INTERACTION BETWEEN HIGH FAT DIET, ADRENALECTOMY AND/ OR EXERCISE ON PANCREATIC B-CELL FUNCTION IN OBESE ZUCKER (fa/fa) RATS.

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University of Prince Edward Island.

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ABSTRACT.

Young adult obese fa/fa Zucker rats are hyperinsulinemic, hyperlipidemic and insulin resistant. Pancreatic islets of fa/fa rats exhibit increased glucose sensitivity (half maximal effective glucose concentration EC_{50}), glucokinase sensitivity (K_m) and lack of mannoheptulose (MH) inhibitory action on glucose stimulated insulin secretion(GSIS). These defects are normalized by adrenalectomy (ADX) in rat chow fed rats within 2 weeks of surgery. The current study was carried out to test the hypothesis that feeding ADX rats a high fat diet would inhibit the effects of ADX on the pancreatic islet B-cells in fa/fa rats and induce adaptive changes in the lean rats. Animals were either ADX or sham operated on and after recovery for one week, they were either fed rat chow or a high fat diet (16% fat) for 4 wk. *In vivo* and *in vitro* glucose and insulin responses, islet glucose phosphorylation and islet free fatty acid (FFA) oxidation were measured in both lean and fa/fa rats.

In pancreatic islets isolated from rat chow fed fa/fa rats, ADX significantly reduced glucokinase K_m and glucose phosphorylating rate (V_{max}) compared to those of sham rats. Although pancreatic islet B-cell EC_{50} to glucose was not modified by ADX, basal insulin secretion from sham fa/fa rats was higher than that of ADX fa/fa and lean rats indicating that islets from sham fa/fa rats were more responsive to low glucose concentrations.

The MH inhibitory response was restored by ADX in fa/fa rat islets. Pancreatic triglyceride and insulin content were higher in fa/fa rat islets than in the lean rat islets and were not affected by ADX. However, palmitic acid oxidation was 3-fold higher in sham fa/fa than in sham lean rats in the presence of both low and high glucose concentrations compared to that from the lean rats and this was significantly reduced by ADX.

Feeding a high fat diet to ADX fa/fa rats significantly increased glucokinase V_{max} ($p<0.05$).The restored MH inhibitory action by ADX in fa/fa rat islets was reduced by increased dietary fat in that a higher MH concentration was required to inhibit GSIS. Pancreatic islet triglyceride content of ADX fa/fa rats was significantly reduced after a high fat diet and this was accompanied a 27%-40% reduction in FFA acid oxidation. In sham fa/fa rats high fat diet significantly lowered islet EC_{50} for glucose. *In vivo* a high fat diet significantly increased integrated insulin and glucose responses during OGTT in ADX and sham rats ($p<0.05$), indicative of increased insulin resistance. From this study it is concluded that feeding ADX a high fat diet modulated but did not eliminate ADX action on pancreatic B-cell function in fa/fa rats.

A second study was carried out to test the hypothesis that exercise would have similar effects on pancreatic B-cell function of fa/fa rats as those induced by ADX, the rationale being that both ADX and exercise increase the sympathetic nervous system activity in the peripheral tissues including the endocrine pancreas. Animals were either fed a low fat diet or a high fat diet and half of them were exercised by swimming 1 hour/day for 5 days/ week for 4 weeks.

Exercise significantly reduced both hexokinase K_m and V_{max} in low fat fed

fa/fa rats. In low fat fed lean rats, exercise reduced glucokinase and hexokinase K_m without affecting the V_{max}. Unlike ADX, exercise had no significant effects on insulin secretion responses to glucose or MH in either phenotype. Insulin and glucose responses during OGTT were decreased in exercised rats compared to sedentary rats suggesting increased insulin sensitivity of target tissues.

Feeding a high fat diet to exercised fa/fa rats significantly increased glucokinase K_m and reduced both hexokinase K_m and V_{max}. The MH response was partially restored in islets isolated from high fat exercised fa/fa rats.

In sedentary lean rats, high fat diet decreased glucokinase K_m, increased pancreatic islet insulin content and FFA oxidation at the lower glucose concentration.

Exercise modified both fa/fa and lean rats *in vivo* responses but induced only small changes *in vitro* pancreatic islet function in fa/fa rat islets. Feeding a high fat diet induced adaptive changes in lean rats similar to those observed in obese rats and worsened insulin resistance in fa/fa rats. Compared to ADX, moderate exercise induced minimal beneficial effects in fa/fa rats and this could be attributed to the presence of an abnormally regulated HPA axis. However exercise lowered body weight and caloric intake when animals were fed a diet with low fat content and may therefore provide beneficial effects distinct from any effects on islet function.

From these studies it is concluded that ADX is more effective than exercise in preventing pancreatic islet dysfunction in obese rats due to the induction of bigger changes in glucokinase activity and islet FFA oxidation. High fat diet was able to block some of the effects of ADX and exercise. However, changes in the B-cell biochemistry and insulin secretion were small and unlikely to account for the changes observed *in vivo*.

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ABBREVIATIONS.

Abbreviation	Term
ACTH	Adrenocorticotrophic hormone
ADX	Adrenalectomy or adrenalectomized
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
AVC	Atlantic Veterinary College
BAT	Brown adipose tissue
BMI	Body mass index
BSA	Bovine serum albumin
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
CHD	Coronary heart disease
CO_2	Carbon dioxide
CRH	Corticotrophin releasing hormone
d	Day(s)
DME	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
GAT	Nucleotide bases (G=guanine, A= adenine T=thymine)
$GLUT_{1-4}$	Glucose transporters one through to four
ICV	Intracerebroventricular
IP	Intraperitoneal
IV	Intravenous
h	Hour(s)
HBSS	Hank's balanced salt solution
HDL	High density lipoprotein
HEPES	N-{2-hydroxyethyl}piperazine-N'-2-ethanesulfonic acid}
HPA	Hypothalamo-pituitary-adrenal axis
HSL	Hormone sensitive lipase
kb	Kilobase pairs
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
min	Minutes
MH	Mannoheptulose
mM	millimolar
MODY	Mature-onset diabetes of the young
mRNA	Messenger ribonucleic acid
MW	Molecular weight
NaCl	Sodium chloride
NEFA	Nonesterified free fatty acids
NIDDM	Non-insulin-dependent diabetes mellitus
NPY	Neuropeptide Y

OLETF	Otsuka-Long-Evans-Tukushima fatty
PE	Polyethylene catheter
RNA	Ribonucleic acid
SEM	Standard error of the mean
SNK	Student-Newman-Keuls test
TG	Triglycerides
VMH	Ventromedial nucleus of the hypothalamus
wk	Week(s)

1. GENERAL INTRODUCTION

1.1. Obesity and hyperinsulinemia

Obesity is defined as an excess of body fat of more than 30% of the ideal body weight (Ashwell, 1994; Kuczmarski *et al.*, 1994) For diagnostic and statistical purposes, an individual with a body mass index (BMI) (kg/m^2) equal to or greater than 30 is considered obese (Maillard *et al.*, 1999). By this definition, over 30% of the population in most industrialized countries is either overweight or obese (Kuczmarski *et al.*, 1994). However, world wide, at least 7 % of the adult population is considered obese while 21% are considered overweight (Seidell, 1999). Obesity is a costly condition both in human lives due to early death and in the cost of treating obesity related illnesses (Wolfe and Colditz, 1998).

The association between hyperinsulinemia and obesity is well documented (Elahi *et al.*, 1989). However, the cause-effect relationship still needs to be firmly established. Plasma insulin level is proportional to adipocyte volume (Eriksson *et al.*, 1998) and body fatness humans (Everson *et al.*, 1998). There is a strong relationship between body weight, the development of insulin resistance and non-insulin dependent diabetes mellitus (NIDDM) (Després *et al.*, 1995; Sigal and Warren, 1996). Figure 1 shows the relative risk of developing NIDDM in men and women as the BMI increases.

Obesity and hyperinsulinemia are major risk factors for the development of NIDDM (Chan *et al.*, 1994). At least 90% of NIDDM patients are either overweight or obese (Colditz *et al.*, 1995). Within the obese population, the incidence of insulin

resistance and coronary heart disease is high in individuals with increased accumulation of body fat in the internal abdominal regions (visceral obesity) (Pi-Sunyer, 1993; Lemieux and Després, 1994). In addition to fasting hyperinsulinemia, obese individuals exhibit fasting hypertriglyceridemia and impaired glucose tolerance, which increase the risk of developing high blood pressure (Pi-Sunyer, 1993; Després *et al.*, 1995; Huang *et al.*, 1996). Obesity also predisposes susceptible individuals to develop certain types of neoplasias such as breast, endometrial, colon and prostate cancers (Folsom *et al.*, 1989; Nomura *et al.*, 1985; Schapira *et al.*, 1991; Greenwald ,1999). Obesity is incriminated in cases of gout and gall bladder disease (Pi-Sunyer, 1993; Oria , 1998). Obesity also aggravates conditions like osteoarthritis (Oliveria *et al.*, 1999; Sandmark *et al.*, 1999) and respiratory diseases, and increases the risk of complications during surgery (Pi-Sunyer, 1993). There is an increased rate of unexplained sudden death in individuals with a BMI greater than 29 compared to those with normal weight individuals (Stevens *et al.*, 1998).

1.2. Factors that regulate energy balance

Insulin secretion and metabolism of nutrients are closely entwined. Plasma insulin concentrations are elevated after mixed fuel consumption indicating that all fuel metabolites participate in the stimulation of insulin release from pancreatic B-cells. In turn the secreted insulin accelerates the nutrient processing by most body tissues

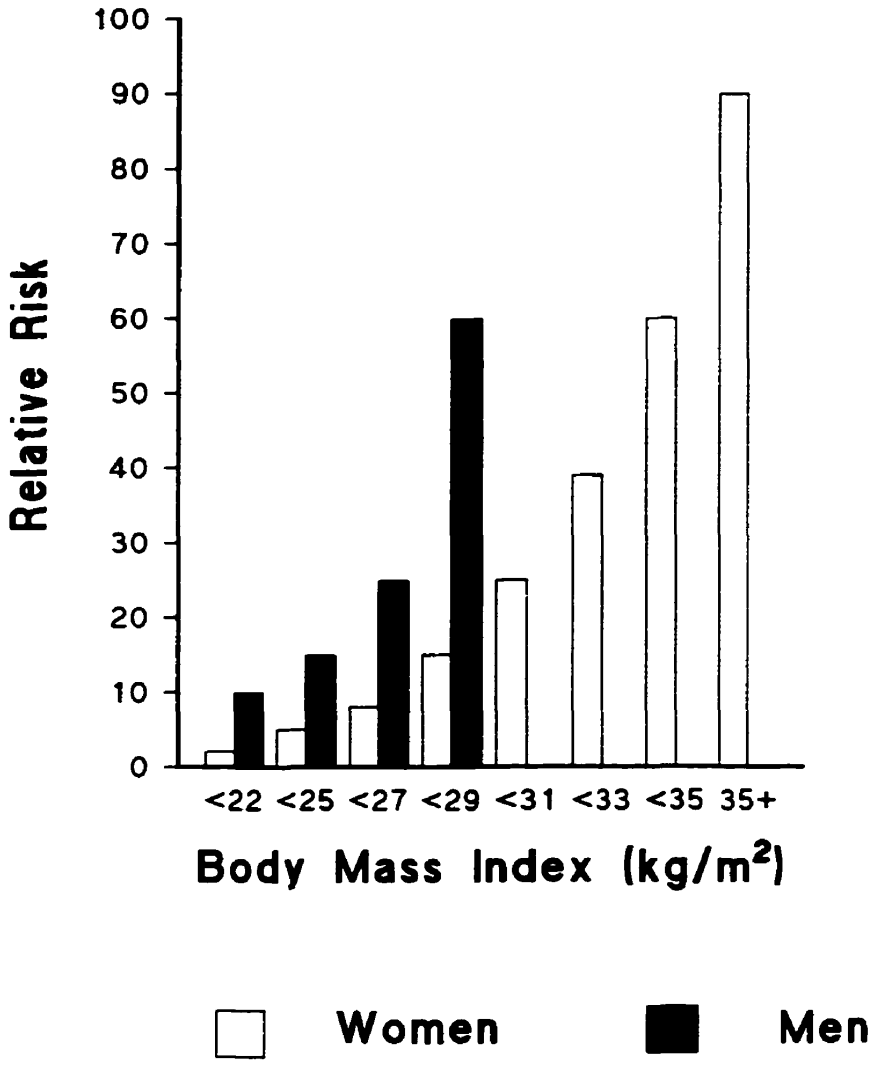

Figure 1. Relative risk of developing diabetes in men and women.

(Adapted from Chan *et al.*, 1994 for men and Colditz *et al.*, 1995, for women).

(Efendic *et al.*, 1991; Malaisse *et al.*, 1991). Obesity is an energy imbalance disorder which develops due to increased energy supply coupled with reduced energy expenditure. The excess energy is stored as triglycerides (TG) (neutral fat) in the adipose tissue (Bouchard, 1996). In order for an individual to maintain a stable body mass, energy intake has to equal to energy expenditure (Rosenbaum *et al.*, 1997). Body mass (especially body fat stores) has been suggested to be closely regulated at a certain set point within an individual, whether lean or obese, when monitored for a relatively short time (Hirsh *et al.*, 1998). The set point is maintained either by increasing food intake during periods of elevated energy expenditure, or by reducing basal metabolism during food restriction (Seeley *et al.*, 1996). During an overfeeding period, people or animals will exhibit an increase in basal metabolism and subsequently reduce their food intake when they resume *ad libitum* eating behavior (Hirsh *et al.*, 1998).

The body weight set point is not necessarily fixed; it can be increased or decreased to a different level in response to aging, sickness, altered eating habits and diet composition (Frederich *et al.*, 1995), reduced activity or abnormal neurohormonal regulation (Levin and Routh, 1996). Several factors that are associated with increased energy storage leading to positive energy balance include age (old subjects are more susceptible to weigh gain than young individuals), gender (female animals or humans tend to store more energy as fat), socioeconomic status (less education and poverty), diet composition (high fat diets or calorie dense foods), reduced physical activity and change of life style (becoming

more urbanized) (Hodge *et al.*, 1995; Grundy, 1998). Because not all individuals exposed to these same environmental conditions become obese, the genetic makeup of an individual appears to play a crucial role in determining the development of obesity (West and York, 1998). Inherited factors such as low resting metabolic rate (RMR) and tendency to store fat rather than use it as energy (Bouchard, 1995) can all lead to positive energy balance. Figure 2 lists some of the factors that can lead to positive energy balance whereby a single factor or in combination of factors could result in abnormal energy balance and the development of the cluster of conditions (such as obesity, hypertension, coronary heart disease, cardiocerobrovascular diseases, hyperlipidemia, hypercholesterolinemia, insulin resistance and NIDDM) also known as " the metabolic syndrome" or "syndrome X".

1.2.1. Environmental factors

The increase in obesity that has occurred in the last 20 years is blamed on a change of environmental factors rather than on change of the genetic makeup of the human population (Kuczmarski *et al.*, 1994). National and World Health Organization studies on obesity show that the weight of most populations has increased by 3.6 kg while the height has changed by less than 1 cm in the last 20 years (Kuczmarski *et al.*, 1994; Sigal and Warram, 1996).

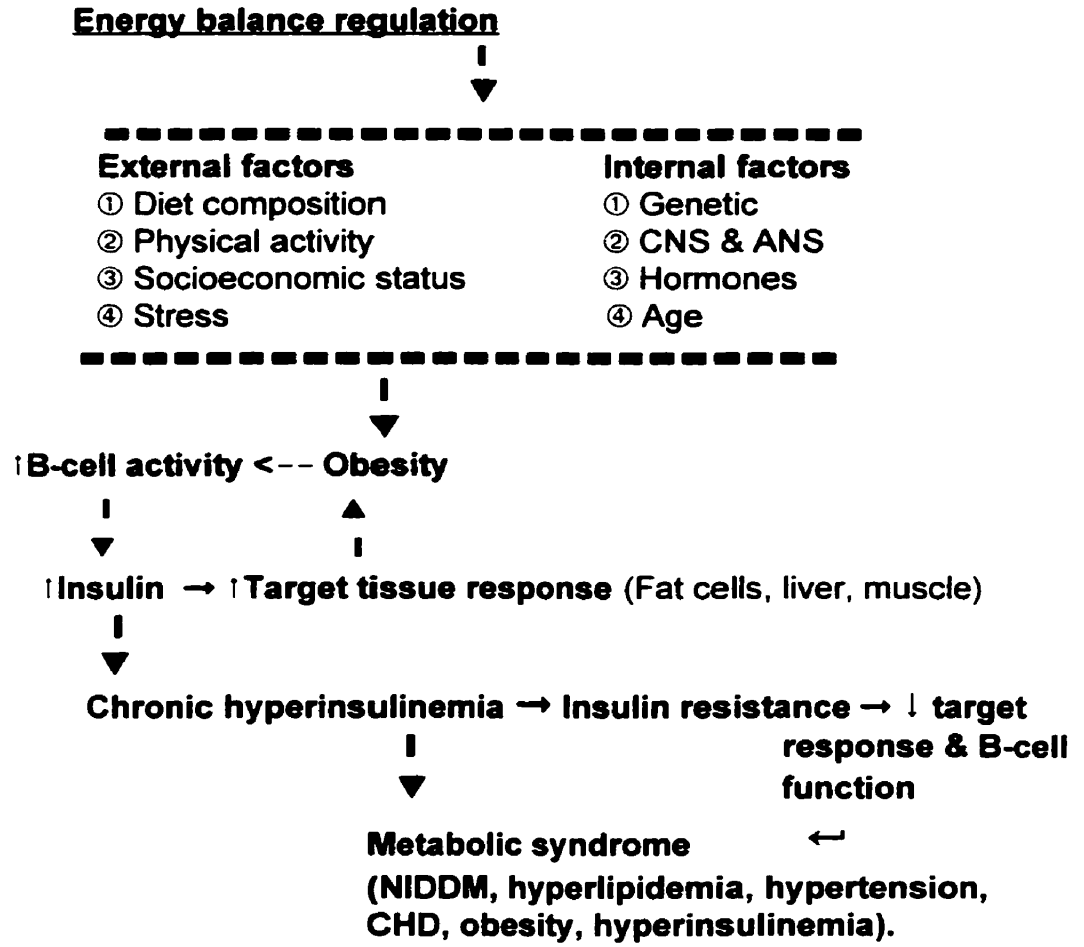

Figure 2. Some of the factors which may influence energy balance and development of the "Metabolic Syndrome". ↓= reduced, ↑ = increased, coronary heart disease (CHD), central nervous system (CNS), autonomic nervous system (ANS) .

Within the same period an increase in overweight and/or obesity in adults (from 24% to 33%) and in children (from 15% to 24%) in Canada (Millar and Stephens, 1993), England (Ashwell, 1994) and the USA (Kuczmarski *et al.*, 1994; Still, 1999) has been observed. Since World War Two, the food supply of most countries has become abundant, physically demanding tasks have been taken over by machines, and intentional physical activity has declined both in adults and young people (Heath *et al.*, 1994). Also with increased immigration from developing countries to industrialized countries there has been a change of population demographics (Le Marchand *et al.*, 1997). The children of immigrants tend to be heavier than the population at large due to a change of diet but not genetic makeup (Popkin and Udry, 1998). This point is well illustrated by the difference between the Arizona Pima Natives, where the majority are overweight compared to their relatives in Mexico (Ravussin *et al.*, 1994), and in rural and urban Asian Indian populations (Dhawan, 1996). Thus two environmental factors that have a recognizable impact on weight maintenance are changes in diet and reduced physical activity (Hill and Peters, 1998). Both increased dietary fat consumption and reduced physical activity may contribute to the increase of the world populations' body mass, insulin resistance, hypertension, coronary heart diseases, hypertriglyceridemia and cerebrovascular diseases. This cluster of conditions is now referred to as "metabolic syndrome" or "syndrome X" and all are associated with hyperinsulinemia (Eriksson *et al.*, 1997).

1.2.1.1. Diet composition

From experimental animal studies, diets with a high fat content and simple sugars induce faster weight gain than do complex carbohydrate based diets (Pagliassotti *et al* ., 1997; West and York, 1998). Dietary obesity is induced by overeating of high fat/high energy foods coupled with insufficient physical activity. Even a small amount of energy surplus, regardless of the diet composition, will result in weight gain as seen with age-related weight gain (McDowell *et al.*, 1994; Bouchard, 1996). Fat content is the main determinant of energy density of a given diet since one gram of fat provides 9 kilocalories (kcal) compared to 4 kcal for a gram of protein or carbohydrate (Golay and Bobbioni, 1997). In rat and mouse strains that are prone to developing dietary obesity, the degree of obesity strongly correlates with the length of exposure to the high fat diet, the age of feeding initiation (Kanerek and Hirsch, 1977), the type of fat and the fat content of the diet (Hill *et al.*, 1992; West *et al.*, 1995). This is also true in human subjects in both controlled and free-living situations. In a controlled human trial, diets providing 40% of the total calories from fat caused intermediate weight gain while diets with 60% fat content caused greater weight gain. In free-living individuals fed the same diets for 7-14 days, only the diet with 60% fat induced weight gain in normal lean healthy subjects (Stubbs *et al.*, 1995; Prentice, 1998), while those who were fed diets with 20% or 40% fat lost some weight. Although there are conflicting results on whether obese subjects eat more fat than do lean subjects, in monitored situations obese subjects tend to choose more high fat foods than do normal weight individuals when

presented with a variety of foods (Blundell *et al.*, 1993). Obese subjects are reported to have a preference for high fat/high sugar foods versus low fat/high complex carbohydrate foods. Similar patterns have been observed in Zucker obese (fa/fa) rats (Castonguay *et al.*, 1982), obesity-prone Sprague-Dawley rats (Wang *et al.*, 1998) and AKR/J mice (Smith *et al.*, 1997).

Conflicting results are observed when fat intake and BMI in populations are evaluated, however studies that closely followed changing eating habits in certain populations have shown that as the diet changed from a low fat diet to a high fat diet, the BMI in those populations increased (Al-Isa, 1997) and in countries like South Korea where fat intake has not increased following economic growth the rate of obesity is low (Kim *et al.*, 2000). In studies where the obesity rate has increased, in addition to increased dietary fat content, reduced physical activity was reported. Therefore interaction between environmental factors as well as genetic factors can lead to the development of obesity and related conditions (Allison *et al.*, 1995). Although there is strong evidence that high dietary fat plays a major role in the development of obesity in immigrants from countries with low fat intake when they move to the USA (Kato *et al.*, 1973), dietary fat may not play a significant role in well established populations. In the USA, reported fat intake appears to have been reduced in the past 2 decades while the obesity rate is on the rise (Kuczmarski *et al.*, 1994; Willett, 1998), however, fat intake could be under reported in the most surveys similar to under reporting of caloric intake in obese individuals (Tomoyasu *et al.*, 2000). Overeating in America is reported to be associated with increased

restaurant food consumption which is normally high in fat content (McCrory *et al.*, 1999).

