zucker lean rat, but the fatty has a smaller lean body mass. Presumably, less food would need to be consumed to support this smaller lean body mass.

4.2 GLUCOSE TOLERANCE

There was no overall diet effect for fasting blood glucose levels as shown in table 4 (time 0 minutes). A 1 x 2 ANOVA for each strain indicated that fasting blood glucose levels were not affected by dietary treatment in Zucker fatty or lean rats or in Sprague-Dawley rats. Several researchers have reported similar results for various strains of lean rats (Cohen and Teitelbaum, 1964; Bruckdorfer et al., 1974; Waterman et al., 1975) although reports to the contrary also have been published (Blazquec and Quijada, 1969; Hallfrisch et al., 1979). In the present study, there was similar response to dietary treatment in each strain investigated.

An overall strain effect (p < 0.03) was observed for fasting blood glucose concentration (table 4). Using a 1 x 3 ANOVA for each dietary treatment, the strain differences within each dietary treatment were investigated. For the groups fed the cornstarch diet, Zucker fatty rats had significantly higher fasting blood glucose levels than Zucker
TABLE 4
Glucose Tolerance Values of Zucker and Sprague-Dawley Rats
Fed Sucrose and Cornstarch Diets

<table>
<thead>
<tr>
<th>Strain or Genotype</th>
<th>Dietary Treatment</th>
<th>0 min.</th>
<th>15 min.</th>
<th>30 min.</th>
<th>90 min.</th>
<th>150 min.</th>
<th>180 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucker Fatty</td>
<td>Cornstarch</td>
<td>84 ± 10</td>
<td>146 ± 28</td>
<td>164 ± 25</td>
<td>142 ± 23</td>
<td>134 ± 33</td>
<td>138 ± 27a</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Cornstarch</td>
<td>71 ± 9</td>
<td>148 ± 20</td>
<td>150 ± 22b</td>
<td>116 ± 16</td>
<td>93 ± 15</td>
<td>102 ± 18</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Cornstarch</td>
<td>70 ± 9</td>
<td>121 ± 14c</td>
<td>115 ± 19</td>
<td>84 ± 13c</td>
<td>78 ± 12</td>
<td>83 ± 14</td>
</tr>
<tr>
<td>Zucker Fatty</td>
<td>Sucrose</td>
<td>78 ± 15</td>
<td>156 ± 26</td>
<td>192 ± 44</td>
<td>164 ± 36</td>
<td>144 ± 28</td>
<td>171 ± 26a</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Sucrose</td>
<td>75 ± 17</td>
<td>164 ± 36</td>
<td>185 ± 34b</td>
<td>113 ± 20</td>
<td>100 ± 20</td>
<td>104 ± 20</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Sucrose</td>
<td>72 ± 7</td>
<td>166 ± 58c</td>
<td>136 ± 26</td>
<td>99 ± 15c</td>
<td>85 ± 13</td>
<td>86 ± 12</td>
</tr>
</tbody>
</table>

Diet
NS p < 0.01 p < 0.005 NS NS p < 0.05

Strain or Genotype p < 0.03 NS p < 0.0001 p < 0.0001 p < 0.0001 p < 0.0001

Diet x Strain or Genotype NS NS NS NS NS p < 0.05

1Values are means ± SD. Diet effects within strain or genotype based on a 1 x 2 ANOVA are indicated as follows: means with common superscripts within a column are significantly different (p < 0.05).
lean or Sprague-Dawley rats ($p < 0.004$). The latter 2 groups had fasting blood glucose levels that were not different, however. Fasting blood glucose concentrations were similar for all groups fed the sucrose diet. Fasting blood glucose levels of Zucker fatty rats were not excessive on either diet, however. The mean fasting blood glucose level for Zucker fatty rats was 84 mg/dl on the cornstarch diet and 78 mg/dl on the sucrose diet. Therefore, Zucker fatty rats had fasting blood glucose levels within the normal range, although the levels were higher than those of Zucker lean and Sprague-Dawley rats. This is in agreement with reports that Zucker fatty rats are characterized by normal or slightly elevated fasting blood glucose levels (Zucker and Zucker, 1962; Zucker and Antoniades, 1972; Eaton et al., 1976; Bach et al., 1980; Wade et al., 1980). Zucker and Zucker (1962) reported a fasting blood glucose concentration of $97 \pm 9$ mg/dl for adult fatty rats, a value that was considered within the normal physiological range. Wade (1980) reported that average fasting serum glucose concentrations were not different in lean and obese Zucker rats. He observed glucose values of $91 \pm 2.3$ mg/dl and $116 \pm 12.6$ mg/dl for lean and obese rats, respectively.

The results of this experiment indicate that diet may be a complicating factor when comparing fasting blood glu-
cose levels of Zucker fatty and lean rats. As stated previously, fasting blood glucose concentrations of lean and obese rats were similar when rats were fed the sucrose diet and different when rats were fed the cornstarch diet. The reason for this can be seen in table 4. The obese Zucker rats had a lower mean glucose concentration when fed the sucrose diet compared to the cornstarch diet and the lean Zucker rats had a slightly higher mean blood glucose concentration when fed the sucrose diet compared to the cornstarch diet. Although these dietary differences were not significant, they can account for strain differences observed within each dietary treatment.

Blood glucose concentrations at 0, 15, 30, 90, 150, and 180 minutes after an intraperitoneal glucose injection are presented in table 4. These data are also presented graphically in figure 2. The overall ANOVA indicated a significant effect of diet on blood glucose concentration at 15, 30 and 180 minutes after injection. The effect of diet for each strain or genotype was also investigated. At various points along the glucose tolerance curve, there were dietary differences for each strain. For all differences noted, within strain or genotype, rats fed the sucrose diet had higher blood glucose concentrations than those fed the cornstarch diet.
Figure 2: Glucose Tolerance Curves of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets
Fatty Sucrose,○—; Lean Sucrose,⁺—;
Sprague-Dawley Sucrose,×—; Fatty Starch,○---;
Lean Sucrose,⁺---; Sprague-Dawley Starch,×---.
Sprague-Dawley rats fed sucrose had significantly higher blood glucose levels than those fed cornstarch at 15 minutes and 90 minutes after glucose injection. For Zucker lean rats the only point at which there was a significant difference in blood glucose levels due to diet was 30 minutes after injection. The glucose tolerance curves of Sprague-Dawley and Zucker lean rats peaked at 15 minutes and 30 minutes, respectively. Therefore, in both of these strains sucrose feeding caused an increased peak of the glucose tolerance curve and a similar decline of the curve compared to cornstarch feeding. This was not observed for Zucker fatty rats. For these animals, the only point at which a dietary difference was observed was 180 minutes after glucose injection. At this point, the blood glucose concentration for fatty rats fed sucrose increased from 144 mg/dl at 150 minutes to 171 mg/dl at 180 minutes. An explanation for this increase is not readily apparent and may be due to extraneous factors, such as stress imposed by the glucose tolerance test.

The results for Sprague-Dawley and Zucker lean rats demonstrated some impairment of glucose tolerance due to sucrose feeding. Hallfrisch et al. (1979) reported somewhat impaired glucose tolerance in Wistar rats fed diets of 54% sucrose compared to those fed 54% cooked cornstarch. Su-
crose fed rats had significantly higher blood glucose levels at 2 hours after glucose injection compared to starch fed rats. The peak of the glucose tolerance curve for these rats was 1/2 half hour after glucose injection, although glucose concentration was not measured at 15 minutes. A greater impairment of glucose tolerance due to sucrose feeding was reported by Vrana et al. (1974a). In this study, female Wistar rats were fed diets of 70% sucrose or wheat starch for 6 to 10 weeks. Rats fed sucrose had a greater rise in blood glucose levels after an intraperitoneal injection of glucose and blood glucose levels remained elevated for a longer period of time compared to rats fed wheat starch. Similar results were also reported by Cohen and Teitelbaum (1964).

Strain differences within each dietary treatment were also investigated. For rats fed the cornstarch diet, Zucker obese rats had higher blood glucose concentrations than Sprague-Dawley rats at every time measured (p < 0.05). At 15, 30 and 90 minutes after glucose injection, Zucker lean rats fed cornstarch had higher glucose concentrations than Sprague-Dawley rats fed the same diet (p < 0.05). Therefore, the Zucker strain appears to have a different glucose tolerance profile than the Sprague-Dawley strain. These differences have not been previously investigated directly
within a single study. Considering lean and obese Zucker rats fed the cornstarch diet, obese rats had higher blood glucose concentrations at 0, 90, 150 and 180 minutes after glucose injection ($p < 0.05$), indicating somewhat impaired glucose tolerance of the obese compared to lean Zucker rats.