1.2.1.1.1. Effects of dietary fat on appetite

One mechanism by which high fat/high energy diets lead to weight gain is by inducing hyperphagia which results in excess energy intake (Gayles *et al.*, 1997). It is also assumed that dietary fat has a lower satiating effect than do carbohydrates and proteins and this can lead to both passive and/or active hyperphagia (Blundell *et al.*, 1994). However, the satiety effect of fat can be influenced by the metabolic fate of fat as well as hormonal status of the individual. Satiety effects of dietary fat are increased if the fat is oxidized instead of being directed to storage in adipose tissue (Parker *et al.*, 1995). Nutrient partitioning depends on the hormonal status, especially insulin concentrations. For example, injection of insulin to normal and diabetic rats will increase fat storage and hyperphagia if the rats are fed a high fat diet (Willing *et al.*, 1994). While increased carbohydrate consumption can lead to increased tissue carbohydrate oxidation, increased fat intake does not stimulate fat oxidation (Horton *et al.*, 1995). Reduction of fat oxidation therefore results in increased fat storage and development of obesity (Gayles *et al.*, 1997; Friedman, 1998).

1.2.1.1.2. Effects of dietary fat on insulin resistance

Insulin resistance is a common feature of obesity. High fat feeding induces

whole body insulin resistance in all target tissues in experimental animal studies (Kraegen *et al.*, 1991; Kim *et al.*, 1996). In rats, insulin resistance in response to high fat feeding occurs before any change in body weight. Dietary fat induces insulin resistance which starts in the liver after only 3 days (d) of high fat exposure (Kraegen *et al.*, 1991). Hepatic insulin clearance and glucose uptake are reduced but lipogenesis is increased (Jeanrenaud, 1985). Insulin resistance then progresses to the skeletal muscles and lastly the adipose tissues are affected. In both skeletal muscles and adipose tissues, glucose uptake and oxidation are impaired (Zierath *et al* ., 1997) and as in the liver lipogenesis is not impaired in the adipose tissue (Jeanrenaud, 1985). In these tissues dietary fat impairs insulin-stimulated glucose uptake by decreasing expression of the glucose transporter (GLUT4) (Kahn, 1994) and impairing its translocation to the plasma membrane thus interfering with signal transduction (Zierath *et al.*, 1997). This early defect in signal transduction in insulin-mediated glucose uptake is shown to be associated with reduced insulin receptor substrate-1 associated phosphatidylinositol 3-kinase activity (Zierath *et al.*, 1997).

The development of insulin resistance in response to high fat diets is also dependent on the free fatty acids (FFA) carbon chain length and the degree of saturation of the dietary fat. Feeding rats a high fat diet rich in long chain polyunsaturated (ω-3, fish oil) FFA or saturated FFA with short carbon chains (less than 14 carbons) did not induce insulin resistance although greater weight gain was induced than with feeding of the diets rich in complex carbohydrates (Kraegen *et*

al., 1986). Diets rich in long chain saturated (tallow), monosaturated (ω-9, olive oil) or polyunsaturated (ω-6, safflower oil) FFA lead to development of insulin resistance in all rat tissues (Storlein *et al.*, 1991). In that study it was also shown that muscle insulin resistance was associated with increased accumulation of stored muscle triglyceride (mTG) (Storlein *et al.*, 1991) and low concentrations of long chain ω-3 FFA in the phospholipid of the skeletal muscle (Storlein *et al.*,1991). The association between high mTG and insulin resistance has also been shown in humans (Pan *et al.*, 1997).

Development of insulin resistance is associated with body fat distribution as well as total adiposity (Carey *et al.*, 1996a). Insulin resistance is more common in individuals with visceral fat accumulation than those with peripheral and/or subcutaneous obesity (Carey *et al.*, 1996b). Visceral obesity is also strongly associated with hypertriglyceridemia and low high density lipoprotein (HDL)-cholesterol (Bonora *et al.*,1998) which increases the probability of developing coronary heart disease (Manninen *et al.*, 1992). Even though the distribution of body fat is genetically determined (Stunkard *et al.*, 1986; Lemieux, 1997), high dietary fat content can contribute to the problem.

1.2.1.1.3. Glycaemic index of nutrients in a diet

Although dietary fat plays a significant role in the development of insulin resistance, the Glycaemic index (GI) of the diet has a contributory role (Liljeberg and Bjorck, 2000). Glycaemic index of the diet predicts the magnitude in the rise

of plasma glucose concentration and insulin response following ingestion of the diet. Glucose is used as the standard with a GI of 100. The Glycaemic index of a mixed meal is influenced by individual components of the diet and the carbohydrate source (Le Floch *et al.*, 1992). Diets that are rich simple carbohydrates have a high GI and also induce high insulin response and low HDL-cholesterol (Ullrich and Albrink, 1989), while those diets with low GI but rich in complex carbohydrates improve glucose tolerance in both healthy (Liljeberg *et al.*, 1999) and diabetic patients (Lafrance *et al.*, 1998). High carbohydrate diets have a tendency to increase plasma triglyceride and cholesterol concentration unless they are supplemented with a high fiber content (Frost *et al.*, 1999). Soluble fibre such as oat bran gum and guar gum decrease both plasma insulin and glucose concentration while non soluble fibre has no effect in NIDDM patients (Braaten *et al.*, 1991; Fairchild *et al.*, 1996). Although a diet with high complex carbohydrate and low fat content improve insulin and glucose responses in NIDDM patients, similar benefits can also be induced by a low calorie diet that is low in carbohydrate and fat but a high protein content (Heilbronn *et al.*,1999).

High protein diets induce less insulin secretion compared to glucose in normal humans (Krezowski *et al.*, 1986) while in NIDDM patients high protein diets elicit a high insulin response (Peters and Davidson, 1993). Short-time exposure to low protein diet in both normal and NIDDM human subjects has no significant effect on basal hepatic glucose production. However reduction of whole body protein catabolism, postabsorptive and daily blood glucose concentration was observed in

both healthy and obese NIDDM patients (Hoffer *et al.*, 1998). In normal rats, feeding low protein diets (less than 10%) results in reduced food intake, fasting plasma glucose and insulin concentrations and abnormal oral glucose tolerance because of lack of pancreatic insulin content (Okitolonda *et al.*, 1987, 1988). However, in Zucker lean rats low protein diets result in an increased caloric intake without affecting caloric intake in fa/fa rats (Specter *et al.*,1995). Also islet size (Tse *et al.*,1997) and insulin secretion was reduced in both phenotypes, but fa/fa rats remain hyperinsulinemic (Tse *et al.*,1995). In rodents and humans a diet which supplies at least 15% of calories as protein maintains normal growth and glucose metabolism and normal pancreatic B-cell function (Colombo *et al.*, 1992; Hoffer *et al.*, 1998).

1.2.1.2. Physical activity

The role of physical activity in energy balance or prevention of obesity is controversial because of the difficulty in monitoring activity as well as in determining the minimal amount and the types of activity, that would be beneficial under ordinary living conditions (Saris, 1996). Compared to reducing caloric intake, the energy deficit induced by exercise is minimal and requires a long time and dedication to see positive results (King, 1999). However, from longitudinal and cross-sectional studies, physically active individuals show lower body weight and reduced risk of death from obesity- and old age-related illnesses (Barlow *et al.*, 1995). Intervention studies by Skender and colleagues showed that even though weight loss due to

exercise alone is very small compared to diet alone or diet in combination with exercise, maintenance of weight loss is better in exercised groups (Skender *et al.*, 1996). A recent survey showed that people who lost weight and have maintained normal weight for over 7 yr were more physically active than those who regained the lost weight (McGuire *et al.*, 1999). Failure of weight loss induced by exercise alone might be due increased caloric intake after physical activity (Scheurink *et al.*, 1999).

1.2.1.2.1. Control of caloric intake and energy expenditure by exercise

The main contributor to energy expenditure is resting metabolic rate (RMR), which is genetically determined (Bouchard *et al.*, 1993). Resting metabolic rate is the energy required to maintain the integrated systems of the body at rest and it accounts for 60-80% of the energy expenditure over 24 hours (h). Resting metabolic rate is reported to be high in physically active individuals and can decline within 3 d of stopping exercise (Tremblay *et al.*, 1988). The remaining 20-40% of total energy expenditure is the thermic effects of food and of exercise (TEF and TEE), which are the energies utilized to process the ingested food and during exercise, respectively. Physical activity increases energy expenditure during exercise by utilizing stored energy (fat) and during rest by conserving lean mass and increasing RMR. During dieting alone, weight loss is comprised of both fat mass and fat free mass. Exercise tends to prevent the loss of the latter (Westerterp *et al.*, 1992). Exercise in combination with diet produces greater weight loss and better weight

management (Eriksson *et al.*, 1997).

One mechanism by which exercise is able to regulate energy balance is by reducing food intake, through exercise's ability to stimulate secretion of corticotropin releasing hormone (CRH) from the hypothalamus. Exercise has been shown to stimulate the secretion of CRH-mediated adrenocorticotropic hormone (ACTH) in rats (Watanabe *et al.*, 1991) and humans (Luger *et al.*, 1991). The anorexic effects of exercise are abolished by administration of the CRH antagonist α-helical CRH_{9-41} to animals before exercise (Richard, 1995). The effects of CRH on regulation of food intake are demonstrated in genetically obese Zucker fa/fa rats (Arase *et al.*, 1989). The CRH effect on food intake is short lived, similar to that induced by exercise (Luger *et al.*, 1991; Cabanac and Morrissette, 1992).

Another mechanism by which exercise increases energy expenditure via CRH is by increasing thermogenesis, which is defective in most genetically obese rodents including Zucker fa/fa rats (Holt and York, 1989). Corticotropin releasing hormone has also recently been shown to mediate the anorexic effects of leptin within the hypothalamus (Uehara *et al.*, 1998).

1.2.1.2.2. Effect of exercise on cardiovascular function

In addition to regulation of energy balance, increased physical activity has other health benefits such as lowering of blood pressure (Dunn *et al.*, 1999; Arvola *et al.*, 1999), and prevention of insulin resistance in healthy individuals (Rogers *et al.*, 1990), in obese non-diabetics (DeFronzo *et al.*, 1987) and in NIDDM patients

(Pedersen *et al.*, 1980). These benefits can be observed with or without any changes in body weight or composition in older persons (Kahn *et al.*, 1990) and with low physical activity intensity (Pescatello and Murphy, 1998). Exercise reduces death from coronary heart disease (CHD) and related illnesses (Després *et al.*, 1995) by reducing plasma lipid concentrations and improving the lipoprotein profile (Després *et al.*, 1991; Després and Lamarche, 1994). Indeed, exercise reduces low density lipoprotein (LDL) and increases HDL-cholesterol (Seip *et al.*, 1995) whether the exercise is performed at low or high intensity (Duncan *et al.*, 1991) and in the absence of concurrent weight loss (Lamarche *et al.*, 1992).

Direct effects of exercise on blood vessels have also been reported in non-diabetic and diabetic patients. Decreased capillary basement membrane thickness is seen in humans (Williamson, 1996). Exercise prevented endothelial dysfunction in Otsuka-Long-Evans-Tukushima fatty (OLETF) rats by increasing nitric oxide (Sakamoto *et al.*, 1998). Exercise also increases blood flow to the exercising muscle during and after an exercise period (Armstrong *et al.*, 1984) by changing vascular resistance (Sexton and Laughlin, 1994) and by altering the response to adrenergic stimulation (Lash, 1998). Exercise also improves vasodilatation, by enhancing relaxation via endogenous and exogenous nitric oxide, and increases endothelial prostacyclin production. The improved control of arterial tone after training could also be attributed to the alleviation of hyperlipidemia and insulin resistance even when hyperinsulinaemia is not eliminated (Arvola *et al* ., 1999).

1.2.1.2.3. Effect of exercise on plasma glucose and insulin

Increased physical activity is recommended as part of the regimen for controlling blood glucose in healthy, overweight and obese people because individuals who are active are less likely to develop NIDDM (Eriksson and Lindgärde, 1991). The Glycaemic benefits can be observed immediately after exercise (Larsen *et al.*, 1997). The use of exercise to prevent development of NIDDM in susceptible individuals is clearly illustrated by the effective prevention of NIDDM development in the OLETF rat which is a model for spontaneous NIDDM. After 17 wk of running on an exercise wheel, the exercised rats had lower body weight and abdominal fat deposits than the control non-diabetic rats and also exhibited normal glucose tolerance (Shima *et al.*, 1997; Man *et al.*, 1997). These effects are in accordance with an increased mobilization of stored fat from the whole body induced by physical activity as has been shown to occur in humans (Balkan *et al.*, 1992; Arner, 1995). Exercise lowers plasma glucose and insulin levels through increased energy expenditure and/or by modification of body composition (Kelly, 1995; Bjorntorp, 1995). Reduced insulin levels in humans are observed during the exercising period (Wasserman *et al.*, 1995) and the reduction is directly related to the intensity and duration of exercise (Larsen *et al.*, 1999; Sigal *et al.*, 1999). Exercise reduces plasma insulin concentrations by suppressing insulin secretion and increasing clearance in healthy humans or NIDDM patients and by increasing insulin clearance in IDDM patients (Tuominen *et al.*, 1997). Exercise increases sympathetic nervous system (SNS) activity, which inhibits insulin

secretion through α_2-adrenergic receptor action while stimulating glucagon secretion through ß-adrenergic receptor action. The increased glucagon in combination with SNS activity in the liver stimulates gluconeogenesis and increases lipolysis and fat oxidation (Wasserman *et al.*, 1993; Kiens *et al.*, 1998). Also, reduced insulin concentrations allow the skeletal muscle to rely on lipid oxidation (Tuominen *et al.*, 1997). Observations that both exercise and reduction of caloric intake have similar effects on insulin secretion and plasma glucose concentrations in NIDDM human patients (Larsen *et al.*, 1997), and in NIDDM OLETF (Man *et al.*, 1997), and prediabetic Zucker diabetic fatty (ZDF) rats (Ohneda *et al* ., 1995) indicate that high insulin secretion is due to excess nutrient availability, glucose inclusive. However, exercise has an added advantage over diet by increasing SNS activity and preventing fat free mass loss thus elevating total energy expenditure (Horton *et al.*, 1998).

1.2.1.2.4. Effects of exercise on skeletal muscle and adipose tissue

Because muscle has a limited supply of stored energy in the form of glycogen, during exercise the increased nutrient requirement is either delivered by increased blood flow or is supplied by stored fat. Indeed exercise increases lipoprotein lipase synthesis and activity in skeletal muscle while decreasing both in adipose tissue thus favoring skeletal muscle fat mobilization (Seip *et al.*, 1995). Cessation of exercise has the opposite effect, favoring fat storage (Simsolo *et al.*, 1993). Exercise increases high density lipoprotein cholesterol (HDL-C) (Ferguson

et al., 1998) and increases FFA oxidation by skeletal muscles (Turcotte *et al.*, 1992). Increased muscle fat oxidation results in reduction of muscle mTG content (Holloszy, 1990). As a result, insulin stimulated glucose disposal is increased since mTG levels are inversely related to insulin action (Pan *et al.*, 1997).

The main observed effect of exercise in the skeletal muscle cells is increased insulin stimulated glucose clearance which is achieved through an increase of GLUT4. Exercise increases GLUT4 gene transcription, protein translation (Neufer and Dohm, 1993) and its translocation (Kennedy *et al.*, 1999) into the plasma membrane. The increased GLUT4 protein induced by exercise returns to control levels within 6 days of stopping exercise (Vukovich *et al.*, 1996). In addition to increasing skeletal muscle GLUT4 activity, exercise can modify body composition by increasing muscle mass (Fabbri *et al.*, 1999). Exercise decreased adipose mass by increasing lipolysis of stored TG and prevention of FFA re-esterification in humans (Hodgetts *et al.*, 1991), through increased lipoprotein lipase (LPL) mRNA expression and activity in skeletal muscle (Seip and Semenkovich, 1998). Rats exercised by swimming had reduced fat cell size and increased glucose uptake and oxidation in fat cells compared to controls (Craig and Foley, 1984). These actions are mediated by increased catecholamines through β-adrenergic receptors (Arner *et al.*,1990). The importance of maintaining a physically active life style in order to prevent the development of NIDDM in diabetes-prone individuals was also demonstrated in these OLETF rats. A period of at least 3 months of continuous exercise was required to prevent the development of

pancreatic changes and it took the same amount of time for the benefits of exercise to disappear after cessation of the exercise program (Shima *et al.*, 1996). Although a physically active life style is recommended to all people, it is more critical for persons who are susceptible to the development of NIDDM.

1.2.1.2.5. Effects of exercise on gene expression

Some effects of exercise on gene expression of enzymes involved in lipid and glucose metabolism are well documented (Griffiths *et al.*, 1993; Fiebig *et al.*, 1997). In the liver, exercise causes differential regulation lipid metabolizing enzymes depending on the type of diet fed. In cornstarch and fructose fed rats, expression of mRNA coding for enzymes involved in lipid metabolism was reduced. These included fatty acid synthase and acetyl CoA-carboxylase (ACC) mRNA. In sucrose-fed trained rats only pyruvate kinase activity was reduced (Fiebig *et al.*, 1997). In high fat diet fed rats exercise increased liver ATP-citrate lyase and malic enzyme (Griffiths *et al.*, 1993). In skeletal muscle both GLUT4 mRNA expression and GLUT4 protein translation are increased (Kuo *et al.*, 1999) and its translocation into the plasma membranes is increased (Kennedy *et al.*, 1999). Exercise also decreased both liver hexokinase and glucokinase mRNA (Zawalich *et al.*, 1982) and muscle hexokinase, pyruvate kinase activities were decreased (Hanissian *et al.*, 1988;). Pancreatic B-cells isolated from exercised rats had reduced expression of proinsulin mRNA, glucokinase mRNA and glucose stimulated insulin secretion (Koranyi *et al.*, 1991).

1.2.2. Genetic factors

Human obesity tends to run in families and ethnic and socioeconomical groups indicating that interaction between genetic and environmental factors is often essential for the expression of obesity (Eck *et al.*, 1992). In experimental animal models, dietary obesity develops in susceptible rodent strains when high fat or high sugar diets are fed (West and York, 1998). Twin and adoptive family studies indicate that there is great variation in the genetic transmission of human obesity, with heritability of BMI, fat mass, skin fold thickness, and leptin levels ranging from 25% to 90% (Stunkard *et al.*, 1986; Allison *et al.*, 1996). Although it is more likely that childhood obesity is genetic in origin (Perusse and Bouchard, 1999), the gradual weight gain that occurs in adults could also be under genetic influence. This is seen in *agouti* mice, *tubby* mice and *fat* mice, which gradually gain weight as they age even when fed normal rat chow (Weigle and Kuijper, 1996). There are only a few examples of single-gene disorders resulting in human obesity. These include Prader-Willi syndrome with defects located on chromosome 15q11-q13 (Mann and Bartolomei, 1999), Bardet-Biedl syndrome due to defects located on chromosome 11q13 (Katsanis *et al.*, 1999) and Alström's syndromes with defects located on chromosome 2p (Collin *et al.*, 1997; Macari *et al.*, 1998). In addition to early development of obesity, unrestrained eating and insulin resistance, the three syndromes are characterized by mental retardation and short stature, while Bardet -Biedl and Alström syndromes are also characterized with retinitis pigmentosa (Collin *et al.*, 1997). Deafness has been reported in Alström syndromes (Collin *et*

al., 1997). The high incidence of human obesity is likely a result of the additive effects of several genes such as the effects of mutations of UCP1 and β_3-adrenergic receptor genes reported in some French Caucasian morbidly obese individuals (Clément *et al.*, 1996b).

Because obesity results from reduced metabolism or an excess of energy intake over expenditure, a gene linked to obesity can be any gene encoding a protein that plays a role in energy homeostasis, from initiating or terminating feeding to nutrient processing. These proteins may include hormones, neurotransmitters, neuropeptides and their receptors that are involved in communications between the periphery and the central nervous system (Clément *et al.*, 1995; Weigle and Kuijper, 1996).

The polygenic nature of human obesity and the interaction of genes with the environment makes identifying the genes responsible for phenotype expression difficult. However, the availability of rodent models of genetic obesity and the knowledge of both human and mouse genomic maps have helped in locating mutations in several genes contributing to obesity. By using both quantitative trait locus (QLT) mapping and positional cloning, murine obesity genes have successfully been located and the impact of homologous genes on human obesity is becoming evident (Roberts and Greenberg, 1996; Comuzzie and Allison, 1998).

Rodent models of obesity with single-gene mutations include the obese (ob/ob) mouse which has a mutation in the leptin gene (Zhang *et al.*, 1994) and the diabetic (db/db) mouse and Zucker (fa/fa) and corpulent (cp/cp) rats which have

mutations of the leptin receptor gene (Friedman and Halaas, 1998). The obesity syndromes of these rodents are characterized by an early onset, hyperphagia, hyperinsulinemia, hyperlipidemia, hypothermia, hypoactivity and varying degrees of insulin resistance (Friedman and Halaas, 1998). The protein leptin is secreted by white adipose tissues in response to the nutritional status and the degree of adiposity of an individual (Cusin *et al* ., 1996). Leptin regulates energy balance by inhibiting food intake and increasing energy expenditure and thermogenesis, thus serving to maintain energy balance by a feedback loop (Ahima *et al.*, 1996; Rouru *et al.*, 1999). To date leptin is the best candidate for the key signal from the peripheral tissues to the central nervous system. Its discovery in 1994 by Zhang and his colleagues has markedly improved our understanding of the regulation of energy balance.

Although plasma leptin levels in humans are positively correlated with the degree of adiposity (Cusin *et al* ., 1996), known polymorphisms of the leptin gene or its receptors have not been observed to lead to obesity (Niki *et al.*, 1996; Maffei *et al.*, 1996), except in a very small number of reported cases (Clément *et al.*, 1998). As with other hormones, a prolonged period of exposure of high plasma leptin concentrations may lead to leptin resistance in the target tissues as observed in obese humans and rodents and this resistance increases with age (Qian *et al.*, 1998). However, long term feeding of a high fat diet also induces obesity and increases plasma leptin levels and eventually leads to leptin resistance in normal mice (Ahren and Scheurink, 1998; Kowalska *et al.*, 1999). Late onset obesity is

seen in the (A^y) agouti mouse, where it is transmitted as a single autosomal dominant trait and is due to ectopic expression of the agouti protein in multiple tissues (Moussa and Claycombe, 1999) and in *fat* (fat) and *tubby* (tub) mice, where obesity is transmitted as a double recessive trait (Weigle and Kuijper, 1996).