Results obtained for the sucrose diet were somewhat similar. At 30, 90, 150, and 180 minutes after glucose injection, Zucker fatty rats had higher blood glucose levels than Sprague-Dawley rats fed the sucrose diet. Lean Zucker rats fed the sucrose diet had blood glucose levels that exceeded Sprague-Dawley rats fed the same diet at only 1 point, 30 minutes after glucose injection. This point corresponds to the peak of the glucose tolerance curve for the lean Zucker rats, which could explain the elevated concentrations observed compared to Sprague-Dawley rats. At 90, 150 and 180 minutes after glucose injection, Zucker fatty rats fed the sucrose diet had higher blood glucose concentrations than Zucker lean rats, which is almost identical to the response of rats fed the cornstarch diet.

Generally, regardless of dietary treatment, Zucker fatty rats had an elevated glucose tolerance curve compared to the lean Zucker and Sprague-Dawley control groups. The Zucker fatty rats did not have an elevated peak of the glucose tolerance curve compared to lean Zucker rats, but blood
glucose levels remained elevated at 90, 150 and 180 minutes in obese compared to lean Zucker rats on either dietary treatment. These results are in conflict with those of Triscari et al. (1979) studying 8 month old female lean and obese Zucker rats fed chow. These researchers report no difference in glucose tolerance curves of lean and obese rats. In addition, the peak of the glucose tolerance curve was reached at 10 minutes and blood glucose concentrations of rats of both genotypes returned to fasting levels by 90 minutes. These researchers used an intravenous glucose injection however, which possibly resulted in the earlier blood glucose peak and clearance.

Zucker and Antoniades (1972) reported similar glucose tolerance of lean and obese Zucker rats at 5 to 6 weeks and 50 weeks of age. Rats were fed a purified diet containing 42% cornstarch and 10.9% sucrose. At 20 weeks of age however, Zucker fatty rats had a slower rate of glucose clearance than Zucker lean rats. The rats of the present study were at an age midway between the 2 older groups of Zucker and Antoniades (1972); however our rats responded to a glucose load in similar manner as the 20 week old rats of Zucker and Antoniades. These differences may have been due to differences in the previous handling of the rats. The rats of the present study were not obtained until needed for the experi-
ment. Therefore handling conditions during growth are not known. In addition, the rats of the present study were obtained from a different laboratory than those of Triscari et al. (1979) and Zucker and Antoniades (1972). It is possible that variations in expression of the genetic trait could have resulted if breeding practices were not similar in both laboratories.

The elevation of the glucose tolerance curve for the Zucker fatty rats may also have been caused by glucose injection being based on metabolic body weight. As mentioned previously, the increase in body weight of Zucker fatty rats is due to only adipose tissue and these rats have less lean body mass than lean controls. Therefore, the glucose injection based on metabolic body weight may have resulted in a greater relative glucose load for the Zucker fatty rats compared to Zucker lean and Sprague-Dawley rats. This in turn may have resulted in the slower clearance of the glucose load that was observed for the Zucker fatty rats.

Glucose tolerance curves were also investigated by estimating the area under the curve (table 5). Only rats with complete glucose tolerance curves were used for these calculations, so means of 4 to 7 rats per group are reported. There was an overall diet effect, as well as, an overall strain effect on glucose area. Within each strain or geno-
type, only Sprague-Dawley rats fed sucrose had a greater glucose area than Sprague-Dawley rats fed cornstarch (p < 0.05). For Zucker lean and fatty rats, there was no effect of the dietary treatment. Looking at strain differences within each dietary treatment, for rats fed the cornstarch diet, obese Zucker rats had greater glucose areas than lean Zucker rats which had greater areas than Sprague-Dawley rats. With sucrose feeding, obese Zucker rats had greater areas than lean Zucker and Sprague-Dawley rats (p < 0.05), which had similar glucose areas. These results correspond generally to the observations made of individual points on the glucose tolerance curve for each strain or genotype and dietary treatment.
TABLE 5

Areas Under Glucose Tolerance Curves of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets

<table>
<thead>
<tr>
<th>Strain or Genotype</th>
<th>Dietary Treatment</th>
<th>Number of Observations</th>
<th>Glucose Area (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucker Fatty</td>
<td>Cornstarch</td>
<td>4</td>
<td>662 ± 32&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Cornstarch</td>
<td>5</td>
<td>591 ± 22</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Cornstarch</td>
<td>7</td>
<td>482 ± 56&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zucker Fatty</td>
<td>Sucrose</td>
<td>4</td>
<td>806 ± 156</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Sucrose</td>
<td>6</td>
<td>616 ± 56</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Sucrose</td>
<td>7</td>
<td>586 ± 90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

2 x 3 ANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>p &lt; 0.003</td>
</tr>
<tr>
<td>Strain or Genotype</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Diet x Strain or Genotype</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SD. Diet effects within strain or genotype based on a 1 x 2 ANOVA are indicated as follows: means with common superscripts are significantly different from each other (p < 0.05).
4.3 PLASMA LIPIDS

Plasma lipid levels of rats fed the sucrose and cornstarch diets are presented in table 6. The overall ANOVA indicated a significant effect of diet on plasma triglyceride concentration. This was not true for very low density lipoprotein (VLDL) triglyceride, however. Considering the effect of dietary treatment within each strain or genotype, Zucker lean and Sprague-Dawley rats fed sucrose had higher plasma triglycerides and VLDL triglycerides than rats of the same genotype or strain fed cornstarch (p < 0.05). This effect has often been observed with the feeding of sucrose to nonobese rats (Cohen et al., 1972; Bruckdorfer, 1974; Naismith and Rana, 1974; Laube et al., 1976; Hallfrisch et al., 1979). It has been suggested (Naismith and Rana, 1974) that the increase in plasma triglyceride concentration caused by sucrose feeding is due to increased hepatic triglyceride production. This idea is supported by a study of Witztum and Schonfeld (1978) who reported greater secretion of triglyceride from perfused livers of Sprague-Dawley rats fed a fructose enriched chow diet compared to those fed only chow.

Although, Zucker lean and Sprague-Dawley rats had increased plasma and VLDL triglycerides after sucrose feeding, this pattern was not observed for Zucker fatty rats. This difference resulted in an overall strain x diet interaction.


**TABLE 6**

Plasma Lipid Values of Zucker and Sprague-Dawley Rats Fed Sucrose and Cornstarch Diets

<table>
<thead>
<tr>
<th>Strain or Genotype</th>
<th>Dietary Treatment</th>
<th>Plasma Triglycerides mg/dl</th>
<th>VLDL Triglycerides mg/dl</th>
<th>Plasma Cholesterol mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucker Fatty</td>
<td>Cornstarch</td>
<td>162 ± 109&lt;sup&gt;1&lt;/sup&gt;</td>
<td>131 ± 91</td>
<td>176 ± 58</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Cornstarch</td>
<td>57 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43 ± 20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71 ± 15</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Cornstarch</td>
<td>80 ± 21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67 ± 17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zucker Fatty</td>
<td>Sucrose</td>
<td>138 ± 53</td>
<td>111 ± 52</td>
<td>174 ± 41</td>
</tr>
<tr>
<td>Zucker Lean</td>
<td>Sucrose</td>
<td>101 ± 35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80 ± 42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>Sprague-Dawley</td>
<td>Sucrose</td>
<td>167 ± 114&lt;sup&gt;b&lt;/sup&gt;</td>
<td>110 ± 48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>110 ± 23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

2 x 3 ANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>p &lt; 0.03</td>
</tr>
<tr>
<td>Strain or Genotype</td>
<td>p &lt; 0.003</td>
</tr>
<tr>
<td>Strain or Genotype x Diet</td>
<td>p &lt; 0.02</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SD. Diet effects within strain or genotype based on a 1 x 2 ANOVA are indicated as follows: Means with common superscripts within a column are significantly different (p < 0.05).
for plasma triglyceride concentration \((p < 0.02)\). Consumption of the diet high in sucrose by the obese rats did not result in plasma or VLDL triglyceride concentrations that differed from obese rats fed the cornstarch diet. These results suggest that lipid metabolism of the obese Zucker rat responds differently to sucrose feeding than does that of the Zucker lean and Sprague-Dawley rat. A recent study (Michaelis et al., 1980) has indicated however, that activity of hepatic lipogenic enzymes of Zucker fatty rats increases with sucrose feeding, as does that of Zucker lean rats. This may not be contradictory to the present study if Zucker fatty rats were able to remove the additional plasma triglycerides resulting from sucrose feeding at a rate greater than that of the Zucker lean and Sprague-Dawley rats. Since Zucker fatty rats have been reported to have increased adipose tissue lipoprotein lipase activity when the mass of the adipose tissue is considered (Stern et al., 1972; DeGasquet et al., 1973; Gruen et al., 1978), an increased rate of plasma triglyceride removal may indeed be possible.