DNA polymorphisms in several genes are associated with significant differences in both body fat mass and fat distribution in human beings. These genes include those encoding apolipoprotein B-48 (Saha *et al.*, 1993); apolipoprotein B-100 and D (Vijayaraghavan *et al.*, 1994); uncoupling protein (UCP) (Clément *et al.*, 1996b); the α_2, β_2 and the β_3-adrenergic receptors (Sakane *et al.*, 1997; Kim-Motoyama *et al.*, 1997) and tumor necrosis factor α (TNF-α) (Norman *et al.*, 1995). Lipoprotein lipase (Clement *et al.*, 1995) and cyclic AMP dependent protein kinase regulatory subunit RIIβ mutations in mice were associated with changes in fat storage in mice (Cummings *et al.*, 1996). DNA polymorphisms in the genes of the α-2 and β subunit of Na^+K^+-ATPase are suggested to affect body fat oxidation and therefore the respiratory quotient (Deriaz *et al.*, 1994). Visceral body fat distribution is also associated with DNA polymorphisms in the insulin, apolipoprotein D, and α_2, β_2 and β_3- adrenergic receptor genes (Clément *et al.*, 1996; Kim-Motoyama *et al.*, 1997; Sakane *et al.*, 1997). Thus the presence and interaction of one or several of these genes could lead to accelerated weight gain in an individual.

1.2.3. Neuroendocrine system

1.2.3.1. Central and autonomic nervous systems

Energy balance and feeding behavior are controlled by both external and internal stimuli that are integrated by the central (CNS) and autonomic nervous systems (ANS). Information processing occurs in the hypothalamic nuclei and the surrounding limbic system (Steffens *et al.*, 1988). The hypothalamus directly or indirectly controls activities of many endocrine glands that play important roles in energy homeostasis via secretion of releasing or inhibitory peptides that regulate synthesis and secretion of pituitary hormones. Food intake is said to be controlled mainly by two centres in the hypothalamus (the dual-centre hypothesis): the feeding centre located in the lateral hypothalamic area (LHA) and the satiety centre located in the ventromedial hypothalamic nucleus (VMH) (Bray, 1991; Rohner-Jeanrenaud, 1995). Figure 3 shows the changes in the regulation of food intake induced by injury to the lateral and ventromedial hypothalamic nuclei in rodents.

Lesions in the LHA lead to reduced food intake and wasting (Milam *et al.*, 1982; Bernardis and Bellinger, 1993) while those in the VMH result in hyperphagia and obesity in experimental animals (Bray *et al.*, 1982; Rohner-Jeanrenaud, 1995), (Figure 3). Indirect connections between the VMH and LHA nuclei and the paraventricular nuclei (PVN) occur through the dorsomedial hypothalamus (DMH) (Ter Horst and Luiten, 1987; Luiten *et al.*, 1987). Connections in the hypothalamus running from the LHA to the VMH to the PVN via the DMH play a very significant role in regulating energy homeostasis (Luiten *et al.*, 1987) and neuroendocrine and

autonomic nervous function (Bernardis and Bellinger, 1998). Both the LHA and VMH are linked to the ANS (Bray *et al.*, 1989; Jeanrenaud 1995). Stimulating the LHA increases the activity of the hepatic vagal nerves while decreasing the activity of the splanchnic nerve as is evidenced by increased liver glycogen synthesis (Bernardis and Bellinger, 1993). Stimulating the VMH increases liver glucose production through gluconeogenesis, an action that is due to increased sympathetic nervous system activity via splanchnic nerve, however, vagal output is reduced (Sudo *et al.*, 1991). LHA lesioned rats exhibit increased sympathetic activity in the peripheral tissues (Holt and York, 1988). LHA lesioned rats have increased noradrenaline (NA) turnover and guanosine 5'-diphosphate (GDP) binding to mitochondria in brown adipose tissue (BAT) (Arase *et al.*, 1987) and increased NA turnover in white adipose tissue (WAT), heart and pancreas (Yoshida *et al.*, 1983).

LHA lesioned rats have increased core body temperature, an effect mediated partly through increasing synthesis of cerebral prostaglandin (Lennie *et al.*, 1995; Monda *et al.*, 1996) and increased oxygen consumption (Yoshida *et al.*, 1983). These LHA lesion effects are mediated through cholinergic neuronal activity (Shimazu *et al.*, 1977).The increased SNS activity also leads to a decrease in insulin secretion (Holt and York, 1988). The chronically elevated catecholamine levels (Opsahl, 1977) are responsible for the reduced plasma insulin levels and food intake (Milam *et al.*, 1982) leading to a permanent low body weight (Mitchel and Keesey, 1977).LHA lesioned rats have a blunted circadian rhythm for corticosterone secretion with lack of the increase in corticosterone levels at the onset of the dark period that occurs

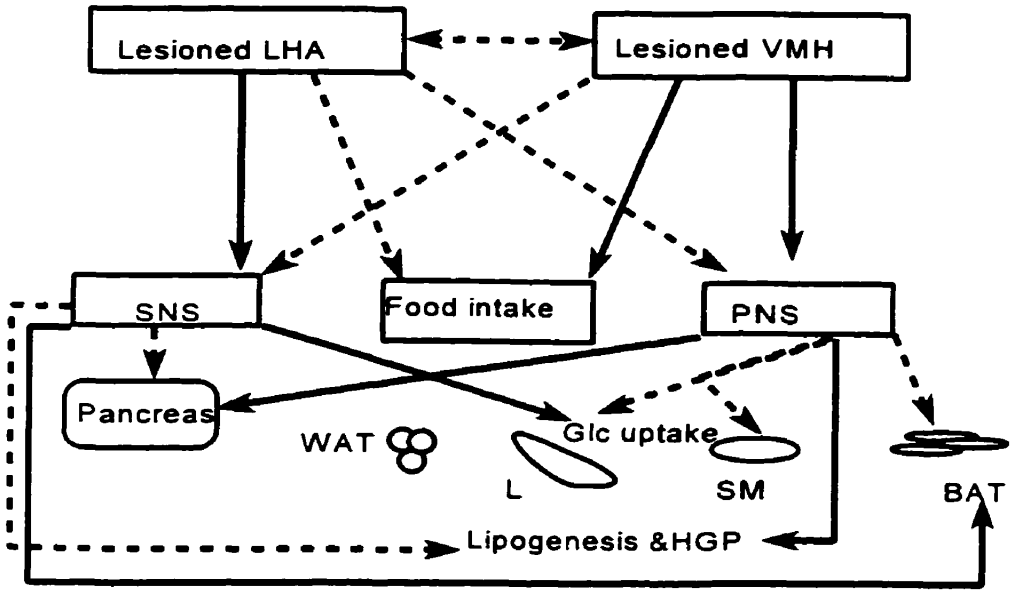

Figure 3. Dual control of energy balance and insulin secretion by lateral (LHA) and ventromedial (VMH) hypothalamic nuclei. - - → decreased, ⟶ increased, sympathetic nervous system (SNS), parasympathetic nervous sysystem (PNS), glucose (Glc), hepatic glucose production (HGP), white adipose tissue (WAT), brown adipose tissue (BAT), skeletal muscle (SM), liver (L). More explanation is in the text. (Modified from Jeanrenaud , 1985; Bray, 1987; 1991; Jeanrenaud, 1995)

in normal animals leads to lack of food seeking ability (Dallman, 1984).

Lesions in the VMH result in obesity with or without hyperphagia in several species including humans (Bray and Campfield, 1975). The immediate observation in VMH lesioned animals is increased plasma leptin (Suga *et al.*, 1999) and oversecretion of insulin and glucagon (Tokunaga *et al.*, 1986) which is corrected by vagotomy or infusion of atropine (Tokunaga *et al.*, 1986) or prevented if vagotomy is performed before the VMH lesions are induced (Cox and Powley, 1981). VMH lesions selectively decrease NE and dopamine in the hypothalamus without affecting serotonin and acetylcholine levels (Takahashi *et al.*, 1995). This leads to an increase in the parasympathetic nervous system activity, but reduced sympathetic nervous system activity in peripheral tissues including BAT (Sakaguchi *et al.*, 1988) where decreased noradrenaline turnover and GDP binding to the mitochondrial membranes are seen (Seydoux *et al.*, 1982). In addition to causing obesity, VMH lesions also disrupt normal circadian rhythms of food intake and energy storage, corticosterone secretion (Egawa *et al.*, 1991a), insulin secretion and lipid handling (Egawa *et al.*, 1984). VMH lesioned rats become obese as a result of ANS-mediated insulin hypersecretion, defective energy expenditure and increased fat accretion, which leads to insulin resistance and abnormal glucose tolerance (Axen *et al.*, 1994) caused by disruption in the neuropeptide Y (NPY) and leptin signaling pathway (Dube *et al.*, 1999).

The metabolic and hormonal abnormalities induced by VMH lesions are similar to those observed in genetically obese rodents, such as ob/ob mice and fa/fa

rats (Jeanrenaud, 1985). Excessive insulin secretion in response to substrates, and reduced BAT thermogenesis are observed in preobese ob/ob mice and fa/fa rat pups (Planche *et al.*, 1983). Because adrenalectomy prevents the development of VMH and genetic obesity, this indicates that both VMH injury (King *et al.*, 1988) and lack of leptin or functional leptin receptors (Chua *et al.*, 1996b) induce abnormal function in the hypothalamo- pituitary-adrenal (HPA) axis.

1.2.3.2. Hypothalamo-pituitary- adrenal axis

The HPA axis is the connection between the CNS, ANS and the periphery. Corticotropin releasing hormone (CRH) and vasopressin (AVP) are synthesized and stored in the cell bodies of neurons mainly located in the PVN and are released in response to internal or external stimuli (Kupfermann, 1991a). CRH and AVP are released into the local hypophyseal-portal blood circulation that drains into the anterior pituitary gland (adenohypophysis) where CRH stimulates the synthesis and secretion of adrenocorticotropic hormone (ACTH) by the corticotropic cells, an action augmented by AVP (Kupfermann, 1991b). ACTH is then released into the general circulation and stimulates the synthesis and secretion of glucocorticoids and mineralocorticoids from the adrenal cortex (Hornsby, 1985; Waterman and Simpson, 1985).

The principal glucocorticoid hormone in rodents (rat and mouse) is corticosterone while in humans and most animals, cortisol is secreted from the zona fasciculata of the adrenal cortex (Hornsby,1985; Waterman and Simpson, 1985). The

principal function of glucocorticoids is the regulation of glucose metabolism, while mineralocorticoids regulate electrolytes and water balance. The HPA axis is under feedback regulation such that low blood corticosterone concentrations signal CRH and ACTH release while high blood glucocorticoid concentrations inhibit CRH and ACTH secretion (Kretz *et al.*, 1999) (Figure 4). However, in some pathophysiological conditions such as chronic stress, the feedback loop may not function as expected (Buwalda *et al.*, 1999). An abnormally regulated HPA axis has been reported in most obese syndromes (Saito and Bray, 1983; Guillaume-Gentil *et al.*, 1990; Plotsky *et al.*, 1992) based on evidence that adrenalectomy (ADX), which disrupts the normal connections of the HPA axis, prevents or retards the development of obesity in these animals (Bray *et al.*, 1989). Glucocorticoid replacement blocks the effects of ADX (Castonguay *et al.*, 1986; Fletcher and MacKenzie, 1988a). The removal of the adrenal gland hormones, especially glucocorticoids, results in events that lead to decreased energy efficiency thus leading to weight loss. This action is centrally mediated because central infusion of glucocorticoids in normal animals leads to the development of obesity and hyperleptinemia, similar to that observed in most genetic obesities (Zakrzewska *et al.*, 1998). Figure 4 shows the shift in ANS activity in the periphery after ADX that leads to reduced food intake and insulin secretion in obesity. In most genetically obese animals such as fa/fa rats, there is increased expression of neuropetide Y (NPY) mRNA (Bchini-Hooft van Huijsduijnn *et al.*, 1993), but reduction of CRH (Timofeeva *et al.*, 1999) in many hypothalamic areas, including the arcuate nucleus and PVN (Beck *et al.*, 1993).

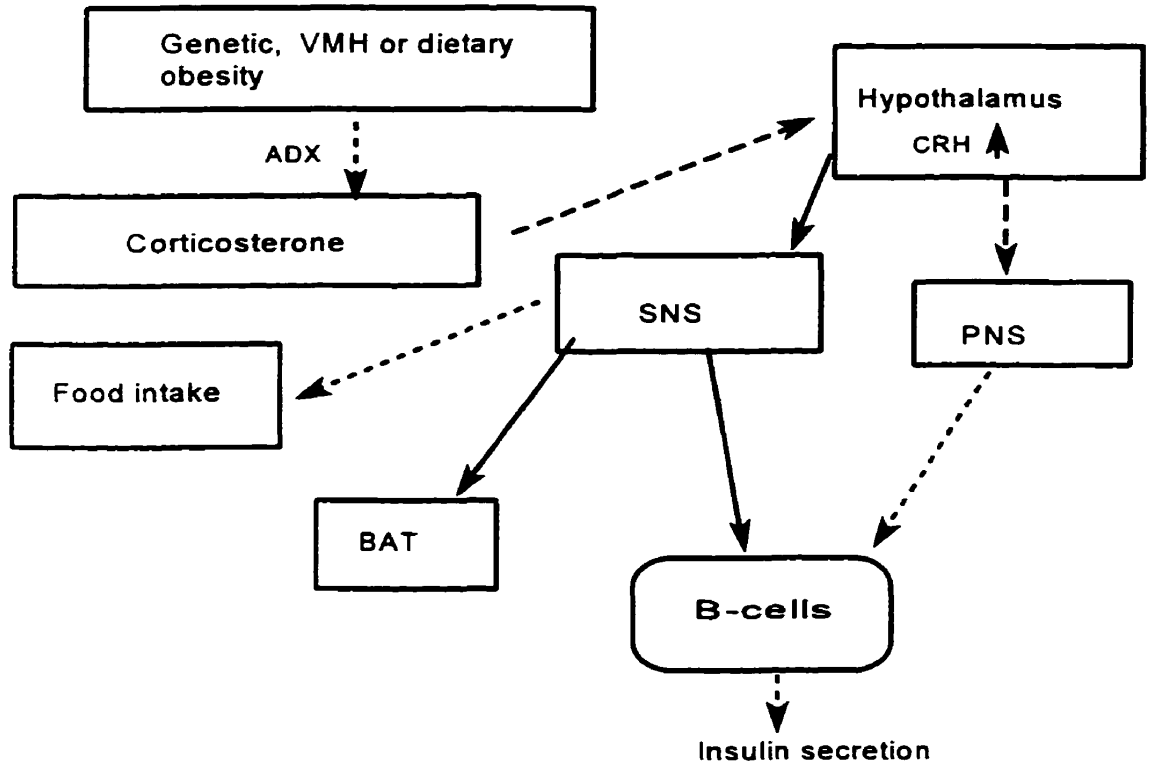

Figure 4. Effect of adrenalectomy (ADX) on food and insulin secretion.Brown adipose tissue (BAT), corticotropin releasing hormone (CRH), sympathetic nervous system (SNS), parasympathetic nervous system (PNS), ventromedial hypothalamic obesity (VMH), ⟶ increased, - - -➤ inhibition . (Modified from Bray, 1987).

Adrenalectomy of fa/fa rats (Plotsky *et al.*, 1992) and leptin administration in ob/ob mice (Huang *et al.*, 1998) reduces the expression of NPY mRNA and synthesis of NPY protein while increasing CRH concentrations in some of these same areas. In some animal models, obesity is due to a lack of leptin protein (obese (ob/ob) mouse), lack of functional leptin receptors (diabetic (db/db) mouse), presence of leptin receptors with reduced activity (Zucker obese (fa/fa) and corpulent (cp/cp) rats), or leptin resistance (dietary obesity) (Friedman and Halaas, 1998). Regardless of the underlying causes of obesity ADX retards or prevents their progression (Bray *et al.*, 1989).

1.2.3.3. Glucocorticoids

Glucocorticoids are necessary for the expression of many obese syndromes as evidenced by the ability of ADX to retard the progression or prevent the development of obesity in many rodent models, depending on the animals' age when ADX is carried out (Fletcher and MacKenzie, 1988a). Adrenalectomy reduces caloric intake, weight gain, plasma lipids, insulin and glucose concentrations and increases sympathetic nervous system activity in BAT in most obese animals. These effects are all reversed by administration of glucocorticoid (King *et al.*, 1992; Feldkircher *et al.*, 1996).

In Zucker fa/fa rats, abnormal HPA axis regulation is demonstrated by the loss of the diurnal rhythm for corticosterone secretion in 5 wk old rats (Fletcher *et al.*, 1986)low CRH concentrations in the portal-hypophyseal blood circulation

(Plotsky *et al.*, 1992), high corticosterone secretion in response to stressful situations (Guillaume-Gentill *et al.*, 1990; Plotsky *et al.*, 1992), increased glucocorticoid binding to its brain receptors (Langley and York, 1992) and increased tissue sensitivity to glucocorticoid (Fletcher and MacKenzie, 1986). However, adult fa/fa rats have plasma corticosterone concentrations that are similar to those of lean rats (Shargill *et al.*, 1987; Kibenge and Chan, 1996).

The inhibitory actions of corticosterone selectively affect CRH regulation of ANS tonicity reaching the periphery, resulting in increased parasympathetic nervous system (PNS) activity and decreased SNS activity, because both ADX and central administration of CRH normalize SNS activity in the periphery (Fletcher and MacKenzie, 1988a; Holt and York, 1989) and glucocorticoid replacement reverses the effects of ADX (Freedman *et al.*, 1986). Glucocorticoid actions on energy regulation have been shown to be centrally mediated (Figure 5), as intracerebroventricular (icv) infusion of low concentrations of the hormone inhibited CRH and induced hyperinsulinemia, hyperphagia and insulin resistance in normal rats (Zakrzewska *et al.*, 1999), and these actions were opposed by leptin (Pralong *et al.*, 1998). Peripheral glucocorticoid administration in normal humans increased energy intake in *ad libitum* fed humans and increased 24-h energy expenditure but reduced noradrenaline concentrations when they were fed a maintenance low fat diet (Tataranni *et al.*, 1996). Within 2 d of peripheral administration of dexamethasone, abnormal oral glucose tolerance without decreased insulin sensitivity in target tissues was also observed in this and another study (Tataranni

et al., 1996; Schneiter and Tappy, 1998).

Glucocorticoids can induce weight gain by reducing energy expenditure through inhibition of the uncoupling protein (UCP-1) in BAT, increasing lipid storage in both BAT and white adipose tissue, and increasing energy intake (Strack *et al.*, 1995a). Glucocorticoids antagonize insulin in some pathways while augmenting it in others based on the ratio of the two hormones (Strack *et al.*, 1995b; Brindley, 1995). Synergistic activities include synthesis of FFA (Al-Sieni *et al.*, 1989) and increased activity of adipose tissue lipoprotein lipase (Rebuffe-Scrive *et al.*, 1988).. Both insulin and glucocorticoids increase energy storage during the nutrient absorptive period (Brindley, 1995), while glucocorticoids also stimulate insulin release indirectly (Dallman *et al.*, 1993). Centrally and peripherally, glucocorticoids increase NPY mRNA, an effect inhibited by insulin in normal animals (Schwartz *et al.*, 1992; Myrsen *et al.*, 1996). During fasting and recovery from exercise, glucocorticoids, in conjunction with increased sympathetic activity, increase energy mobilization and increase glycogen synthesis (Toode *et al.*, 1993). Prolonged exposure to normal or slightly elevated concentrations of glucocorticoids leads to insulin resistance in both the liver and skeletal muscles through inhibition of insulin stimulated glucose uptake (Weinstein *et al.*, 1995) and to an increase in liver glucose production through gluconeogenesis (Brindley, 1995).

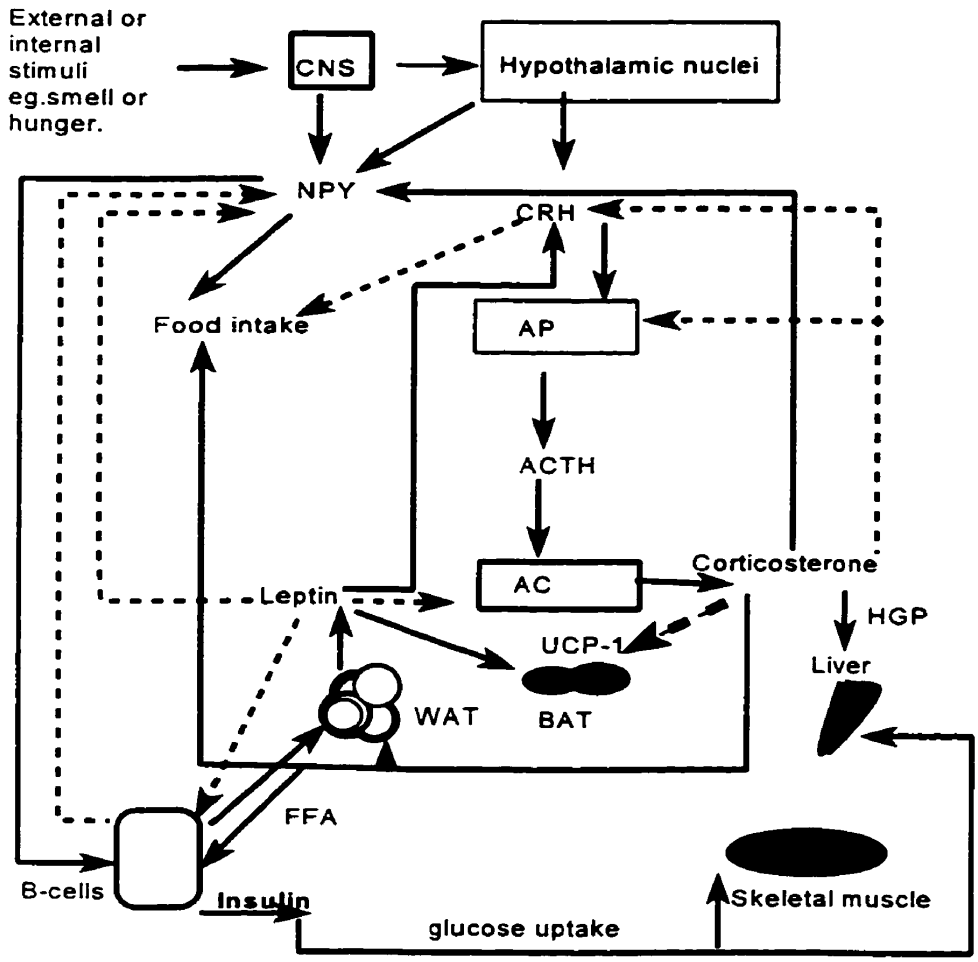

Figure 5. Neuroendocrine regulation of energy balance in normal animals. Leptin, insulin and corticosterone interact with each other and other central and autonomic nervous system neuropeptides to regulate energy balance. Anterior pituitary (AP), corticotropin releasing hormone (CRH), central nervous system (CNS), adrenocorticotropic hormone (ACTH), adrenal cortex (AC), neuropeptide Y (NPY), brown adipose tissue (BAT), white adipose tissue (WAT), uncoupling protein-1 (UCP-1), hepatic glucose production (HGP), → stimulation and ---➤ inhibition.

Although glucocorticoids stimulate leptin mRNA expression (Russell *et al.*, 1998), they oppose the actions of leptin on NPY and CRH mRNA expression in the brain (Zakrewska *et al.*, 1997) and expression of UCP-1 mRNA in the BAT (Arvaniti *et al.*, 1998). Therefore, glucocorticoids promote energy imbalance by opposing leptin and insulin effects in the hypothalamus while augmenting insulin activity in the periphery (Brindley, 1995). Figure 5 summarizes the interaction of hormones, peptides and the nervous system in the regulation of energy balance.

1.2.3.4 Leptin

Genetically obese rodents have defects in the leptin signaling pathway due to the absence of the leptin (Ob) gene or its functional receptors (Friedman and Halaas, 1998). Because all these animal models of genetic obesity exhibit abnormal HPA axis function, leptin is implicated as a HPA axis regulator (Campfield *et al.*, 1995). Leptin is a hormone that is synthesized and secreted by white adipose tissue. It plays a crucial role in the regulation of long term body energy stores. Its importance is demonstrated by its absence in ob/ob mice and the lack of functional receptors in db/db mice and fa/fa and cp/cp rats leading to severe obesity (Friedman and Halaas, 1998). In all the leptin dysfunctional rodents, a similar obese syndrome is seen that is established early and progresses throughout the animals' life span (Bray *et al.*, 1989). All these rodents are severely hyperinsulinemic, hypoactive, hypothermic, insulin resistant and hyperlipidemic (Bray *et al.*, 1989). The functions of leptin are broad, including effects on the reproductive system (Chehab *et al.*,

1997), body temperature, food intake and energy expenditure (Friedman and Halaas, 1998), all of which are normally regulated by the hypothalamic nuclei.

Leptin actions are mediated through receptors belonging to the cytokine family of receptors (Tartaglia *et al.*, 1995), of which five alternatively spliced isoforms have been identified (Tartaglia *et al.*, 1995; Lee *et al.*, 1996). These include Ob-Ra to Ob-Re; Ob-Rb has a long cytoplasmic region containing several motifs required for signal transduction while the other four isoforms lack some of the cytoplasmic domains. The functional Ob-Rb is highly expressed in hypothalamic nuclei including the arcuate nucleus, VMH, LHA, DMH and PVN (Schwartz *et al.*, 1996; Fei *et al.*, 1997). Surrounding areas which are involved in regulating body weight and have neuronal input to the ANS also express leptin receptors (Friedman and Halaas, 1998). Intracerebroventricular injection of leptin in rhesus monkeys suppresses food intake and increases plasma noradrenaline concentrations (Tang-Christensen *et al.*, 1999). Leptin can act centrally to regulate body energy requirements and the activity of the ANS (Wand and Schumann, 1998).

The expression of Ob gene mRNA and protein synthesis is positively regulated by insulin, glucocorticoid (Russell *et al.*, 1998; Spinedi and Gaillard, 1998) and glucose infusion. Leptin gene expression is inhibited by starvation in lean animals but not in genetically or diet-induced obese animals (Trayhurn *et al.*, 1995; Mizuno *et al.*, 1996). In humans plasma leptin concentration is positively correlated with fat mass and plasma insulin concentrations (Caprio *et al.*, 1996). Increased total daily energy expenditure (Nicklas *et al.*, 1997), energy restriction, weight loss (Guven

et al., 1999) and reduction of carbohydrate intake (Jenkins *et al.*, 1997) result in reduced plasma leptin concentrations. In rats leptin resistance increases with age (Qian *et al.*, 1998) and level of adiposity (Cusin *et al.*, 1996). The recent observation that icv. infusion of leptin is able to regulate both food intake and weight gain in VMH lesioned rats (Jacob *et al.*, 1997), fa/fa rats (Zakrezweska *et al.*, 1999) and to a lesser extent in diet-induced obese rats (Widdowson *et al.*, 1997) may offer some hope of its use in treatment of human obesity.

1.2.3.5. Neuropeptide Y

Neuropeptide Y (NPY) is a potent central appetite stimulant and is synthesized in the arcuate nucleus (AC) (Zukowska-Grojec, 1995). The expression of NPY mRNA is increased by glucocorticoids (Larsen *et al.*, 1994; Zukowska-Grojec, 1995), starvation (Tomaszuk *et al.*,1996), lactation (Malabu *et al.*, 1994), exercise (Lewis *et al.*, 1993) and in diabetic animals (Sahu *et al.*, 1990). NPY mRNA is decreased by insulin and leptin administration (Stephens *et al.*, 1995; Mercer *et al.*, 1997) and ADX (Pralong *et al.*, 1993). Concentrations of NPY are elevated in genetically obese rodents such as cp/cp and fa/fa rats (Williams *et al.*, 1992; Bchini-Hooft VanHuijsduijnen *et al.*, 1993) and ob/ob and db/db mice (Stephens *et al.*, 1995). Intracerebroventricular infusion of NPY quickly stimulates food intake and insulin secretion (Zarjeveski *et al.*, 1993) in normal rats (Figure 5) and increases insulin stimulated glucose disposal and glycolytic flux in skeletal muscle without significant effects in subcutaneous adipose tissue (Vettor *et al.*, 1998). Chronically, NPY treated

animals develop insulin resistance in both skeletal muscle and liver and these effects are reversed when NPY infusion is stopped (Vettor *et al.*, 1994). Both exogenous and endogenous NPY stimulation of food intake is specifically mediated through its NPY-subtype Y5 receptors (Schaffhauser *et al.*, 1997) located in the PVN, DMH and AC nucleus (Frankish *et al.*, 1995). Short term icv NPY infusion in rats preferentially increases carbohydrate intake over fat intake (Koopmans and Pi-Sunyer, 1986) while chronic infusion increases intake of both carbohydrate and fat (Stanley *et al.*, 1989). NPY also reduces energy expenditure by reducing sympathetic nerve firing rate in BAT (Egawa *et al.*, 1991b). NPY stimulation of insulin secretion is mediated by increased vagal stimulation in the B-cells (Moltz *et al.*, 1985). Compared to ADX which prevents the NPY-induced obesity (Sainsbury *et al.*, 1997), genetically transmitted obesity of all rodent models, hypothalamic obesity (Bruce *et al.*, 1982) and dietary obesity (Bray *et al.*, 1989), lack of the NPY gene does not completely eliminate fat accumulation in ob/ob mice (Erickson *et al.*, 1996).

1.2.3.6. Insulin

Plasma insulin concentrations are elevated in all obese animals and humans (Bray *et al.*, 1989; Lanzi *et al.*, 1999). During the feeding period, insulin secretion increases declines during the post absorptive period or fasting (Ito *et al.*, 1997). The secreted insulin regulates the metabolism and storage of all nutrients in virtually all body tissues (Campbell *et al.*, 1992). Chronic excess caloric ingestion leads to weight gain, excess insulin secretion and eventually insulin resistance in target

tissues (Reaven, 1988). Insulin resistance can similarly be induced by persistent insulin infusion in normal individuals (Cusin *et al.*, 1992).

Insulin is a powerful inhibitor of food intake, an action that is centrally mediated through inhibition of NPY expression (Figure 5) (Figlewicz *et al.*, 1986; Schwartz *et al.*, 1992). Central infusion of insulin in normal animals in the fed state reduces food intake and weight gain (McGowan *et al.*, 1992; Figlewicz *et al.*, 1995) and increased, ^{3}H-GDP binding to innervated BAT (Muller *et al.*, 1997), effects which can be eliminated by administration of insulin antibodies (McGowan *et al.*, 1992). Intravenous and subcutaneous infusions of insulin also lead to reduced caloric intake as long as the dose is kept low enough not to induce hypoglycemia (Woods *et al.*, 1984). The ability of insulin to inhibit food intake is evidenced by the hyperphagic nature of insulin-deficient diabetic animals (Porte *et al.*, 1988). However, in genetically obese fa/fa rats, which are insulin and leptin resistant, infusion of insulin failed to inhibit food intake or synthesis of NPY (Figlewicz *et al.*, 1996). Peripherally secreted hormones such as insulin, leptin and corticosterone interact with neuropeptides like NPY, CRF, norepineprine, dopamine and seratonin in the central nervous to regulate energy balance and any defect in one pathway could adversely affect the activity of the others (Rosenbaum *et al.*, 1997; Bray and York, 1998).

Insulin induces its biological action after binding to its receptor on plasma cell membranes in target tissues. The insulin receptor is a heterotetrmeric transmembrane glycoprotein with a molecular weight of 350-440 kilodaltons (kDa) and belongs to a family of tyrosine kinases (Lee and Pilch, 1994). It is found in

virtually all body cells at different concentrations ranging from 100 to 200,000 per cell. Insulin receptor is composed of two α-subunits (135 kDa) which are linked to two β -subunits (95kDa) and to each other by disulfide bonds (Lee and Pilch, 1994). The α-subunits, which are entirely extracellular, contain insulin binding sites while the β-subunits are transmembrane proteins that contain the tyrosine kinase activity (Yip *et al.*, 1991; Luo *et al.*, 1999).

In the peripheral tissues, insulin receptors are abundant in hepatocytes, adipocytes, and muscle cells where insulin regulates glucose, fat and protein metabolic pathways. The central nervous system expresses insulin receptors of lower molecular weight, therefore having different structure and probably function from those in peripheral tissues (Heidenreich *et al.*, 1983; Ciaraldi *et al.*, 1985). Central insulin receptors have been located in hypothalamic areas such as the arcuate nucleus, PVN, hippocampus, olfactory bulbi, amygdala and in autonomic brain stem areas (Heidenreich *et al.*, 1983; Unger and Betz, 1998) and these areas are linked to body energy regulation (Melnyk and Martin, 1985).

Peripheral insulin resistance is a common phenomenon in obesity and diabetes (Bjorntorp , 1997). In animals or humans may be caused by defects in the insulin secretion (Okamoto *et al.*, 1986; Haffner *et al.*, 1996), by reduced receptor numbers (Migdalis *et al.*, 1996), or by postreceptor defects like reduced tyrosine kinase activity (Slieker *et al.*, 1990) or reduced insulin receptor substrate (IRS-1) activity (Saad *et al.*, 1992). Abnormal or reduced dissociation of insulin:insulin receptor complexes observed in some non diabetic obese and NIDDM obese

patients might account for the reduced insulin receptors and activity in skeletal muscles of these obese individuals (Goodyear *et al.*, 1995). High fat diets induce insulin resistance by reducing insulin receptor activation of phosphatidylinositol 3-kinase in skeletal muscle and adipose tissue but not in liver tissues (Anai *et al.*, 1999), a similar defect observed in many tissues including blood vessels of fa/fa rats (Jiang *et al.*, 1999).

Insulin content and binding capacity is dependent on the nutritional state of the animal in that both activity and content are increased in the hypothalamic nuclei in the fed state but reduced in the starved state. Maximum insulin binding to the hypothalamic tissue is correlated with pancreatic islet responsiveness to glucose stimulation, which is low during weight loss period, indicating that insulin is involved in central regulation of feeding and body weight (Melnyk and Martin, 1985; Gerozissis *et al.*, 1999). Variability of central nervous insulin activity has also been reported in fa/fa rats. Reduced insulin binding in the hypothalamic tissues with no change in the cerebral cortex receptors was reported in young male fa/fa rats (Melnyk, 1987). Probably the presence of "fa" gene influences the insulin binding activity, since brain and liver reduced insulin binding have also been observed in non obese Fa/fa and Wistar Kyoto rats (Baskin *et al.*, 1985; Figlewicz *et al.*, 1986). However, both insulin binding and binding sites were found to be increased in arcuate and dorsomedial hypothalamic nuclei in adult female fa/fa rats (Wilcox *et al.*, 1989). The reduced central insulin binding correlates with reduced insulin concentrations in these areas (Baskin *et al.*, 1985; Melnyk, 1987). The low insulin

concentration in the brain is as a result of peripheral hyperinsulinemia and insulin resistance of these rats, which causes defects in the transportation mechanism since reduced insulin binding to its blood- brain capillary insulin receptors is observed in fa/fa rats (Schwartz *et al.*,1990). Binding is normalized after reduction of plasma insulin concentration by treatment with diazoxide (Alemzadeh and Holshouser, 1999).

Defects in insulin secretion regulate central insulin action in the central nervous system including HPA axis activity. Insulin infusion in normal human subjects lead to reduced CRH (Walker *et al.*, 1994) but maintenance of high cortisol and ACTH secretion, indicating that hyperinsulinemia regulates the HPA axis centrally at the hypothalamic level and peripherally at the adrenal cortical level (Fruehwald-Schultes *et al.*, 1999). Central insulin resistance in obesity at least in fa/fa rats is manifested in similar manner as that in the peripheral whereby hyperinsulinemia precedes reduced insulin receptor activity (Marfaing-Jallat *et al.*, 1992; Alemzadeh and Holshouser, 1999).

1.3. Regulation of insulin secretion (Figure 6)

1.3.1. Insulin biosynthesis and secretion

Insulin biosynthesis and secretion by pancreatic B-cells are physiologically regulated by glucose (Sjoholm, 1997; Wang *et al.*, 1997), but can be modulated by other nutrients, hormones and neurotransmitters (Efendic *et al.*, 1991). Glucose (Scruel *et al.*, 1999) is the major physiological stimulant of insulin secretion following

its transport into the B-cell and metabolism through the glycolytic pathway and Krebs' cycle. As a consequence of glucose metabolism, ATP is produced which closes the ATP-sensitive potassium (K_{ATP}) channels, which in turn depolarizes the B-cell plasma membrane (Cook *et al.*, 1991). The depolarization opens the voltage-gated calcium channels. Extracellular calcium then enters the cell, and activates Ca^{2+}-calmodulin dependent proteins which regulate the insulin secretory machinery (Efendic *et al.*, 1991). This is referred to as the classical mechanism. However, glucose can stimulate insulin release independent of K_{ATP} channels (Gembal *et al.*, 1993; Chan and MacPhail, 1996), likely via the guanine triphosphate (GTP)-dependent (Meridith *et al.*, 1995; Vadakekalam *et al.*, 1997) protein kinase C and protein kinase A pathways (Aizawa *et al* ., 1994; Tian *et al* ., 1996). The glucose action on B-cells not involving closure of K_{ATP} channels is observed when the pancreatic B-cell membrane potential is clamped at a depolarized level using diazoxide and high K^+ concentrations so that glucose could not induce any electrophysiological changes (Sato and Henquin, 1998). Also insulin secretion in the presence of 12-O-tetradecanoylphorbol-13-acetate (TPA), a protein kinase C agonist, and forskolin, an activator of adenylate cyclase were augmented by physiological and supraphysiological glucose concentrations in the absence of intracellular calcium elevation (Komatsu *et al.*, 1995).

Another mechanism by which glucose induces insulin secretion via a non-ionic pathway is by increasing cytosolic malonyl CoA (Prentki and Corkey, 1996).

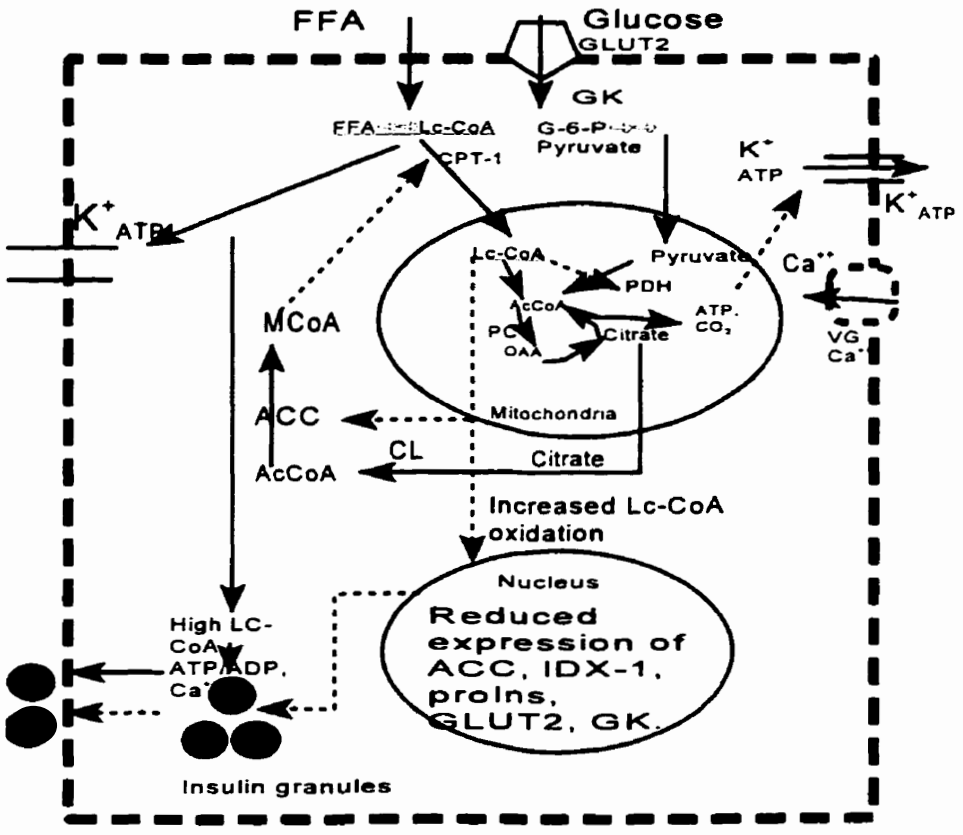

Figure 6. Interaction between FFA and glucose metabolism in B-cells. Increased FFA oxidation results in the inhibition of several enzymes necessary for glucose metabolism resulting in GSIS. ➔, increased,----➤decreased metabolism, acetyl CoA carboxylase (ACC), pyruvate carboxylase (PC), citrate lyase (CL), carnitine palmitoyltransferase-1 (CPT-1), malonyl-CoA (MCoA), glucose transporter- 2 (GLUT2), oxaloacetate (OAA), long chain acyl CoA (LC-CoA), acetyl CoA (AcCoA), glucose 6-phosphate (G-6-P),Voltage gated calcium channels (VG Ca^{++}), ATP sensitive potassium channels (K_{ATP}), glucokinase (GK), pancreatic/duodenal transcription/ translation factor (IDX-1), proinsulin (ProIns). Modified from McGarry and Dobbins, 1999)

Increased malonyl CoA inhibits activity of carnitine-palmitoyltransferase-1 (CPT-1) an enzyme required for the metabolism of long- chain FFA as shown in section 1.3.3 (Figure 6). The inhibition of CPT-1 results in accumulation of cytosolic long-chain free fatty acyl Coenzyme A (L-C acyl CoA) in the B-cells, which then augments basal insulin secretion and glucose-stimulated insulin secretion (GSIS) (Prentki and Corkey, 1996; Komatsu *et al.*, 1998; McGarry and Dobbins, 1999). However, increased FFA metabolism by the B-cells or insulin secreting cell lines results in the inhibition of acetyl CoA carboxylase, an enzyme that catalyzes the synthesis of malonyl CoA. With reduced malonyl CoA synthesis, an increase of CPT-1 activity would lead to increased ß-oxidation of long chain FFA (Zhou *et al.*, 1994) resulting in reduced glucose metabolism and GSIS (Zhang and Kim, 1998).

1.3.2. Glucokinase

Metabolism of glucose by the pancreatic islet B-cells initiates events that lead to insulin secretion (Malaisse *et al.*, 1976; Zawalich, 1979). The first rate limiting reaction in the metabolism of glucose is the phosphorylation of glucose to glucose-6-phosphate as it enters the B-cells, which is catalyzed by a low K_m hexokinase and a high K_m glucokinase (Malaisse-Lagae and Malaisse, 1988). In contrast to hexokinase, which phosphorylates other hexose sugars and is the predominantly isoenzyme in exocrine cells and in islet cell populations not including B-cells (Schuit *et al.*, 1999), glucokinase has high specificity for glucose (Xu *et al.*, 1995) and is predominantly located in the islet B-cells (Jetton and Magnuson, 1992). Also unlike

hexokinase, glucokinase is not inhibited by its end product glucose-6-phosphate), thus enabling continuous phosphorylation of glucose at physiological glucose concentrations (Giroix *et al.*, 1984). The two tissues where glucokinase expression and activity have been detected are pancreatic islets and hepatocytes (Matschinsky, 1990). Glucokinase and GLUT2 are reported to make up part of the primary pancreatic islet B-cell glucose sensor and the pacemaker for the liver (German 1993; Matschinsky, 1996). Glucose metabolism by islet B-cells leads to insulin secretion while in the liver glucose phosphorylation facilitates glucose removal from the circulation (Magnuson, 1992). Both the liver and the endocrine pancreas play very significant roles in whole body glucose homeostasis (Magnuson, 1990).

Because of the presence of two separate promoters on a single gene, glucokinase gene expression in the liver and the pancreatic B-cells are regulated differently. The downstream promoter is used in the liver while the upstream promoter is used by the B-cells (Magnuson and Shelton, 1989). In the liver, glucokinase expression and activity are positively regulated by insulin and tri-iodothyronine but negatively regulated by glucagon (Bedoya *et al.*, 1986; Iynedjian *et al.*, 1989; Narkewicz *et al.*, 1990; Nouspikel and Iynedjian, 1992). In pancreatic B-cells, glucose regulates the post-transcriptional expression and activity of glucokinase (Liang *et al.*,1992; Tiedge and Lenzen, 1995). Glucokinase activity in the pancreatic islets increases in the presence of high glucose concentrations and decreases in the presence of low glucose concentrations (Liang *et al.*, 1992; Purrello *et al.*, 1993). Starvation reduces plasma glucose concentrations thus reducing

glucokinase, proinsulin, voltage-gated calcium channels, GLUT2 mRNA expression (Iwashima *et al.*, 1994), activity of glucokinase and insulin secretion (Burch *et al.*, 1981). This should be expected since glucokinase activity, insulin biosynthesis, and insulin secretion are all regulated by glucose (Philippe *et al.*, 1994).

The importance of liver and pancreatic B-cell glucokinase in the regulation of glucose metabolism, insulin secretion and prevention of diabetes is shown by the finding that mutation of the glucokinase gene results in maturity-onset diabetes of the young (MODY) (Stoffel *et al.*, 1992). Also, knockout mice for the pancreatic glucokinase gene (Terauchi *et al.*, 1995) or both islet B-cell and liver glucokinase (Postic *et al.*, 1999) develop severe hyperinsulinemia and diabetes and die within a week of birth while those that are globally heterozygous for the glucokinase gene (for both liver and B-cells) or only for the pancreatic B-cell glucokinase gene are moderately hyperglycemic. Those without the liver glucokinase gene are mildly hyperglycemic and display defects in glycogen synthesis and glucose turnover rates and impaired GSIS during hyperglycemic clamp (Postic *et al.*, 1999). Replacement of the B-cell glucokinase gene reverses the hyperglycemia and prevents death (Grupe *et al.*, 1995). Also, increased human hepatic glucokinase expression in mouse livers reduced fasting plasma glucose, insulin and lactate concentrations and reduced the body weight of transgenic mice (Hariharan *et al.*, 1997).