Another explanation of the lack of dietary effect on plasma and VLDL triglyceride concentration in the Zucker fatty rats is that these animals did not respond to sucrose feeding by increased hepatic VLDL synthesis. Investigation
of hepatic lipogenic enzyme activity or hepatic perfusion and measurement of triglyceride and VLDL production may have been helpful in determining the cause of the differences of the dietary effect between Zucker fatty and lean rats.

Zucker lean rats had fasting plasma and VLDL triglyceride levels similar to Sprague-Dawley rats, regardless of dietary treatment. Similar results have been reported by Schonfeld et al. (1974) and Bach et al. (1977). As expected, Zucker fatty rats fed the cornstarch diet had higher plasma and VLDL triglyceride concentrations than Zucker lean or Sprague-Dawley rats (p < 0.05). Increased plasma triglyceride levels are unanimously reported in Zucker fatty rats compared to lean controls (Zucker and Zucker, 1962; Barry and Bray, 1969; Schonfeld and Pflegar, 1971; Zucker, 1972; Schonfeld et al., 1974; Bach et al., 1977; Bach et al., 1980). With sucrose feeding however, since this diet resulted in increased triglyceride levels of Zucker lean and Sprague-Dawley rats, there was no difference in plasma or VLDL triglyceride concentrations due to strain or genotype. This same pattern was observed for fasting blood glucose concentrations. Therefore, for fasting plasma triglyceride concentration, as well as, fasting blood glucose concentration, diet may be a complicating factor when comparing Zucker fatty and lean rats.
No overall dietary effect was observed for plasma cholesterol concentration (table 6). Investigation of each strain individually using a 1 x 2 ANOVA indicated no difference due to diet in plasma cholesterol concentration of Zucker fatty and lean rats. Sprague-Dawley rats fed sucrose had higher plasma cholesterol levels than those fed cornstarch (p < 0.01). Most research indicates that feeding of high sucrose diets to rats does not affect plasma cholesterol concentrations (Laube et al., 1973; Michaelis and Szepesi, 1974; Waterman et al., 1975) however, reports to the contrary have also been published (Naismith, 1971; Naismith and Rana, 1974) despite similarities of experimental design.

A significant strain effect for plasma cholesterol was observed (p < 0.0001). Zucker fatty rats had higher fasting plasma cholesterol levels than Zucker lean or Sprague-Dawley rats, regardless of dietary treatment (p < 0.05). This is in agreement with reports of increased plasma cholesterol concentrations of Zucker fatty rats compared to lean controls (Barry and Bray, 1969; Zucker, 1972; Schonfeld et al., 1974; Bach et al., 1977; Bach et al., 1980). Zucker lean rats had plasma cholesterol levels significantly lower than Sprague-Dawley rats when fed the sucrose diet (p < 0.05), but not different from Sprague-Dawley rats when fed the
cornstarch diet. This difference is due to the increase in fasting plasma cholesterol concentrations observed in Sprague-Dawley rats fed the sucrose diet.

4.4 VLDL SIZE DISTRIBUTION

No differences in plasma VLDL size distribution were observed among the groups studied. Examples of VLDL chromatograms are presented in figures 3, 4, and 5. For each sample, 3 peaks were generally observed. For random samples however, either peak 1 or peak 2 was absent. The first peak eluted approximately at the void volume, tubes 16 to 21. The elution volume of the second peaks was approximately tube 33 to 42. For the third peak, the elution volume was observed at tubes 57 to 61 for all samples. There appeared to be as much variation within groups in elution volume of the 3 peaks as among groups.