Reduction of glucokinase protein or its activity leads to reduced insulin secretion by the pancreatic B-cells or reduced glucose clearance by the liver, thus resulting in hyperglycemia (Barzilla and Rossetti, 1993; Clément *et al.*, 1996). A

moderate increase in glucokinase gene expression and glucokinase protein activity (Wang *et al.*, 1998) results in increased glucose usage by the pancreatic islet B-cells leading to higher GSIS, which in turn increases the rate of glucose clearance by the liver (Becker *et al.*, 1996). However, over-expression of glucokinase gene results in higher glucokinase activity in insulinoma cells (INS-r3-GK27) and reduces GSIS in response to high glucose concentrations (Wang and Iynedjian, 1997). The cells over-expressing glucokinase show a high capacity for accumulating glucose-6-phosphate, reduction of ATP and reduced viability. In order for loss of cell viability to occur, both overexpression of glucokinase and high glucose concentration are required since cells with overexpressed glucokinase maintained at low (2.5 mM) glucose concentrations or normal cells maintained at high glucose (24 mM) concentrations remain normal (Wang and Iynedjian, 1997). Therefore both increased and reduced islet B-cell glucokinase activity can lead to expression of NIDDM.

Because high concentrations of a given hormone tend to down regulate its receptor to protect tissues from over-stimulation (Darnell *et al.*, 1991), excess insulin secretion results in insulin resistance in target tissues, a common phenomenon observed in obesity and NIDDM (Jeanrenaud, 1985; McGarry, 1992). Also insulin is capable of inhibiting its own biosynthesis at the transcriptional level through a negative feedback inhibitory action on its own gene expression, such that prolonged hyperinsulinemia leads to suppression of normal islet B-cell responses to stimuli (Koranyi *et al.*, 1992b), thus contributing to development of NIDDM.

Glucose-stimulated insulin secretion can be blocked by inhibitors of glucose

metabolism such as mannoheptulose (Scruel *et al.*, 1998), starvation (Burch *et al.*, 1981) or increased ß-oxidation of FFA (Zhou *et al.*, 1996b). Mannoheptulose (a 7-carbon non-metabolizable sugar) inhibits GSIS by competing with glucose as a substrate for glucokinase (Scruel *et al.*, 1998), however, it does not block glucose-induced increase in glucokinase content in human pancreatic islets (Gasa *et al.*, 2000). Glucose-stimulated insulin secretion from pancreatic islet cells of cp/cp and fa/fa rats is refractory to inhibition by fasting and mannoheptulose (MH) as compared to GSIS from lean rat pancreatic islet cells (Timmers *et al.*, 1992; Chan *et al.*, 1995; Kibenge and Chan,1995). A reduced MH response is observed in 5 wk old fa/fa rats which is totally lost by the time the rats reach adulthood (Kibenge and Chan, 1995). Increased GSIS was observed in perfused pancreases of 2 d old fa/fa rat pups (Atef *et al.*, 1991) and was later confirmed in 17 d old pre-obese rat pups (Rohner-Jeanrenaud *et al.*, 1983). Also at 2 d of age defective brown adipose tissue (BAT) thermogenesis was observed (Moore *et al.*, 1985). Although reduced SNS and increased PNS activity are evident in genetic and hypothalamic obesity (Jeanrenaud 1985), the early increased response to glucose in pancreatic tissue of these animals could also be due to abnormal glucokinase activity since glucokinase mRNA expression can be detected in the neonatal pancreatic islets from rats as young as 2 d old (Tiedge and Lenzen, 1993) and is similarly regulated by glucose as in islets from adult rats (Tu *et al.*, 1999).

1.3.3. Effects of FFA in pancreatic B-cells

Because most obese humans and animals have high concentrations of fasting FFA (Elks, 1990; McGarry, 1992), lipid regulation of insulin secretion has stimulated great interest. Feeding high fat diets to experimental animals raises their plasma FFA, TG and insulin concentrations (Bray *et al.*, 1992; Buettner *et al.*, 2000), and total body insulin resistance (Kraegen *et al.*, 1991). The inhibitory effect of lipid availability on glucose oxidation was first recognized by Randle *et al.* (1963) in heart muscle; however, this effect has recently also been demonstrated in skeletal muscle (Boden *et al.*,1994) and has also been observed to occur in pancreatic islets by inhibiting pyruvate dehydrogenase (Lee *et al.*, 1994).

In vitro and *in vivo* studies have shown that short term exposure of pancreatic islets from normal animals to high concentrations of FFA renders them hypersensitive to both low and high glucose stimulation (Elks, 1993; Milburn *et al.*, 1995; Hosokawa *et al.*, 1996). This state is common during the dynamic phase of weight gain (McGarry, 1992). Prolonged exposure of islets to high concentrations of FFA reduces insulin secretion at high glucose concentrations while maintaining basal hypersecretion (Lee *et al.*, 1994), a phenomenon observed in glucose intolerant animals (Lee *et al.*, 1994; Hirose *et al.*, 1996). Figure 6 summarizes the interaction between FFA and glucose metabolism in the B-cells.

The mechanism by which FFA increase basal insulin secretion is partly attributed to enhancement of hexokinase activity (Milburn *et al.*, 1995; Hirose *et al.*, 1996; Cockburn *et al.*, 1997), and increased basal insulin secretion is observed with

over-expression of hexokinase I in pancreatic islets (Becker *et al.*, 1994). However, the chronic effects of FFA on GSIS in the islets are thought to be due to increased FFA utilization and inhibition of glucose metabolism (Chen *et al.*, 1994) because inhibitors of long-chain FFA oxidation such as 2-bromostearate and etomoxir eliminated the inhibitory effects of FFA on GSIS (Chen *et al.*, 1994; Zhou *et al.*, 1996a).

Long-chain FFA inhibit synthesis and activities of key enzymes involved in glucose metabolism in pancreatic islet B-cells (Figure 6). Long chain FFA increases the activity of pyruvate dehydrogenase kinase which in turn phosphorylates pyruvate dehydrogenase (PDH) thus rendering it inactive (Zhou *et al.*, 1996b). Acetyl-CoA carboxylase gene expression is also inhibited by long chain FFA (Brun *et al.*, 1997). Enzymes involved in lipid metabolism are enhanced. FFA induced CPT-1 gene expression in the pancreatic B-cell line INS-1 (Assimacopoulos-Jeannet *et al.*, 1997) and pancreatic lipase gene expression and activity are increased in the pancreatic tissues (Wicker and Puigsever, 1990). In pancreatic B-cells and INS-1 cells, FFA also stimulated K_{ATP} and calcium channels (Warnotte *et al.*, 1994; Larsson *et al.*, 1996), rendering the cell membrane insensitive to changes induced by glucose's depolarizing action.

Long chain acyl CoA are linked to inhibition of glucokinase activity in the liver (Tippet and Neet, 1982), and their involvement in the inhibition of acetyl CoA carboxylase may be due to their action on glucokinase activity in clonal B-cell lines (Brun *et al.*, 1997). This is evidenced by a 78% reduction of glucokinase mRNA and

protein in B-cells treated with palmitic acid (Gremlich *et al.*, 1997). Chronic feeding of a high fat diet to rats or mice reduces proinsulin, glucokinase and GLUT2 mRNA and reduces GSIS (Capito *et al*,. 1992; Kim *et al.*, 1995).

The depression of gene expression in pancreatic cells has been traced to reduction of the transcription and translation of the duodenal and pancreatic B- and D- cell transcription factor IDX-1 (Gremlich *et al.*, 1997) in the presence of high FFA concentrations. IDX-1 is a 283-amino acid transcription factor protein that recognizes a TAAAT(T/G) sequence present in the promoters of insulin (Petersen *et al.*, 1994) and somatostatin genes. In B cell-derived MIN6 cells, suppression of IDX-1 gene expression using antisense oligodeoxynucleotide failed to decrease insulin, glucokinase and islet amyloid polypeptide gene transcription (Kajimoto *et al.*, 1997). This might indicate that in addition to suppression of pancreatic IDX-1, FFA may act through other mechanisms, not yet clear, to suppress the expression of several pancreatic genes involved with insulin secretion.

The necessity of FFA for GSIS after prolonged fasting in humans (Dobbins *et al.*, 1998), rats (Stein *et al.*, 1996) and mice (Fernandez and Valdeolmillos, 1998) illustrates the beneficial effects of FFA under some metabolic conditions. When basal plasma FFA concentrations are reduced by administering nicotinic acid, both basal and GSIS are inhibited but can be restored by elevation of FFA. This action is attributed to reduction of the pancreatic B-cell cytosolic long-chain-acyl CoA esters required for normal pancreatic islet B-cell function (McGarry and Dobbins, 1999).

1.4. Animal model: the Zucker fa/fa rat

The Zucker obese (fa/fa) rat is hyperphagic, hyperinsulinemic, hypertriglyceridemic, hypothermic, hypoactive, insulin resistant and in most cases infertile (Zucker and Zucker, 1961; Moore *et al.*, 1985; Krief and Bazin; 1991). By 12 days of age they exhibit lower VMH serotonergic activity and higher concentrations of plasma leptin than do lean rats (Routh *et al.*, 1994; Horwitz *et al.*, 1998). The obese syndrome is established early during suckling stages with the presence of increased lipogenic capacity, as indicated by increased lipogenic enzymes in the white adipose tissues (Pènicaud *et al.*, 1991; Phillips *et al.*, 1994). Animals continue to gain weight throughout their adult life (Bray, 1977). Because of the early onset and the mild insulin resistance, Zucker fa/fa rats are used to study the causes of human childhood obesity (Cleary *et al.*, 1980) and the early pathological changes that occur before the onset of overt NIDDM (Jeanrenaud, 1988; Sharfir, 1992).

The syndrome of Zucker obesity is genetically transmitted as an autosomal recessive trait (fa/fa) which arose from the crossing of Merck stock M and Sherman rats (Zucker and Zucker, 1961) . The obese rats are visually identifiable by 4-5 wk of age by their body shape and weight (Zucker and Zucker, 1961). The "fa" gene, which maps to rat chromosome 5, has been shown to be homologous to the mouse diabetic "db" gene that is mapped on mouse chromosome 4 (Truett *et al.*, 1991). Both genes encode for dysfunctional hypothalamic leptin receptors due to missense mutations within the leptin receptor gene (Chen *et al.*, 1996; Lee *et al.*, 1996). In the db/db mouse the mutation creates a new splice donor that inserts a premature stop

codon into the normal receptor gene 3'-end, thus resulting in a short isoform (Ob-Ra) without the second intracellular domain (Chen *et al.*, 1996; Lee *et al.*, 1996). Zucker fa/fa rats express a leptin receptor in which the glutamine at position 269 is replaced by a proline due a missense mutation (Chua *et al.*, 1996b). This receptor isoform is shown to be less abundant on the cell membrane surface, and has reduced leptin binding, defective signaling to the janus kinase-signal transducers and activator of transcription (JAK-STAT) pathway and markedly diminished ability to activate transcription of early growth response gene (*egr-1*) promoter in transfected mammalian cell lines (daSilva *et al.*, 1998). This defective responsiveness explains the early exhibition of hyperleptinemia (Cusin *et al.*, 1995; Horwitz *et al.*, 1998) and the persistence of hyperphagia that accompanies this obese syndrome (Bray, 1979). Despite the defective leptin receptor, it was recently shown that central leptin infusion in fa/fa rats results in reduced food intake and weight loss at a reduced rate (al-Barazanji *et al.*, 1997), or similar to that of lean rats (Wang *et al.*, 1998).

Fasting hyperinsulinemia is one of the main characteristics of fa/fa rats and is observed concurrently with the onset of overt obesity in 5 wk old rats (Kuffert *et al.*, 1988). Glucose-stimulated insulin secretion (GSIS) from preobese rat pancreases is greatly enhanced by parasympathetic nervous system (PNS) stimulation at the very young age of 2 d (Atef *et al.*, 1991).

Possible causes of hyperinsulinemia include factors such as hyperphagia, insulin resistance, overactive entero-insular axis, abnormal regulation by the central nervous system, and pancreatic islet B-cell defects (Kibenge, 1994). Any of these

factors, when chronic, may lead to adaptations in B-cell biochemistry that persist and lead to further disruption of insulin secretion regulation.

Pancreatic islets of fa/fa rats, compared with those of lean rats, are hypertrophic and hyperplastic (Chan *et al.*, 1985) and the enlarged islets were recently shown to be responsible for the increased glucose sensitivity observed in these rats (Chan *et al.*, 1998). However, *in vivo* other factors such elevated plasma FFA and TG (Castonguay *et al.*, 1986), increased pancreatic glucose sensitivity (Kuffert *et al.*, 1988; Chan *et al.*, 1993), abnormal pancreatic glucokinase activity regulation (Chan, 1993; Kibenge and Chan, 1995), and reduced leptin receptor function (Chua *et al.*, 1996; daSilva *et al.*, 1998) may contribute to the persistent insulin hypersecretion exhibited *in vitro*.

Zucker fa/fa rats exhibit an abnormally regulated HPA axis (Guillaume-Gentil *et al.*, 1990; Plotsky *et al.*, 1992) and ADX of preobese fa/fa rats completely prevents the development of obesity, while ADX of young adults normalizes most of the metabolic, behavioral, and hormonal abnormalities of these animals (Fletcher and MacKenzie, 1986; Castonguay *et al.*, 1986). Glucocorticoid replacement reverses ADX effects (Fletcher and MacKenzie 1986; Castonguay *et al.*, 1986; Fletcher and MacKenzie, 1988). The implications of abnormal HPA axis regulation include increased PNS and reduced SNS activity (Fletcher and Mackenzie, 1988a), increased pancreatic B-cell glucose sensitivity and increased glucokinase activity (Kibenge and Chan, 1996).

Pancreatic islet B-cell function of fa/fa rats is normalized by ADX to that of

lean rats by 2 wk post surgery. ADX decreases pancreatic islet glucose sensitivity and restores the reduced MH inhibitory action on GSIS observed in fa/fa rats (Kibenge and Chan, 1996). A regulatory role of the HPA axis in the development and maintenance of hyperinsulinemia and in the control of glucokinase function of fa/fa rats is therefore indicated. Other benefits of ADX include reduced TG, FFA and insulin resistance and reduced food intake and weight gain in the fa/fa rats (Castonguay *et al.*, 1986).

Feeding diets with high fat content increases plasma lipid concentration, induces insulin resistance, increases weight gain (Mantha *et al.*, 1999) and stimulates the HPA axis (Tannenbaum *et al.*, 1997), effects that are similar to those seen with glucocorticoid replacement in ADX animals (Freedman *et al.*, 1986, Mantha *et al.*, 1999). Feeding a high fat diet to ADX adult fa/fa and lean rats was reported to reduce the ADX effect (Bray *et al.*, 1992) . In ob/ob mice, high fat diets reversed ADX effects on energy efficiency and pancreatic islet glucose sensitivity (Kim and Romsos, 1987; Chen *et al.*, 1994).

Pancreatic islet exposure to high FFA both *in vivo* and *in vitro* induces insulin secretion abnormalities associated with obesity and NIDDM, which have been attributed to increased lipid metabolism by the B-cells (Lee *et al.*, 1994). Feeding rats a diet with moderate fat content (20% of total caloric content) (Tannenbaum *et al.*, 1997) and raising plasma FFA by Intralipid infusion (Widmaier *et al.*, 1995) were both reported to stimulate secretion of corticosterone, indicating that a rise in plasma FFA concentrations is able to regulate HPA axis activity. Also it indicates that a diet

which may not cause any ill effects in human (Stubbs *et al.*, 1995) might be harmful to rats.

The actions of ADX on energy regulation and insulin secretion, are due to the removal of inhibitory effects of glucocorticoids on CRH in the hypothalamic nuclei on the modulation of the balance of autonomic nervous system impulses reaching the peripheral tissues (Lehnert *et al.*, 1998; Jacobson , 1999). As a result of ADX an increase in hypothalamic CRH leads to decrease in parasympathetic activity while increasing SNS activity in the peripheral tissues as measured by increased noradrenaline turnover, GDP binding to UCP-1 and increased thermogenesis in the BAT of fa/fa rats (Arase *et al.*, 1988;), ob/ob mice (Vander Tuig *et al.*, 1984) and cafeteria-fed rats (Rothwell *et al.*, 1984). Exercise is a SNS stimulator (Arner *et al.*, 1990) and might produce benefits similar to ADX on pancreatic islet function.

1.5. Hypotheses and objectives

With reports that high plasma FFA concentrations are able to regulate HPA axis function (Tannenbaum *et al.*, 1997; Widmaier *et al.*, 1992; 1995) and pancreatic islet B-cell function (McGarry and Dobbin, 1999), and that feeding a high fat diet reduces effects of ADX in adult fa/fa rats (Bray *et al.*, 1992) and abolishes them in ob/ob mice (Kim and Romsos, 1987; Chen *et al.*, 1994), we hypothesized that:

1. Post-surgical feeding of a high fat diet would negate the previously identified beneficial effects of ADX (Kibenge and Chan, 1996) on pancreatic islet B-cell function in fa/fa rats and would induce adaptive changes in pancreatic islets

from lean rats.

2. Since ADX effects are thought to as a result of increasing the activity of SNS in the periphery, and exercise is an activator of SNS, regular exercise would produce similar pancreatic islet B-cell benefits as does ADX in fa/fa rats. Further, that a high fat diet would have the same effects on exercised and ADX rats.

3. ADX, exercise and diet would produce their effects on the pancreatic islet B-cell function in fa/fa rats via key regulatory enzymes including glucokinase or by regulating FFA oxidation levels.

The objectives of the study were:

a. To determine whether time was a major factor on the pancreatic islet functional changes after ADX by keeping the animals for 5 wk instead of 2 wk after surgery.

b. To investigate the interaction between ADX and dietary fat on pancreatic islet B-cell function in Zucker rats.

c. To investigate effects of ADX and dietary fat on pancreatic islet FFA oxidation and islet TG content.

d. To determine whether physical activity would have similar effects on pancreatic islet function as those produced by ADX in both rat chow and high fat fed Zucker rats.

e. To investigate the interaction between exercise and dietary fat effects on pancreatic B-cell function.

2. GENERAL METHODS AND MATERIALS

2.1. Experimental animals

Zucker lean and obese 5 wk old female rats were either obtained from Charles River Laboratories (St. Constant, Que) or bred at the Atlantic Veterinary College (AVC) (University of Prince Edward Island, Charlottetown PEI). Animals bred at the AVC were routinely weaned and sexed at 21 d, and only females were used in these studies. All animals were fed Purina rat chow and tap water *ad libitum* until they were put on their respective diets. In additional to the regular tap water the ADX and sham-operated control rats had free access to saline (0.9% NaCl)/4% sucrose solution to prevent electrolyte imbalance due to absence of mineralocorticoid (see Chapter 3). During the study all animals were housed individually in an artificially lit room with a 12 h dark/light cycle at a temperature of 22-25°C. All protocols were approved by the local Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

2.2. Diet composition

The high fat diet was made by mixing ground rat chow, Eagle's brand sweetened condensed milk and Mazola corn oil in the ratio of 4.25:3.96:0.75 by weight according to Triscari *et al.* (1985) and stored at 4°C. The regular Purina rat chow (LF) provided 3.74 kcal/g and 4.5 % fat while the high fat (HD) diet provided 3.96 kcal/g and 15.8 % fat (Triscari *et al.*, 1985) as shown in Table 1. Additional of corn oil and condensed sweetened milk renders the diet a high fat diet and also

diluted out other nutrients, however dietary protein of 14.7% is still with in the normal range for adequate growth and metabolism (Okitolonda *et al.*, 1987). Also this diet had reduced fiber content, which could induce abnormal glucose as well lipid metabolism and vitamin deficiency especially thiamine (Bakker *et al.*, 1998). Fiber content of the diet in humans predicts insulin levels, weight gain, and other CVD risk factors more strongly than total or saturated fat consumption, thus high-fiber diets may protect against obesity and CVD by lowering insulin levels (Ludwig *et al.*, 1999). Together the low dietary fiber, reduced vitamin concentration and high fat content of this diet reflects the unhealthy diets of many human subjects.

2.2.1. Food intake and weight measurement

In the ADX study, all animals were fed rat chow for one wk after surgery and in the exercise study animals were also maintained on rat chow until they were 6 wk old. Animals were then assigned to either high fat or low fat diet and remained on their respective diets for 4 weeks. Daily average caloric intake was obtained by giving rats a known amount of food (g) and then the difference between the amount given and food left on three consecutive days each was converted to kilocalories consumed per rat per day for that week. Animals were weighed once a week. Both food and animal were weighed between 9.00 -10.00 a.m.

Table 1. Diet composition of the low fat (Purina rats chow) and high fat (condensed milk) diet fed Zucker rats (Triscari *et al.*, 1985)

Components	Low fat diet % by weight	High fat diet % by weight
Crude protein	23.4	14.7
Carbohydrate	49	44.2
Lipid	4.5	15.8
Fiber	5.8	2.5
Vitamin mix/ Ash	7.3	1.2
Water	10	19

2.3. Oral glucose tolerance test

After 28-30 days of the experiments , the animals were fasted overnight but had *ad libitum* access to water. The animals were weighed the next day and a 0.3 - 0.5 ml blood sample was collected in a heparinized tube from the tail vein of conscious rats using minimal gentle restraint (this was referred to as 0 min). Glucose (40%w/v solution) at a dose of 1 g/Kg body weight was administered using a gastric feeding tube and blood samples were collected after 10, 20, 30, 40 and 60 min. Plasma was collected by centrifugation and stored at -20°C for insulin, glucose and corticosterone determination.

2.4. Pancreatic islet studies

2.4.1. Islet isolation and culture

Immediately after the last blood collection the animals were anesthetized by intraperitoneal injection with sodium pentobarbital (65 mg/kg body weight). Before the pancreas was removed a large sample of blood (3 ml) was collected by cardiac puncture, processed as above and stored at -70°C until assayed for FFA and triglycerides (TG). Pancreatic islets were isolated by collagenase digestion and dextran density step gradient, a modification of the method of Van Der Vliet *et al.* (1988) as described by Kibenge and Chan (1995). Siliconized glassware was used to prevent islets from sticking and all other instruments used were sterilized to maintain aseptic conditions.

After opening the abdominal cavity and identification of the pancreas, the bile

duct was ligated at both the distal (duodenal) and the proximal (hilus of the liver) ends. A polyethylene tubing catheter (P50) was used to cannulate the duct distal to the ligature at the hilus of the liver. Through the catheter 10-15 ml of ice cold collagenase type XI (0.32 mg/ml) dissolved in Hank's balanced salt solution (HBSS) (supplemented with 10 mM N-{2-Hydroxyethyl}piperazine-N'-{2-ethanesulfonic acid}(Hepes), 0.2% bovine serum albumin (BSA) and 2 mM L-glutamine) was injected into the pancreas. The collagenase distended pancreas was dissected from the animal, put into a petri dish and chopped into small pieces. About 10-15 ml extra collagenase solution was added and the tissue transferred to a 50 ml flask and incubated in an orbital water bath at 37°C (150 rpm). Because of different sensitivity of lean and fa/fa rat pancreatic tissues to collagenase, the incubation time was adjusted to obtain the maximum islet yield (Kibenge and Chan, 1995) (Table 2). Collagenase digestion was stopped by adding cold HBSS and the digest was centrifuged (Beckman J-6M/E centrifuge, rotor TY JS4.2 at 1500 rpm (463 g) for 5 min at 4°C). The supernatant was discarded and the pellet was resuspended in 25 ml of collagenase solution for a second incubation (Table 2) after which the centrifugation was repeated as above. The pellet was suspended in HBSS and filtered through a 800 μm Nitex screen to remove undigested tissues. The filtrate was centrifuged as above and the supernatant was discarded. The pellet was suspended in 10 ml of 27% dextran and a cushion of 6 ml of 27% dextran was layered below. Two layers of 10 ml of each of 23% and 14% dextran were added on top. This gradient preparation was centrifuged at 1500 rpm (463 g) at 4°C for 15 min resulting

Table 2. Collagenase exposure times (min) of the pancreatic tissues from lean and fa/fa rats.

Treatment	Phenotype	First incubation	Second incubation
Intact/sham	lean	20	10
ADX	lean	22	10
Intact/sham	fa/fa	10	7
ADX	fa/fa	15	10

ADX = adrenalectomized (Chapter 3), Sham = sham operated rats (Chapter 3) and Intact rats = all rats in the exercise study (Chapter 4).

in enrichment of islets in the interface between 23% and 14% dextran. This layer was collected and washed with HBSS by centrifugation for 5 min as above. After the supernatant was discarded the pellet was suspended in HBSS, put in a sterile petri dish, and islets were hand picked under a dissecting microscope. For overnight culture, islets were picked into sterile culture wells containing 1 ml of Dulbecco's modified Eagle's medium (DME) supplemented with 12.5 mM glucose, 10 mM Hepes, 1% antibiotic-antimycotic solution (10,000 units/ml penicillin, 10 mg/ml streptomycin and 25 µg/ml amphotericin B) and 10% calf serum. Islets were cultured overnight in a humidified incubator at 37°C in 95% air and 5% CO_2 atmosphere. For determination of FFA oxidation, 20 islets were picked into 2 ml vials and the experiment was carried out immediately. For determination of pancreatic islet TG content, 100-300 islets were picked and frozen in 50 µl of 2 mM NaCl/20 mM EDTA/50 mM sodium phosphate and stored at -70°C until assayed.

2.4.2. Insulin release

Insulin release was measured by replacing the culture medium with 1.0 ml of fresh DME containing various glucose concentrations (0-25 mM) and 0.1% gelatin. In order to measure the effect of mannoheptulose (MH) (glucokinase inhibitor) on GSIS, concentrations of 1-100 mM MH were added to some samples incubated in the presence of 16.5 mM glucose. Samples were statically incubated for 90 min at 37°C (95% air, 5% CO_2, saturated with water vapor). At the end of 90 min the supernatant was collected by aspiration following centrifugation at 2000 rpm (824 g)

for 5 min at 20 °C . The pellet was boiled for 5 min in 3% acetic acid and together with the supernatant was stored at -20°C until assayed for insulin.

2.4.3. Insulin radioimmunoassay

Immunoreactive insulin was measured using a radioimmunoassay (RIA) employing iodinated ^{125}I porcine insulin as the tracer, which was diluted to give approximately 10,000 cpm per 100 µl. Rat insulin was the standard (150-4800 pM). The insulin antiserum (Gp 02) was raised against porcine insulin in guinea pigs in 1993 in our laboratory according to Makulu and Wright (1971) as described by Chan (1985) and was used at a final dilution of 1:40,000. The standard curve was measured in triplicate while samples were measured in duplicate. The free and bound insulin was separated by centrifugation by dextran-coated charcoal and, after discarding the supernatant, the free insulin was measured using a Packard Riastar gamma counter and lowest detection limit (75 pM) provided by the machine was used as the cut off for our reported values. Due to variability in pancreatic islet size of the obese rats, insulin release was routinely expressed as a percent of total islet insulin content.

2.5. Determination of plasma concentrations of hormones and nutrients

2.5.1. Corticosterone

Corticosterone was determined by double antibody RIA following the kit manufacturer's manual (ICN Biomedical Inc, Montreal, Quebec). The standard curve

(25 -1000 ng/ml), controls of low and high corticosterone concentrations (78-112 ng/ml and high 517-777 ng/ml), respectively and samples were measured in duplicate and all were diluted 1:200 which allowed the sample values to be read directly against the standard curve. The assay employed a highly specific antiserum to corticosterone 3-carboxymethyloxime and [^{125} I] corticosterone as the tracer. Bound corticosterone was measured by the Riastar gamma counter after precipitation by polyethylene glycol (PEG) and goat anti-rabbit gamma globulins. Adrenalectomized animals with plasma corticosterone values exceeding the lowest value of the standard curve (25ng/ml) were considered incompletely ADX and were omitted from the analysis.

2.5.2. Plasma glucose determination

Plasma glucose concentrations were measured by the glucose oxidase method using a Beckman glucose analyzer 2 (Beckman Instruments, Fullerton CA, USA). This method is based on oxygen consumption rate with oxygen monitored by a Beckman Oxygen Electrode. Ten microlitres of the plasma sample was added to the enzyme glucose oxidase reagent in a cup containing the electrode that responds to oxygen concentration. The rate of oxygen consumption is directly proportional to the concentration of glucose in the samples (Kadish *et al.*, 1968) and lower detection limit is 10 mg/dl while the high detection limit is 450mg/dl. Each sample was done in duplicate and then the readings were averaged. We did not encounter differences in our reading from the same samples.

2.5.4. Insulin determination

Plasma insulin was determined by RIA as described above except the insulin antibody used was Gp01 obtained from Dr Pederson (University of British Columbia, Vancouver, Canada) because it was sufficiently sensitive to detect insulin in plasma without extraction. In order to assess the overall responses to glucose and insulin in the Zucker rats during OGTT, integrated (or the accumulated insulin or glucose concentrations from the beginning to the end of OGTT test) responses were calculated as described by Chan (1985) using the formula below

$$X_1 = \frac{(x_{t0} + x_{t1})}{2}(t1 - t0) + \frac{(x_{t1} + x_{t2})}{2}(t2 - t1).....- t_{n-1}(X_{t0})$$

where X_1= integrated release of the measured parameter, x = concentration of the measured parameter at time t.

2.5.5. Plasma triglycerides

Measurement of plasma triglyceride concentrations was carried out according to the kit manufacturer's manual. The assay involves sequential enzymatic reactions (Buccolo and David, 1973) and is a modification of the method of McGowan *et al.* (1983). Plasma TG are hydrolysed by lipoprotein lipase to glycerol and FFA. Glycerol is then phosphorylated by glycerol kinase and ATP to form glycerol 1-phosphate and ADP. Glycerol phosphate is oxidized to dihydroxyacetone phosphate and hydrogen peroxide by glycerol phosphate oxidase. Hydrogen peroxide converts 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl) m-aniside (ESPA) to a quinoneimine dye and water in the presence of peroxidase. The absorbency of

quinoneimine dye is then measured by spectrophometer at wavelength 540 nm. The absorbency is directly proportional to the TG concentration in the sample and the and diagonistic test is linear up to 1000 mg/dl (Trinder, 1969; Barham and Trinder, 1972).

2.5.6. Free fatty acid determination

The free fatty acid assay is based on the same principle as that of TG. FFA are converted to acyl CoA by acyl-CoA synthetase in the presence of coenzyme A and ATP. Acyl-CoA then reacts with oxygen in the presence of acyl-CoA oxidase to form 2,3-enoyl coenzyme A and hydrogen peroxide. The peroxide then converts 2,4,6-tribromo-3-hydroxybenzoic acid and 4-aminoantipyrine to a red dye in the presence of a peroxidase. The dye is measured in the visible range at 540 nm and the method shows linearity up to a concentration of 1.5 mmol/l FFA (Shimizi *et al.*, 1980).

2.6. Pancreatic islet enyzme activity

2.6.1. Glucose phosphorylating (hexokinase and glucokinase) activity

After 16-24 h culture, isolated pancreatic islets in batches of 20-30 were washed in Krebs-Ringer bicarbonate buffer (KRB) containing 0.05 mM glucose, supplemented with 10 mM Hepes (pH 7.4). After pelleting by centrifugation, the islets were re-suspended in 225 µl of glucokinase assay buffer, which was KRB buffer supplemented with various glucose concentrations (0.05-16 mM), 10 mM Hepes (pH 7.4), 5 mM MgATP and 10 mM NaF. The samples were then subjected

to three freeze-thaw cycles at -70° C to disrupt the islet cell membranes. At zero time D-[U-^{14}C] glucose (250 mCi/mol) was added and the disrupted islets were incubated for 20 min at 37°C . The reaction was terminated by adding 30 µl of 0.25 mM EDTA in 2 M glucose, and duplicate 60 µl samples were then spotted on diethylaminoethyl (DEAE) cellulose filters (Whatman DE-81, VWR Scientific, Toronto, Ont) that retained the phosphoric esters. After washing and drying the filters the radioactivity retained was counted by liquid scintillation. The protein content of the remaining sample was determined by the Lowry method using bovine serum albumin as standard. Samples without islets served as the blank controls. Hexokinase activity was determined by using glucose concentrations from 0.05- 0.5 mM while 6-16 mM glucose were used for glucokinase activity measurements. Glucokinase activity in islets increases with glucose concentration in the media and in most studies measuring glucokinase kinetics, glucose concentration up 100mM have been employed (Sweet *et al.*, 1996.) Initial studies conducted in our laboratory using high glucose concentrations resulted values that were not different from the blanks (Chan, personal communication). Using glucose concentrations between 6 - and 16 mmol gave the best linear fit, thus the reason for employing these glucose concentrations in this study. Velocities were calculated after correcting for specific activity and normalized by comparing to islet protein (Lowry method, Sigma). For glucokinase, values were corrected for hexokinase activity by subtracting $V_{HEXOKINASE}$ at 0.5 mM (Chan *et al.*, 1995). Enzyme kinetics were derived from Eadie-Hofstee plots (V vs V/S) to assess K_m and V_{max}.

2.7. Fatty acid oxidation in isolated islets

Pancreatic islet fatty acid metabolism was measured as described by Chen *et al.* (1994). Briefly, 20 islets were picked into 2 ml glass vials containing 200 µl previously gassed (95% O_2, 5% CO_2) ice cold KRB buffer supplemented with 3 mM glucose, 0.3% BSA and 10 mM Hepes (pH 7.4) and incubated on ice for 30 min. The medium was decanted after brief centrifugation and fresh KRB buffer containing either 3 mM or 25 mM glucose supplemented with 1% BSA, 0.8 mM carnitine and 0.5 mM [1-^{14}C]- palmitic acid was added whereupon the 2 ml vials were placed inside 20 ml scintillation vials, closed with a rubber stopper and incubated in a shaking water bath (100 rpm) at 37°C for 2 h. The reaction was stopped by injecting 100 µl of 6 % perchloric acid into the small vial. Hydroxide of hyamine 10-X (Packard) (300 µl) was injected into the outer vial to capture the CO_2 produced and the vials were incubated at 37°C for another 2 h. The small vials were removed, and 4 ml of scintillation fluid was added to contents of the outer vial and the DPM counted using a TriCarb liquid scintillation analyzer (Packard). Blank samples without islets were included with each glucose concentration.

CO_2 production was calculated as

$$\frac{\text{Sample DPM-blank DPM}}{\text{Specific activity of [1 -}^{14}\text{ C] palmitic acid X time X Islet \#}}$$

and is expressed in fmol/h/islet.

2.8. Islet triglyceride content

Islet triglyceride content was determined as described by Lee *et al.* (1994).

Briefly, 100-300 islets were hand-picked into microcentrifuge tubes, centrifuged (1500 rpm) and the pellet re-suspended in 50 µl of 2 mM NaCl/ 20 mM EDTA/ 50 mM sodium phosphate buffer (pH 7.4). The islets were then freeze/ thawed 3 times and 10 µl of the homogenate was mixed with 10 µl tert-butyl alcohol and 5 µl Triton X-100/methyl alcohol mixture (1:1 by volume) to extract the lipids (Lee *et al.*, 1994). Triglycerides were then determined as described for plasma samples (section 2.5.5).

2.9. Statistical analysis

Data are expressed as means ± SEM and (N) refers to number of donor animals in each group. Data were analyzed with two way ANOVA (general linear model due to unequal data sets which also allowed to test for the effects of treatment, phenotype, age and diet effects on the measured responses). The repeated measure aspects of the data was analyzed by using the SAS scientific software (SAS Institute Inc SAS/STAT Guide for personal computers Version 6 Edition, 1985, Hatcher and Stepanski, 1994), followed by Student-Newman-Keuls (SNK) test. In some cases unpaired t-test was used. In both studies the effect of a phenotype is denoted by [a] whereby obese fa/fa rats are compared with lean rats receiving similar treatment; [b] effect of age whereby old rats are compared to young rats within the same rat group and phenotype; comparison of sham -operated or sedentary controls to the ADX or exercised groups is denoted by [c] for animals fed a similar diet; [d] denotes comparison between high fat and low fat diet in animals treated similarly (eg: ADX.HF compared to ADX.LF or HF.EX is compared to LF. EX). All results were considered significant at $p \leq 0.05$.

2.10. Materials and sources.

Materials	Sources
Acetic acid	Baxter
Albumin fraction 5	Boehringer Mannheim
Animals (Zucker rats)	Charles River Laboratories, St Constant, Quebec
Antibiotic/antimycotic solution	Sigma
Calf serum	Gibco
Carbon decolorizing neutral	Fisher
Carnitine	Sigma
Collagenase Type XI	Sigma
Corticosterone assay kit	ICN Biomedicals
DEAE (Whatman DE-81 filters)	VWR Scientific
4.0 Dexon	Cyanamid Canada
4.0 Dermalon	Cyanamid Canada
Dextran Industrial grade	Sigma
Dextran Clinical grade	Sigma
Dextran T70	Pharmacia
Diazepam	Sabex Quebec.
Dulbecco's modified Eagle's medium	Gibco
Ethanol 95%	Consolidated Alcohol Ltd
Free fatty acid test kit	Boehringer Mannheim
Gelatin	Difco
^{14}C-glucose	Mandel (Dupont NEN)
Glucose	Sigma
L-Glutamine	Gibco
Lowry protein assay kit	Sigma
Hank's Balanced salt solution	Gibco
Heparinized tubes	Fisher
Hepes	Sigma
Hyamine hydroxide	Packard
^{125} Iodine	Amersham (Canada)
Insulin (rat, standard)	Novo Biolabs
Insulin antibody (GP 01)	Dr.R.A.Pederson, Vancouver, Canada.
Insulin antibody (GP 02)	Atlantic Veterinary College
Insulin (porcine, tracer)	Novo Biolabs
Mannoheptulose	Sigma
Nylon mesh screen nitex (800 µm)	B&SH Thompson Bros
(Somnotol) Sodium Pentobarbital	MTC Pharmaceuticals
Scintillation fluid (ultra gold)	Packard
^{14}C- palmitic acid	Mandel (Du Pont NEN)
Perchloric acid	Mallinckrodt
Polyethylene tubing (50 and 10)	Fisher

Sodium chloride	Fisher
Sodium bicarbonate	Fisher
Sodium fluoride	Fisher
Sodium iodide	Fisher
Sodium phosphate diphasic	Fisher
Triglyceride (GPO-trinder) kit	Sigma
Triton X-100	Sigma
Tert-butyl alcohol	Sigma

All reagents and chemicals used were of cell culture grade or molecular biology grade.

Chapter 3.

INTERACTION BETWEEN ADRENALECTOMY AND HIGH FAT DIET IN ZUCKER *fa/fa* RATS.

3.1. Introduction

3.1.1. Pancreatic B-cell glucose sensitivity

Simple obesity in both humans and rodent models is associated with insulin resistance, fasting hyperinsulinemia and hyperlipidemia but normal or only slightly elevated blood glucose levels. Perfused pancreases from genetically obese Zucker (*fa/fa*) rats exhibit increased sensitivity to glucose at an early age (Kuffert *et al.*, 1988; Atef *et al.*, 1991) and this sensitivity persists in adult rats (Curry and Stern, 1985). The increased sensitivity correlates with development of fasting hyperinsulinemia in weanling fa/fa rats (Chan *et al.*, 1985) and with B-cell insensitivity to mannoheptulose, an index of glucokinase dysfunction (Kibenge and Chan, 1995). The fasting hyperinsulinemia has been attributed to increased sensitivity of pancreatic islets to low levels of glucose as seen in most genetically obese rodents (Kuffert *et al.*, 1988; Timmers *et al.*, 1992; Chan *et al.*, 1993; Chen *et al.*, 1993). The lowered glucose threshold could be caused by changes within the B-cell glucose sensing machinery such as increased glucokinase catalytic activity (Chan 1993; Chen *et al.*, 1994b; Chan *et al.*, 1995) and/or increased activity of hexokinase (Chan *et al.*, 1995; Hirose *et al.*, 1996; Cockburn *et al.*, 1997). Overall, enhanced glucose phosphorylation leads to increased glucose flux through glycolysis and promotes insulin secretion. However, what regulates glucokinase

function has not been firmly established.

Because in simple obesity the animals or humans have elevated plasma fasting FFA and TG, it has been postulated that the abnormal plasma lipid profiles may contribute to the changes seen in the *B*-cell response to glucose stimulation (McGarry 1992). Both *in vivo* (Elks, 1993) and *in vitro* (Zhou and Grill, 1994) short term exposure of pancreatic islets to high levels of FFA enhances both basal and glucose-stimulated insulin secretion (GSIS). However prolonged exposure decreases GSIS as a result of inhibition of glucose metabolism in the pancreatic B-cells (Zhou and Grill, 1995). The action of long chain FFA is multifaceted (Prentki and Corkey,1996; McGarry and Dobbins, 1999) as described in detail in Chapter 1.

3.1.3. HPA axis regulation of B-cell function

The hypothalamus regulates energy balance and the functions of many endocrine glands either through releasing or inhibiting hormones and/ or neurotransmitters. The hypothalamic control on pancreatic islet beta cell function is illustrated by the immediate insulin hypersecretion that follows injury to ventromedial hypothalamic nuclei in most experimental animals (Jeanrenaud, 1985). This control is through neural mechanisms since disruption of neural connections to the pancreas prevents the development of obesity due to VMH lesions in rats (Lee *et al.*, 1989).The excessive insulin secretion in response to nutrients (glucose and arginine) and the hypothalamic obesity that develops is prevented by ADX (Bruce *et al.*, 1982). Also, ADX blocks the progression or

development of dietary obesity (King *et al.*, 1985) and genetically transmitted obese syndromes, such as that of Zucker *fa/fa* rats (Fletcher, 1986), and most of the metabolic, hormonal, behavioral abnormalities that accompany these syndromes (Bray *et al.*,1989). Pancreatic islet glucose sensitivity, glucokinase kinetics and mannoheptulose inhibitory action on glucokinase have been shown to be normalized by ADX in fa/fa rats (Kibenge and Chan, 1996) when maintained on normal rat chow for two weeks post operation. The actions of ADX on insulin secretion are mediated through reduction of caloric intake, reduced glucose production by gluconeogenesis, and/or increased sympathetic nervous system activity in the peripheral tissues of the rats (Arase *et al.*, 1989; Rohner-Jeanrenaud *et al.*, 1989). In addition to reduction of plasma insulin and leptin concentrations, ADX reduces expression of type 2 corticotropin releasing hormone receptors (CRHR-2) mRNA in the VMH, an action that is reversed by corticosterone replacement in rats (Makino *et al.*, 1998). Leptin actions on food intake reduction are also via these same CRHR-2 receptors in the PVN and VMH since its administration results in an increased expression of CRHR-2 mRNA in both areas (Nishiyama *et al.*, 1999). ADX does not alter pancreatic B-cell ultra structure (Borelli *et al.*, 1982; Gomez Dumm *et al.*, 1984) or insulin content (Fiedoreck and Permutt, 1989) but reduces islet glucose metabolism (Giddings *et al.*, 1995), reduces pancreatic islet glucose sensitivity and increases glucokinase K_m (Kibenge and Chan, 1996). Glucocorticoids centrally mediate the endocrine pancreatic secretory activity by increasing parasympathetic drive to the B-cells in obese animals as is

evidenced by atropine administration or by ADX blocking glucocorticoid actions. Therefore, ADX normalizes ANS tone reaching the B-cells of the obese animals. (Fletcher and MacKenzie, 1988a; Stubbs and York, 1991).

3.1.3. Direct effects of glucocorticoid in pancreatic B-cells

The direct action of glucocorticoids on nutrient- stimulated insulin secretion is not very clear. Glucocorticoids are required for early fetal development of B-cells in rats (12-15 d of gestation), but their absence in late pregnancy does not alter B-cell development (Komatsu *et al.*, 1996). Because actions of most glucocorticoids are permissive, glucocorticoids (corticosterone in rodents or cortisol in man) might enhance insulin secretion by regulating the synthesis of several enzymes involved in glucose, lipid and protein metabolism in tissues such as liver, muscle, adipose tissue and pancreatic islets (Baxter and Tyrrell, 1987). In order to understand the direct actions of glucocorticoids in different tissues, dexamethasone, a synthetic glucocorticoid, is widely used in experimental studies. *In vivo*, dexamethasone increases proinsulin mRNA (Koranyi *et al.*, 1992a; Ludvik *et al.*, 1993) in both rodent and human pancreas. *In vitro* actions of dexamethasone in pancreatic islets and insulin secreting cell lines include increased proinsulin mRNA and glucokinase mRNA (Philippe *et al.*, 1992; Fernandex-Meija and Davidson, 1992) and inhibition of insulin secretion in response to several stimulants (Lambillotte *et al.*, 1997), an effect attributed to glucocorticoids actions at the genomic level in the B-cells that lead to a decrease in the efficacy of cytoplasmic Ca^{2+} on the exocytotic process

(Lambillotte *et al.*, 1997). In ob/ob mouse islets, dexamethasone increases both glucose 6-phosphatase protein and its activity (Ling *et al.*, 1998) thereby enhancing glucose cycling, which reduces further glucose metabolism through Krebs's cycle and results in a reduction of insulin secretion (Khan *et al.*,1992).

3.1.4. Interaction between HPA axis and diet composition in genetic obesity

Adrenalectomy-mediated reduction of circulating plasma lipids might also play an important role in normalizing plasma insulin levels (Castonguay *et al.*, 1986, Bray *et al.*, 1989). High fat feeding (Triscari *et al.*,1985) or lipid infusion increases plasma lipids in both animals and human subjects (Boden *et al.*, 1995) and this has a stimulatory effect on the HPA axis to increase corticosterone secretion in rats (Widmaier *et al.*, 1992; Tannenbaum *et al.*, 1997). Corticosterone then increases adipose mobilization and contributes to hyperinsulinemia and insulin resistance.

A high fat diet reduces the effects of ADX on the amelioration of obesity in adult *fa/fa* rats (Bray *et al.*, 1992), but not when ADX is performed in preobese fa/fa pups (Fletcher and MacKenzie, 1988b). In ob/ob mice both high fat and high glucose diets reduce the effects of ADX on energy balance (Kang *et al.*, 1992; Mistry *et al.*, 1995). Adrenalectomy-mediated lowering of energy efficiency in starch-fed animals is centrally mediated and is associated with restoration of SNS activity in the peripheral tissues (i.e. BAT, pancreas, liver, heart and skeletal muscles) of most obese animals (Vander Tuig *et al.*, 1984; Shargill *et al.*, 1987; Arase *et al.*, 1988). Feeding a high fat and/or high glucose diet seems to interfere with this

mechanism (Kim and Romsos, 1988) by reducing sympathetic nervous system activity in BAT (Sakaguchi *et al.*, 1989) and also this effect can be centrally mediated by reducing effects of leptin (Lu *et al.*, 1998), thus restoring the imbalance of autonomic nervous system activity in the peripheral tissues.