Several attempts were made to characterize the peaks eluted from the column. Agarose electrophoresis was attempted to assess the mobility of the chromatographic peaks. Unfortunately, too little material was present in peaks 1 and 3 for this analysis. In addition, peak 2 could not be sufficiently concentrated to clearly determine its electrophoretic mobility.
Figure 3: VLDL Size Distribution of Zucker Fatty Rats Fed Sucrose or Cornstarch. VLDL equivalent to 4.5 ml plasma from 3 rats was applied to the column. See page 54 for column conditions.
Figure 4: VLDL Size Distribution of Zucker Lean Rats Fed Sucrose or Cornstarch. VLDL equivalent to 4.0 ml plasma from 4 rats and 4.5 ml plasma from 3 rats was applied for rats fed sucrose and cornstarch, respectively. See page 54 for column conditions.
Figure 5: VLDL Size Distribution of Sprague-Dawley Rats Fed Sucrose or Cornstarch. VLDL equivalent to 4.0 ml plasma from 4 rats and 4.5 ml plasma from 3 rats was applied for rats fed sucrose and cornstarch, respectively. See page 54 for column conditions.
Elution patterns observed for VLDL in this study do not indicate larger VLDL in Zucker fatty rats compared to lean rats. In addition, rats fed sucrose did not exhibit larger VLDL than cornstarch fed rats, since elution patterns were similar for all groups studied. It was expected that rats fed sucrose and Zucker fatty rats would have larger VLDL than rats fed cornstarch and lean rats, respectively. Witztum and Schonfeld (1979) reported larger VLDL of plasma and liver perfusate of Zucker fatty rats compared to lean rats. In addition, Witztum and Schonfeld (1978) studied liver perfusate of rats fed chow or chow plus fructose (60:40). Perfusate VLDL of rats fed the high carbohydrate diet were of a larger size than those of rats fed chow. In both of the above studies, 2 to 3 elution peaks were observed. The first 2 peaks were identified as VLDL, whereas the third peak was not fully characterized in either study.

The lack of VLDL size differences observed in this study may be due to the relative VLDL concentrations present. No group of rats had exceedingly high VLDL triglyceride concentrations. This may have resulted in similar size distributions of VLDL in all groups, since increased triglyceride concentrations are associated with increases in VLDL size. Witztum and Schonfeld (1978, 1979) observed greater VLDL triglyceride concentrations than were observed
in the present study. This may account for why these investigators observed VLDL size differences, whereas no differences were observed in the present study.
Chapter V
SUMMARY AND CONCLUSIONS

The effect of dietary sucrose on glucose and lipid metabolism of obese hypertriglyceridemic animals has not been clearly established. In normal weight rats and humans, sucrose feeding results in increased plasma triglyceride concentrations. Fasting plasma glucose concentrations and glucose tolerance curves may also be altered by sucrose feeding.

In the present study, the Zucker fatty rat was used as a model of obesity accompanied by hypertriglyceridemia. Diets high in sucrose or cornstarch were fed to Zucker fatty, Zucker lean, and Sprague-Dawley rats.

Sprague-Dawley rats fed sucrose had higher final body weights than rats of the same strain fed cornstarch. For lean and obese Zucker rats however, there was no difference in final body weights due to dietary treatment. No rats fed cornstarch gained weight throughout the study. Lean Zucker rats and Sprague-Dawley rats fed sucrose gained weight when fed the sucrose diet, however. This was not observed for Zucker fatty rats.

Zucker lean and Sprague-Dawley rats fed sucrose consumed more food throughout the study than animals of the
same strain or genotype fed cornstarch. Zucker fatty rats however, consumed the same amount of food regardless of dietary treatment. The food intake data help to explain the weight gain results observed above.

Sucrose feeding did not result in elevated blood glucose concentrations in any strain investigated. Glucose tolerance curves of Zucker lean and Sprague-Dawley rats were somewhat altered due to sucrose feeding. In both strains, sucrose fed rats had a higher glucose tolerance peak than cornstarch fed rats. For Zucker fatty rats, sucrose consumption resulted in elevated blood glucose concentrations at 180 minutes after glucose injection.

Generally Zucker fatty rats had an elevated glucose tolerance curve compared to Zucker lean and Sprague-Dawley rats. In addition, for rats fed the cornstarch diet, Zucker lean rats had an elevated glucose tolerance curve compared to Sprague-Dawley rats.