Adrenalectomized obese rodents reduce their food intake immediately such that within a week caloric intake and weight gain is significantly reduced in ADX animals compared to sham controls (Bligh *et al.*, 1993). Our previous study demonstrated that in pancreatic islets the glucose-induced insulin response to mannoheptulose inhibition was restored in fa/fa rat islets 2 wk post surgery, which was correlated to glucokinase sensitivity (K_m) to glucose (Kibenge and Chan, 1996). In that study we did not determine food intake or measure other *in vivo* changes other than weight gain. With the current literature regarding the important role of high plasma lipid concentration on regulation of islet B-cell function and the evidence that ADX reduces plasma TG and FFA concentration we considered that lipids might also play a role in the restoration of mannoheptulose response observed in ADX rats.

Dietary manipulation can also induce physiological changes in normal rats in less than 2 wk exposure. High fat/protein diets resulted in weight gain and insulin resistance when after the rats were exposed to the diet for only 10 d (Ramirez *et al.*, 1990). Feeding rats a high fat diet to ADX rats for 4 wk should induce measurable changes in both lean and obese Zucker rats as has been shown in ob/ob mice (Kim and Romsos 1998). Since there are no reported studies to date that have directly addressed the effects of ADX combined with increased dietary fat on insulin secretion and islet B-cell function in fa/fa rats, this study was carried out to address

this issue.

3.1.5. Hypothesis and study objectives

Given the apparent importance of lipids in the regulation of B-cell function (Prentki and Corkey, 1996; Larsson *et al.*, 1996; Fernandez and Valdeolmillos , 1998), the ability of ADX in prepubertal fa/fa rats to normalize several insulin secretion parameters (Kibenge and Chan, 1996), and the observation that ADX reduces plasma triglycerides (Castonguay *et al.*, 1986), we carried out this study to investigate the hypothesis outlined below.

3.1.5.1. Hypothesis

The hypothesis of this study was that post-surgical feeding of a high fat diet would negate the benefits of ADX on B-cell function previously identified in obese fa/fa rats (Kibenge and Chan, 1996) and would induce adaptive changes in pancreatic islets from lean rats.

3.1.5.2. Objectives

The objectives were (1) to compare both the *in vivo* and *in vitro* insulin responses to glucose stimulation in ADX fa/fa rats fed low or high fat diets for 5 wk post surgery. The rationale for ADX fa/fa rats at 5 wk of age, was that at this stage in their development, fa/fa rats begin to exhibit hormonal abnormalities fasting hyperinsulinemia (Chan *et al.*, 1985) and an abnormal HPA axis regulation

evidenced by a loss of diurnal rthym for corticosterone secretion (Fletcher *et al.*, 1986). At the same time hyperphagia is evident at this age and these defects are normalized by ADX (Fletcher and MacKenzie, 1986). (2) to investigate any interactions between ADX and high fat diet on pancreatic islet glucose sensitivity, islet B-cell TG content and FFA metabolism, and glucokinase activity. Similar experiments were carried out in age matched lean rats. Sham-operated lean and fa/fa rats served as controls for each phenotype. A third objective was to compare the results with ADX experiments terminated 2 wk. post surgery (Kibenge and Chan, 1996).

3.2. Materials and Methods

3.2.1. Animals

Zucker lean and obese 5 week old rats were either obtained from Charles River Laboratories (St. Constant, Que) or bred at the Atlantic Veterinary College (AVC) from Charles River stock. Animals bred at AVC were routinely weaned and sexed at 21 d of age. All animals were fed Purina rat chow and tap water *ad libitum* before and one week after surgery, when they were put on their respective diets.

3.2.2. Adrenalectomy

Bilateral ADX or sham operations were performed on lean and fa/fa rats between 38-39 days of age using a ventral approach as previously reported (Kibenge and Chan 1996). The animals were anesthetized with a mixture of sodium

pentobarbital (65 mg/ml) and diazepam (5 mg/ml) in saline (0.9% NaCl) ip. at a dose of 0.1-0.15 ml/100 g body weight. Before surgery a 0.5 ml blood sample was collected from the tail vein for measuring plasma glucose and insulin levels. Control animals underwent laparotomy and their adrenal glands were teased and left in place and these animals are referred as "sham"-operated (SH). The success of the surgical procedure was evaluated by the number of animals that recovered from anesthesia where 20 (25%) of obese and 4 (6%) lean rats died during recovery from anesthesia. Of the 80 fa/fa and 64 lean rats that underwent ADX or sham- operation 60 obese and 60 lean rats completely recovered from surgery. After surgery the animals were housed singly in plastic cages. Tap water containing NaCl (0.9%) and sucrose (40 g/L) was available to all post-surgical animals *ad libitum*. Animals were considered completely ADX if their plasma corticosterone concentrations were below 25 ng/ml. The success rate of ADX was above 60% for both phenotypes.

3.2.3. Experimental design

One week after surgery, the ADX and SH animals were divided into groups and were fed either a sweetened condensed milk diet with elevated fat (HF) content (3.96 kcal/g, 15.8% fat) or low fat (LF) rat chow (3.74 kcal/g, 4.5% fat) (Triscari *et al.*, 1985). In addition to their diets and tap water animals had access to a saline (0.9% NaCl)/sucrose (4%) solution *ad libitum* throughout the study in order to prevent side-effects of mineralocorticoid or glucocorticoid deficiency. The animals remained on their diets for 4 wk after which the experiment was terminated. Food

intake was measured on three consecutive days every week and then averaged to find daily food intake. Average food consumed in grams was converted to calories by multiplying by the caloric content /g of each diet (Table 1). All protocols were approved by the local Animal Care Committee and met the guidelines of the Canadian Council on Animal Care.

3.2.4. OGTT, *in vivo* metabolic assessment, pancreatic islet isolation and *in vitro* B-cell function measurements

After 28-30 d of diet, the animals were fasted overnight and the saline/ sucrose solution was replaced with saline and OGTT was carried out as described in the methods. It is recommended to give animals saline to maintain blood volume during OGTT, however, due to the short period this test was carried out (one hour instead of 2d) and the small blood volume of 2.4 -3 ml (or 10-13%) obtained from 170-400 g rats during OGTT we did not deem this necessary. A loss of 20-30% of total blood volume was shown to result in high plasma glucose, epinephrine and norepinephrine concentrations but not insulin concentrations (McCoy *et al.*, 1995). Thus the plasma glucose and insulin obtained in this study reflect the response to glucose administration. In the *in vitro* studies, in order to use one animal for all the experiments requiring isolated islets, a minimum of 600 islets would be required. However, this was not possible in the ADX rats because the pancreatic tissue became more difficult to digest with collagenase, possibly due to increased extracellular matrix protein, leading to a low islet yield. Therefore not all experiments

could be completed with the same animal and this accounts for the variations in the number of animals reported in the Results sections. The rest of the experiments were carried out as described in the General Methods (Chapter 2).

3.2.5. Statistical analysis

Data are expressed as means ± SEM and (N) refers to number of donor animals in each group. Weight change, food intake and insulin secretion were analyzed by analysis of variance using general linear model for unbalanced data sets and for repeated measures (using SAS mixed procedure), followed by Student-Newman-Keuls (SNK) test. Unpaired t-test was used when comparing responses by the same treatment between ADX and control within phenotype and between phenotypes and comparison of data was carried out as described in General Methods. All results were considered significant at $p \leq 0.05$.

3.3. Results

3.3.1. *In vivo* results

3.3.1.1. Energy intake

After surgery all rats were fed regular rat chow for one week and at 6 wk of age, some of the rats were fed a high fat diet for the next 4 wk. Sham fa/fa rats were hyperphagic during the recovery period, consuming more than twice as many calories as did the lean rats ($p < 0.005$). Adrenalectomy significantly reduced caloric intake during the first week in both fa/fa and lean rats. This was more

apparent in the fa/fa group where sham rats consumed 72% - 110% more calories than did ADX rats while the sham lean rats consumed 29% -34% more calories than did the ADX rats during the recovery week (Figure 7).

In obese rats, introduction of a high fat diet increased caloric intake by 50% in the first week of exposure in sham rats and by 43% in ADX rats ($p < 0.05$), respectively. The excess caloric intake persisted to the same extent (43%) in ADX rats but declined in the sham rats such that the SH.HF rats consumed less calories compared to SH.LF rats by the end of the experimental period. Overall caloric intake remained higher than in the ADX.HF fa/fa rats ($p<0.05$, Figure 7). Sham.LF fa/fa rats also consumed 80% more calories than did ADX.LF rats in the first week and reduced their intake to 62% more calories in the final week of the experiment ($p<0.005$). Introduction of high fat diet in lean rats significantly increased food intake by 40% in ADX.HF rats. Although caloric intake remained slightly higher in HF diet fed lean rats, this difference was not significantly different from the LF fed rats. Adrenalectomy did not influence caloric intake in lean rats fed rat chow. Overall, ADX reduced the caloric intake in fa/fa rats consuming both low and high fat diets ($p<0.0001$). Energy intake also was influenced by the age, phenotype, diet and treatment (all; $p<0.0001$). Interactions between age and phenotype, treatment or diet significantly affected caloric intake (all; $p<0.005$).

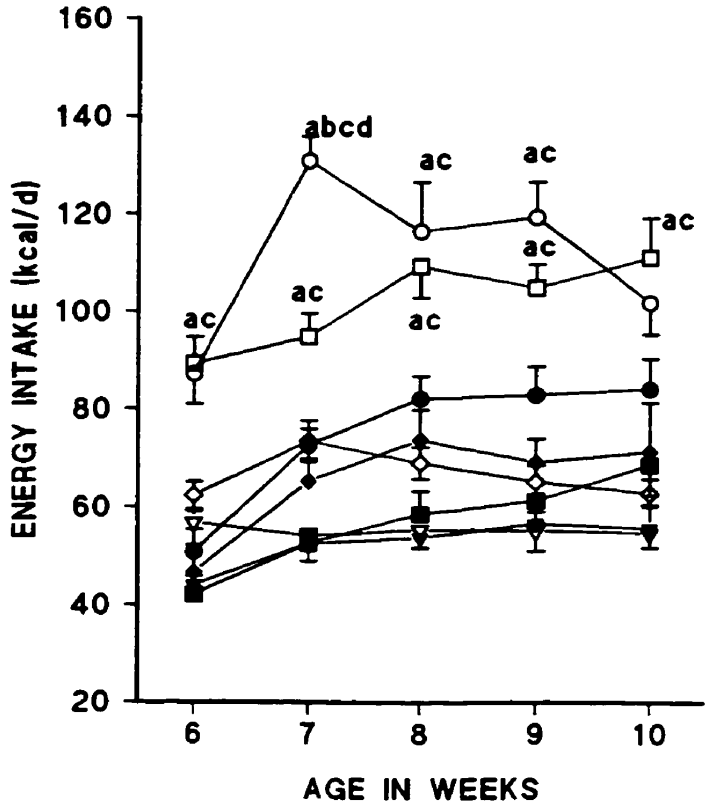

Figure 7. Effects of ADX and high fat diet on caloric intake in Zucker rats.

Data is expressed as means ± SEM of the number of animals shown in Table 3.

Obese rats: ADX.LF ■, n=10, SH.LF □, n=12, ADX.HF ●, n=10, and SH.HF ○, n=12.

Lean rats: ADX.LF ▼, n=10, SH.LF ▽, n=14 , ADX.HF ◆,n= 10, and SH.HF ◇, n=16. [a] $p<0.05$ fa/fa compared to lean rats, [b] $p<0.05$ 7wk compared to 6 wk (SH.HF fa/fa rats) [c] $p<0.05$ Sham operated compared to ADX, [d] $p< 0.05$ high fat compared to low fat, using SNK after ANOVA (with repeated measures).

3.3.1.2. Weight gain

Animals were weighed before surgery (5 wk of age) and weekly thereafter for 4 wk. The weight profiles are shown in Table 3 and Figure 8 A and B. At 5 wk of age fa/fa rats weighed 50% more than the lean rats and this difference increased with age in sham operated rats to more than 70% at the termination of the study ($p < 0.001$) (Table 3).

In fa/fa rats (Figure 8 B), increasing dietary fat content significantly increased weight gain by 19% ($p < 0.05$) in obese SH.HF rats compared to the SH.LF rats, a difference that was reached in the last week of the study. There was no difference in ADX.HF and ADX.LF rats final weights. Adrenalectomy decreased weight gain in both ADX.HF and ADX.LF rats when compared to their respective controls and normalized weight gain of ADX.HF and ADX.LF fa/fa rats to that of sham lean rats fed similar diets. ADX.LF and ADX.HF fa/fa rats gained 45% and 47% less weight such that at the end of 5 wk fa/fa ADX.HF and ADX.LF rats had gained as much weight as the lean SH.HF and SH.LF rats, respectively (Table 3).

In lean rats (Figure 8A), ADX did not affect weight gain in low fat diet fed rats but prevented weight gain induced by feeding a high fat diet ($p < 0.05$) (Table 3). High dietary fat content increased weight gain in lean SH.HF rats by 15% compared with ADX.HF and SH.LF rats ($p<0.05$). The difference in weight between SH.HF and ADX.HF lean rats was noticeable in last 2 wk of the study.

Analysis of the data by ANOVA (with repeated measures) showed that weight was significantly influenced by treatment (ADX/SH), phenotype and age (all,

p<0.0001). Interactions between treatment and phenotype, or age, and phenotype and age significantly influenced weight gain (all , p< 0.0001). A four way interaction between phenotype, diet, age and treatment was also observed (p < 0.0001).

3.3.1.2. Plasma concentrations of corticosterone, insulin, glucose, TG and FFA.

3.3.1.2.1. Corticosterone

After 4 weeks on either high fat or regular chow diet the success of the ADX surgery was confirmed by measuring corticosterone concentrations in rat plasma by RIA. Animals with corticosterone concentrations below the detection limit of the assay (25 ng/ml) were considered fully ADX. With this criterion, the plasma corticosterone of ADX rats was below the assay's detection limit. Although corticosterone level in both lean and fa/fa were within the reported normal range (0.05 to 0.64 ± 0.082 µg/ml) (Shimuzu *et al.*, 1983; Shargil *et al.*, 1987) there was a significant phenotype effect and a diet effect on the fasting concentrations of corticosterone in sham rats. Obese sham rats had significantly higher corticosterone concentrations than did lean sham rats (p< 0.05). Diet had no significant effect on corticosterone concentrations in the sham lean rats (p> 0.05); however, there was a diet effect in fa/fa rats, where SH.LF fa/fa rats had higher fasting corticosterone concentrations than SH.HF fa/fa rats (p< 0.05) (Table 4).

Table 3

The effect of ADX and diet composition on weight (Wt) gain in Zucker rats

Animals	N	Initial Wt (5 wk) (g)	Final Wt (10 wk) (g)	Total Wt gained (g)	% Wt gain vs SH.LF
Lean rats					
ADX.LF	10	104.0 ± 5.0 [ab]	204.2 ± 9.3 [a]	99.8 ± 5.0 [a]	-15.7
ADX.HF	10	96.6 ± 4.1 [ab]	193.1 ± 6.6 [ac]	98.5 ± 4.1 [ac]	-16.9
SH.LF	14	98.4 ± 4.0 [ab]	213.6 ± 6.6 [a]	118.4 ± 4.4	
SH.HF	16	96.3 ± 3.8 [ab]	234.7 ± 9.6 [ad]	136.5 ± 6.9	+15.3
Obese rats					
ADX.LF	10	154.1 ± 6.7 [b]	273.9 ± 7.9 [c]	122.2 ± 10.8 [c]	- 44.7
ADX.HF	10	149.6 ± 7.8 [b]	288.8 ± 10.3 [c]	139.2 ± 8.6 [c]	- 37
SH.LF	12	145.6 ± 4.1 [b]	366.6 ± 9.2 [d]	221.0 ± 7.0 [d]	
SH.HF	12	142.4 ± 5.3 [b]	405.3 ± 13.5	262.6 ± 15.6	+19

Values are means ± SEM, N = animals per group.

[a] $p < 0.0001$, lean compared to fa/fa rats; [b] $p < 0.0001$, 5 wk compared to 10 wk; [c] $p < 0.0001$ ADX compared to sham rats; [d] $p < 0.05$ low fat compared to high fat diet, using SNK after ANOVA.

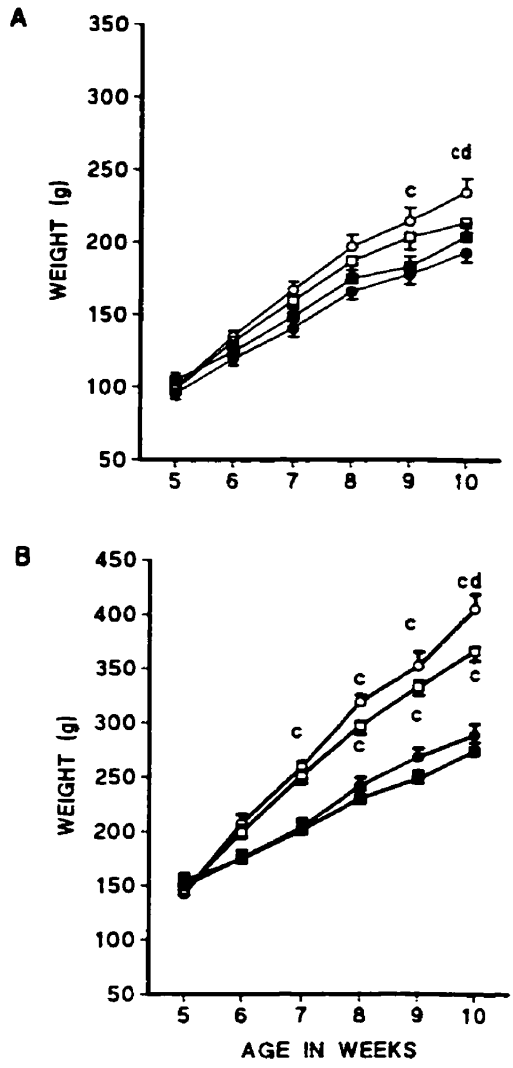

Figure 8. Effects of ADX and a high fat diet on weight gain in Zucker (A) lean and (B) fa/fa rats. Rat were weighed weekly as described in General methods. Values are means ± SEM for number of animals shown in Table 3, ADX.LF ■, SH.LF □, ADX.HF ● and SH. HF ○ rats. ^{c} $p < 0.05$ sham rats compared to ADX rats fed a similar diet, ^{d} $p < 0.05$ high fat compared to low fat diet in rats receiving similar treatment within phenotype using SNK after ANOVA.

3.3.1.2.2. Insulin

Fasting plasma insulin concentrations were measured using a standard curve with a range of 37.5-1200 pmol/l. The lower detection limit as calculated by the RIA software was 18.5 pmol/l. Fasting plasma insulin concentrations were not significantly different among the lean rat groups (Table 4). However, a high dietary fat content increased the insulin response to glucose during OGTT. Improved glucose tolerance induced by ADX required higher insulin output in the lean ADX.HF rats compared to that of the ADX.LF rats indicating that high fat diet reduced tissue insulin sensitivity (Figure 9 A).

Sham fa/fa rats were hyperinsulinemic with fasting plasma insulin concentrations 4-fold higher than in lean rats. Even though ADX decreased the concentrations by 41%-59%, fasting plasma insulin concentrations in ADX fa/fa rats remained 2-fold higher than those in the lean rats. Feeding a high fat diet did not affect fasting insulin in ADX fa/fa rats. Insulin response to glucose administration showed a similar trend to the lean rats except there was wider variability in insulin concentrations such there was no significant difference among the rat groups. However, ADX reduced fasting hyperinsulinemia, which is an indicator of increased tissue insulin sensitivity and improved glucose tolerance (Figure 9 B). From these results we conclude that high fat diets did not block, but reduced, the effects of ADX in fa/fa rats.

In lean rats ADX did not affect the integrated insulin response in the OGTT regardless of the diet composition. However, increased dietary fat content significantly increased the integrated insulin response in lean SH.HF rats compared

to that in SH.LF rats ($p < 0.05$). In fa/fa rats the integrated insulin response was significantly higher in ADX.HF rats than the ADX.LF rats (Figure 10).

3.3.1.2.3. Glucose

In lean rat groups fasting plasma glucose concentrations were not significantly different (Table 4). Plasma glucose concentrations remained lower in ADX rats after oral glucose administration throughout the entire sampling period ($p<0.05$) (Figure 11 A) and high fat diet did not affect glucose concentration in lean rats during OGTT. Feeding a high fat diet induced a mild insulin resistance in lean rats as illustrated by plasma glucose concentrations in SH.HF rats that were higher those than in SH.LF rats ($p < 0.05$) but similar to those of fa/fa SH.LF rats at 60 minutes post glucose administration. ADX reduced the integrated glucose response in lean rats and this was not modified by high dietary fat content. However, increasing dietary fat significantly increased the integrated glucose response in sham lean rats (Figure 12).

Fasting glucose concentrations of fa/fa sham rats were not influenced by dietary fat (Table 4). However, glucose concentrations of fa/fa SH.HF rats were significantly higher than those of the lean SH.HF rats ($p < 0.05$) but there no difference between fa/fa SH.LF and lean SH.LF glucose concentrations (Table 4). The abnormal glucose metabolism of these rats was confirmed by OGTT (Figure 11 B) where a higher dietary fat content worsened the glucose intolerance in fa/fa rats.

Table 4.
Effect of ADX and diet composition on fasting plasma corticosterone, insulin, glucose, TG and FFA concentrations in Zucker rats.

Animals		Corticosterone (ng/ml)		Insulin (pmol)		Glucose (mM)		TG (mg/d)		FFA (mM)
Lean rats	N		N		N		N		N	
ADX.LF	10	NA	6	104 ± 21.5[a]	6	4.69 ± 0.28	7	0.99 ± 0.25 [acd]	7	0.117 ±0.04
ADX.HF	10	NA	6	75.4 ± 29.2[a]	5	4.46 ± 0.26	9	3.63 ±1.21 [ac]	9	0.119 ± 0.06
SH.LF	14	552 ± 52.5 [a]	8	77.8 ± 27.0 [a]	9	6.39 ± 0.71	7	4.38 ± 2.79 [ad]	7	0.085 ± 0.05 [a]
SH.HF	16	573 ± 55.6 [a]	6	75.4 ± 29.2 [a]	8	6.11 ± 0.23 [a]	6	10.22 ± 6.3 [a]	6	0.151 ± 0.05 [a]
obese rats										
ADX.LF	10	NA	8	222 ± 73	8	5.99 ± 0.2 [c]	8	4.75 ± 1.12 [c]	6	0.147 ± 0.05 [c]
ADX.HF	10	NA	8	163 ± 37 [c]	8	5.3 ± 0.65 [c]	10	7.45 ± 1.33 [c]	8	0.23 ± 0.05 [c]
SH.LF	12	762.6 ± 33.3	5	379 ± 87	7	7.03 ± 0.28	7	47.57 ± 12.4	8	1.05 ± 0.46
SH.HF	12	695.9 ± 43.4 [d]	5	398 ±103	7	7.29 ± 0.24	12	47.39 ± 5.24	8	0.67 ± 0.29

Plasma corticosterone, insulin and glucose concentrations were determined in the blood collected before carrying out OGTT while TG and FFA concentrations were measured in blood samples collected at the end of OGTT. The values are means ± SEM, N= animals used. NA = values were below the detection limit of the assay. [a] $p < 0.05$ lean compared to fa/fa rats, [c] $p < 0.05$ ADX compared to SH, [d] $p < 0.05$ low fat compared to high fat using SNK after ANOVA.