Considering plasma lipid concentrations, Zucker lean and Sprague-Dawley rats responded in a similar manner. Sucrose feeding resulted in elevated plasma and VLDL triglyceride levels compared to cornstarch fed animals. This effect has been frequently reported for lean rats and is suggested to be caused by increased hepatic triglyceride synthesis. In contrast to the lean rats, sucrose consump-
tion did not result in increased plasma and VLDL triglycer-ide concentrations for Zucker fatty rats. This may indicate a different response of the obese rat to sucrose consumption compared to the lean rats investigated. The mechanism for this different response is unclear. The Zucker fatty rat may clear excess hepatic triglyceride produced more quickly than lean rats, or may not have had increased hepatic triglyceride synthesis at all. Sucrose consumption resulted in elevated fasting plasma cholesterol concentrations in only the Sprague-Dawley rats. A similar response has been noted by some investigators, but not others.

There were no differences in VLDL size for any diet and strain or genotype combination investigated. When subjected to gel filtration chromatography, VLDL were separated into 2 to 3 size distributions. The lack of difference due to dietary treatment or strain or genotype may be due to the VLDL concentrations observed, since no group had exceedingly high VLDL triglyceride concentrations.

This study indicates that Zucker fatty rats may not respond in the same way as Zucker lean and Sprague-Dawley rats to sucrose consumption. Glucose tolerance curve response and plasma and VLDL triglyceride concentration response were different in Zucker fatty rats compared to the two lean strains studied. This may indicate that obese ani-
mals respond differently to dietary carbohydrate than lean animals.
LITERATURE CITED


The vita has been removed from the scanned document
BLOOD GLUCOSE AND PLASMA LIPIDS OF ZUCKER FATTY AND LEAN RATS FED DIETS CONTAINING CORNSTARCH OR SUCROSE

by

Patricia M. Sheehan

(ABSTRACT)

The effects of consumption of diets high in sucrose and cornstarch on glucose and lipid metabolism in Zucker fatty and lean rats and Sprague-Dawley rats were investigated in the present study. Rats of each strain or genotype, 8 to 10 months of age, were fed diets containing 56% cornstarch or 57% sucrose for 4 weeks, when an intraperitoneal glucose tolerance test was administered. After 2 additional weeks of dietary treatment, the rats were sacrificed and plasma was collected. The plasma was assayed for triglyceride and cholesterol concentration. Plasma VLDL were isolated by ultracentrifugation and were assayed for triglyceride. Pooled VLDL samples from each group were separated by size using a 2% agarose column.

Sprague-Dawley rats fed sucrose had higher final body weights than rats of the same strain fed cornstarch. For lean or obese Zucker rats however, there was no difference in final body weights due to dietary treatment. No rats fed cornstarch gained weight throughout the study. Lean Zucker rats and Sprague-Dawley rats fed sucrose gained weight when fed the sucrose diet, although Zucker fatty rats did not.
Zucker lean and Sprague-Dawley rats fed sucrose consumed more food throughout the study than animals of the same strain or genotype fed cornstarch. Zucker fatty rats however, consumed the same amount of food regardless of dietary treatment.

Fasting blood glucose concentrations were not affected by the dietary treatment within any strain or genotype investigated. However, Zucker fatty rats fed cornstarch did have significantly higher fasting blood glucose levels than Zucker lean or Sprague-Dawley rats fed cornstarch. This strain difference was not noted for groups fed the sucrose diet. Sucrose consumption resulted in an increased glucose tolerance curve peak with a similar decline of the curve for Zucker lean and Sprague-Dawley rats. This pattern was not observed for Zucker fatty rats fed sucrose. These rats had elevated blood glucose levels at 180 minutes after glucose injection, possibly indicating delayed glucose clearance in these rats. Zucker fatty rats generally had an elevated glucose tolerance curve compared to Zucker lean and Sprague-Dawley rats, regardless of dietary treatment.

Plasma lipid concentrations of Zucker fatty rats responded differently to the dietary treatment than those of Zucker lean and Sprague-Dawley rats. Sucrose feeding caused increased plasma and VLDL triglyceride concentrations in
Zucker lean and Sprague-Dawley rats, whereas no elevation of triglyceride levels occurred in Zucker fatty rats. Increased cholesterol levels due to sucrose feeding were observed only in Sprague-Dawley rats. There were no VLDL size differences observed for any strain or genotype and diet combination investigated. This may be due to the similarity of relative concentrations of VLDL triglyceride observed for all groups.

This study demonstrated a difference in response of glucose tolerance and plasma and VLDL triglyceride concentrations of Zucker fatty rats fed sucrose compared to Zucker lean and Sprague-Dawley rats fed the same diet.