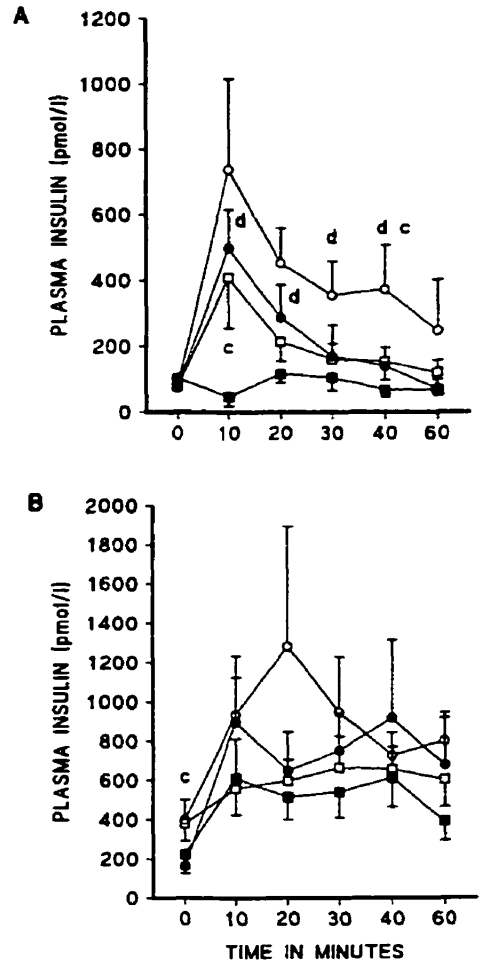

Figure 9. Effects of ADX and high fat diet on plasma insulin concentrations in Zucker lean (A) and fa/fa (B) rats. OGTT was carried out after overnight fasting as outlined in Chapter 2 section 2.3. Data are expressed as means ± SEM for the number of animals shown in Table 4; ADX.LF ■, SH.LF □, ADX.HF ●, and SH.HF○ rats. ^{c} $p < 0.05$ Sham rats compared to ADX rats fed similar diet within phenotype; ^{d} $p < 0.05$ high fat diet compared to low fat diet fed rats that received similar treatment within phenotype using SNK test after ANOVA.

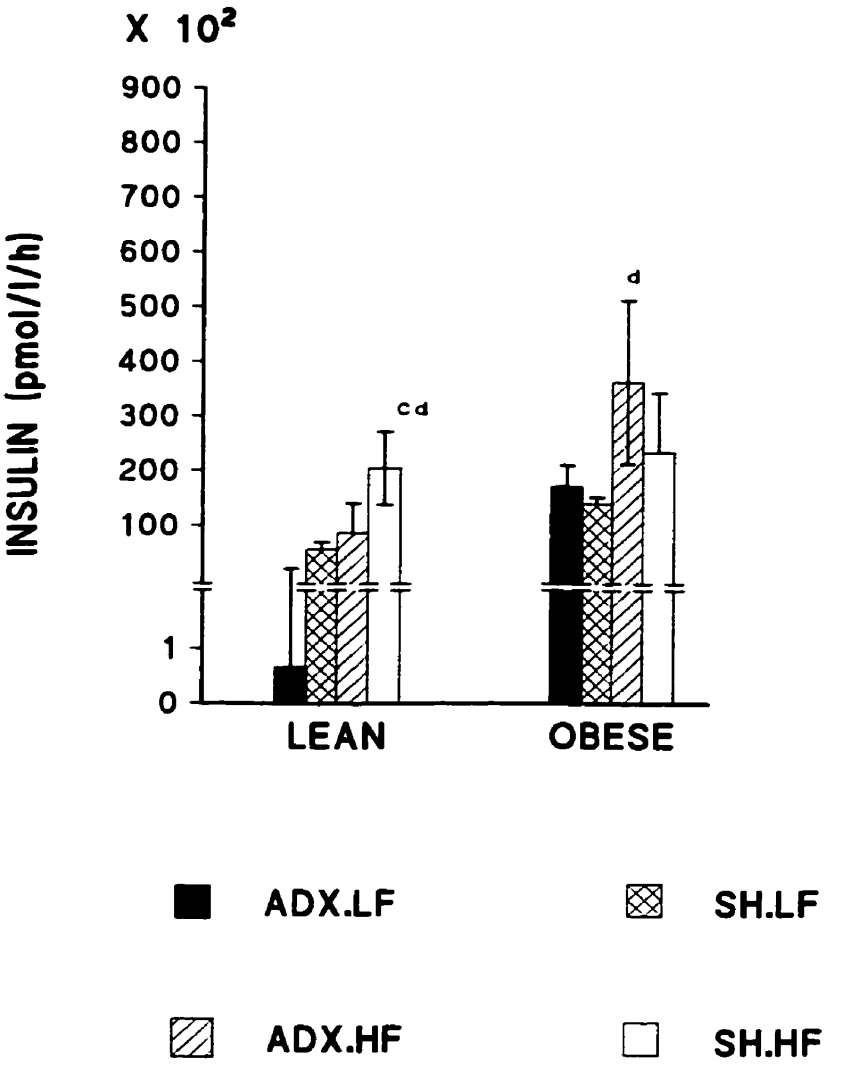

Figure 10. Effects of ADX and high fat diet on integrated insulin response in Zucker rats during OGTT. Values are means of animals shown in Table 4. The values were calculated as described in General methods (Chapter 2).

[c] $p < 0.05$ sham compared to ADX rats fed a similar diet, [d] $p < 0.05$ high fat diet fed rats compared to low fat diet fed rats that received similar treatment, within phenotye using SNK after ANOVA.

In the fa/fa SH.HF group, plasma glucose concentrations continued to rise and remained above 20 mM 60 min post-glucose gavage, indicating severe insulin resistance in the target tissues of these rats. Although mean values for the plasma glucose concentrations were higher in fa/fa SH.HF than those of fa/fa SH.LF rats throughout the entire period, a significant difference was observed only at 60 min post gavage ($p < 0.005$).

Adrenalectomy significantly lowered fasting plasma glucose concentrations of fa/fa rats independent of the diet. However, high dietary fat increased the glycemic response after a glucose challenge in ADX.HF fa/fa rats compared to the response in ADX.LF rats fed regular chow ($p < 0.05$) (Figure 11 B).

The integrated glucose response was significantly influenced by phenotype, ADX (both $p < 0.0001$), diet ($p < 0.02$) and an interaction between phenotype and ADX ($p < 0.02$). In the obese rat groups, sham rats had the highest integrated glucose response and this was significantly reduced by ADX ($p < 0.005$). High dietary fat increased the integrated glucose response in the ADX.HF fa/fa rats ($p < 0.05$).

In lean rats the ADX group had a significantly lower integrated response than the sham controls regardless of the diet, however the difference was greater in the high ADX.HF rats ($p < 0.05$) (Figure 12). Adrenalectomy improved glucose tolerance in both lean and fa/fa rats regardless of the diet composition.

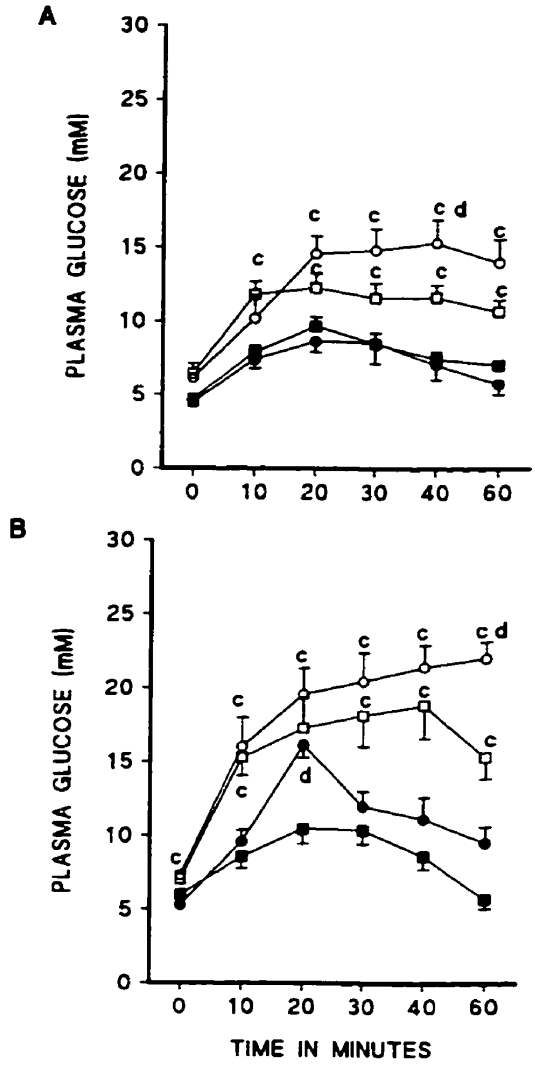

Figure 11. Effects of ADX and high fat diet on plasma glucose concentrations in Zucker (A) lean and (B) fa/fa rats. OGTT was carried out after an overnight fasting as outlined in Chapter 2 section 2.3. Data are expressed as means ± SEM for the number of animals shown in Table 4; ADX.LF ■, SH.LF □, ADX.HF ●, and SH.HFO rats. ^{c} $p < 0.05$ sham rats compared to ADX rats fed similar diet within phenotype; ^{d} $p < 0.05$ high fat diet fed rats compared to low fat diet fed rats that received similar treatment using SNK test after ANOVA.

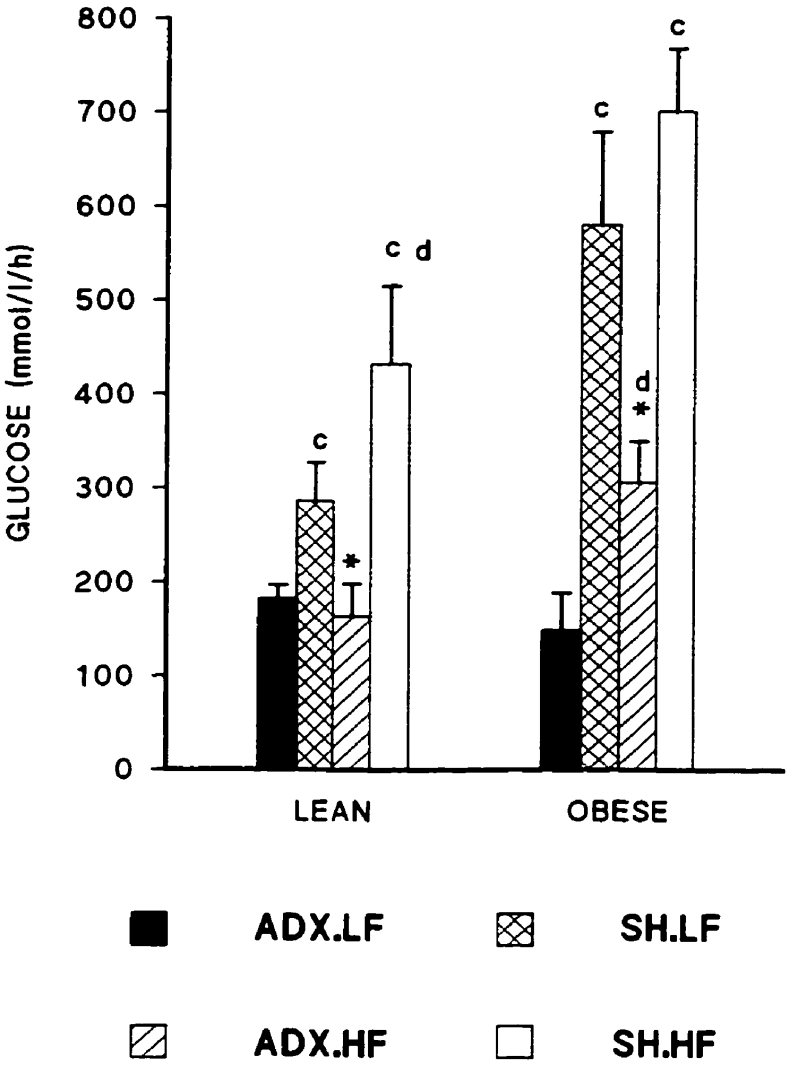

Figure 12. Effects of ADX and high fat diet on integrated glucose response in Zucker rats during OGTT. Values are means of animals shown in Table 4. The values were calculated as described in General methods (Chapter 2).

[c] $p < 0.05$ sham compared to ADX rats fed a similar diet, [d] $p < 0.05$ high fat diet fed rats compared to low fat diet fed rats that received same treatment, * $p < 0.05$ ADX.HF compared to SH.LF rats within phenotype using SNK after ANOVA.

3.3.1.2.3. Plasma triglycerides

At the end of the 4 wk feeding regimen, SH.HF lean rats had 57% higher plasma TG concentrations than SH.LF rats. ADX significantly reduced TG concentrations by more than 70% in rats on either diet ($p < 0.05$). However, plasma TG concentrations in ADX.HF rats remained 2.7- fold higher than in the ADX.LF rats; thus, feeding a high dietary fat content significantly increased plasma TG concentrations in both ADX and sham lean rats.

Obese sham rats exhibited plasma TG concentrations 4- to 10-fold higher than lean sham rats reflecting the already abnormal lipid metabolism in these rats which was not affected by feeding a high fat diet (Table 4). This difference was reduced but not eliminated by ADX, such that the ADX fa/fa rats' final plasma TG concentrations were similar to those of the sham lean rats fed the same diet ($p > 0.05$), but were still higher than those of lean ADX rats. Within the obese phenotype, ADX significantly reduced TG concentrations by approximately 84%-90% from the sham TG concentrations ($p < 0.0001$) (Table 4). High dietary fat content increased plasma TG concentrations by 56% and 266% in ADX.HF fa/fa and lean rats compared to their respective low fat fed ADX rats. Feeding a high fat diet modulated but did not negate ADX effects on TG, since in both phenotypes the TG concentrations of ADX.HF rats were still significantly lower than those of their respective sham rats; these results are similar to those reported by others (Castonguay *et al.*, 1986).

3.3.1.2.4. Plasma free fatty acids

At the end of 4 wk of diet FFA concentrations in lean rats were not different between groups. However, SH.HF rats tended to have elevated FFA concentrations compared with the other lean groups (Table 4).

Obese sham rats exhibited 4-fold higher FFA concentrations than the lean sham rats ($p < 0.05$). Adrenalectomy decreased FFA concentrations of fa/fa rats to concentrations similar to those in lean rats and increasing dietary fat did not affect FFA in ADX fa/fa rats (Table 4).

3.3.1.3.5. Summary of *in vivo* response

The results of Zucker lean and fa/fa rat *in vivo* responses to ADX and dietary fat are summarized in Table 5. The phenotypic differences are predominantly in the regulation of caloric intake, weight gain and fasting insulin and FFA concentrations. In lean rats ADX had no significant effect on these parameters, however in fa/fa rats, all these parameters were significantly reduced by ADX. In addition, ADX reduced fasting glucose concentrations in fa/fa rats, indicating the dependency of metabolic abnormalities on the presence of an intact HPA axis. In both phenotypes ADX significantly reduced TG regardless of the diet but a high dietary fat increased TG concentrations only in sham lean rats.

Table 5.

Summary of the *in vivo* results of ADX and sham Zucker rats.

Animals	Total kcal Intake	Wt gain	Corticosterone	Insulin	Glucose	TG	FFA	Integrated OGTT Response	
Lean rats								**Insulin**	**glucose**
ADX.LF	–	–	↓	–	–	↓	–	–	↓
ADX.HF	↑	–	↓	–	↓	–	–	–	↓
SH.HF	↑	↑	–	–	–	↑	–	↑	↑
Obese rats									
ADX.LF	↓	↓	↓	–	↓	↓	↓	–	↓
ADX.HF	↓	↓	↓	↓	↓	↓	↓	-	↓
SH.HF	–	↑	↓	–	–	–	–	–	–

The *in vivo* parameters are compared to SH. LF for each phenotype: _ no change, ↓ decreased and ↑ increased.

3.3.2. *In vitro* results

3.3.2.1. Glucose response of isolated islets

Glucose stimulated-insulin secretion (GSIS) was measured by statically incubating isolated pancreatic islets with various glucose concentrations (0-25 mM) as described in the Methods section. Percent insulin release and the half maximal effective glucose concentration (EC_{50}) were calculated.

Both basal secretion and GSIS were lower in islets isolated from sham and ADX lean rats compared to that in sham fa/fa rats, however the difference in the basal insulin secretion was not significant. There was no difference in % insulin output in the lean groups at any glucose concentrations except in ADX.LF rat islets where GSIS was generally higher and at 25 mM was approximately 67% more than in islets from sham rats ($p< 0.05$) (Figure 13 A). Neither ADX nor feeding a high fat diet had any significant difference in the calculated EC_{50} of islets from all lean rat groups or pancreatic insulin content in lean rats ($p> 0.05$) (Table 6).

In fa/fa rats, basal secretion and GSIS were higher in sham rats compared to ADX rats but this was significant only between the high fat diet fed rats ($p<0.05$). Adrenalectomized rat islets exhibited low insulin release which was further depressed by feeding high fat diet (Figure 13 B). Adrenalectomy did not affect islet glucose sensitivity in fa/fa rats since there was no difference in the calculated between EC_{50} of ADX.LF and SH.LF nor ADX.HF and SH.HF rat islets. However, feeding sham rats a high fat diet significantly increased islet glucose sensitivity by 60% ($p< 0.05$).

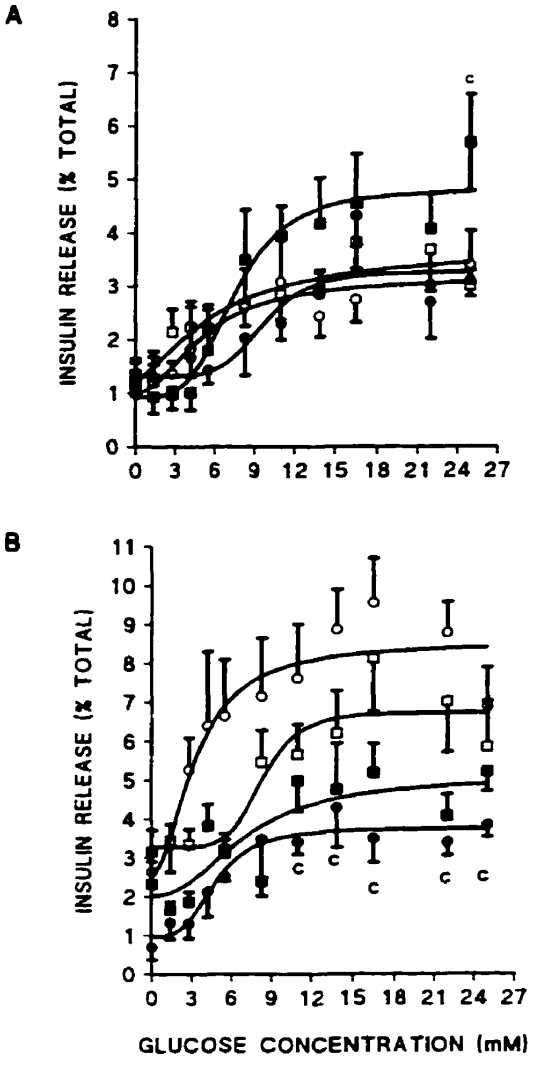

Figure 13. Effects of ADX and high fat diet on the glucose response in isolated islets of Zucker (A) lean and (B) fa/fa rats. Insulin release in response to several glucose concentrations was measured as described in Chapter 2 (sections 2.4.2 and 2.4.3). Total islet insulin content and EC_{50} are reported in Table 6. Data are expressed as means ± SEM for the number of animals shown in Table 6; ADX.LF ■; SH.LF □; ADX.HF ● and SH.HF ○ rats. ^{c} $P<0.05$ ADX.HF rats compared with SH.HF rats with in phenotype using SNK after ANOVA.

Table 6.

Effect of ADX and diet composition on glucose sensitivity (half maximal concentration, EC_{50}) and insulin content of isolated pancreatic islets from Zucker rats.

Animals	N	EC_{50} (mM)	Insulin content (nmol/islet)
Lean rats			
ADX.LF	6	7.57 ± 0.96	11.46 ± 2.87 [a]
ADX.HF	7	9.72 ± 1.84 [a]	13.42 ± 2.22 [a]
SH.LF	11	6.15 ± 3.27	13.77 ± 2.07 [a]
SH.HF	9	5.21 ± 1.23 [a]	14.10 ± 1.85 [a]
Obese rats			
ADX.LF	6	7.15 ± 3.76	34.97 ± 7.26
ADX.HF	4	4.83 ± 0.62	31.76 ± 4.68
SH.LF	6	8.34 ± 1.28	43.19 ± 5.75
SH.HF	6	3.32 ± 0.91 [d]	48.6 ± 10.6

The data are expressed as means ± SEM, N= animal donors. [a] $p< 0.05$ lean vs fa/fa rats, [d] $p <0.05$ high fat vs low fat using SNK after ANOVA.

Pancreatic islet insulin content of fa/fa rats was approximately 3-fold higher than in the lean rats ($p<0.05$). Neither ADX nor high fat diet induced significant changes in pancreatic islet insulin content of these rats ($p<0.05$).

3.3.2.2. Mannoheptulose response

In this study we wanted to investigate whether the ADX effects on MH inhibitory action on GSIS, which is a probe for measuring glucokinase activity in B-cells, would be antagonized by chronic feeding of a high fat diet. Data was analysed using one way ANOVA and SNK t-test.

Glucose (16.5 mM)-stimulated insulin release was measured in the absence (referred to as 0) or in the presence of different MH concentrations (1-100 mM). The MH responses are reported for each rat group, whereby GSIS in the absence of MH is compared to the other points on the same curve. Where applicable the MH response between groups is also compared. Mannoheptulose response was significantly affected by phenotype, ADX and diet (all, $p< 0.005$) and interaction between ADX and phenotye ($p< 0.05$). All MH concentrations used significantly inhibited GSIS release from lean rat islets ($p < 0.05$, Figure 14 A). And as expected MH at all concentrations used did not have a significant inhibitory effect on GSIS in islets isolated from sham fa/fa rats on either diet ($p >0.05$). In the ADX group, GSIS in pancreatic islets from ADX.LF rats was significantly inhibited by MH (3 mM and higher) ($p< 0.05$).

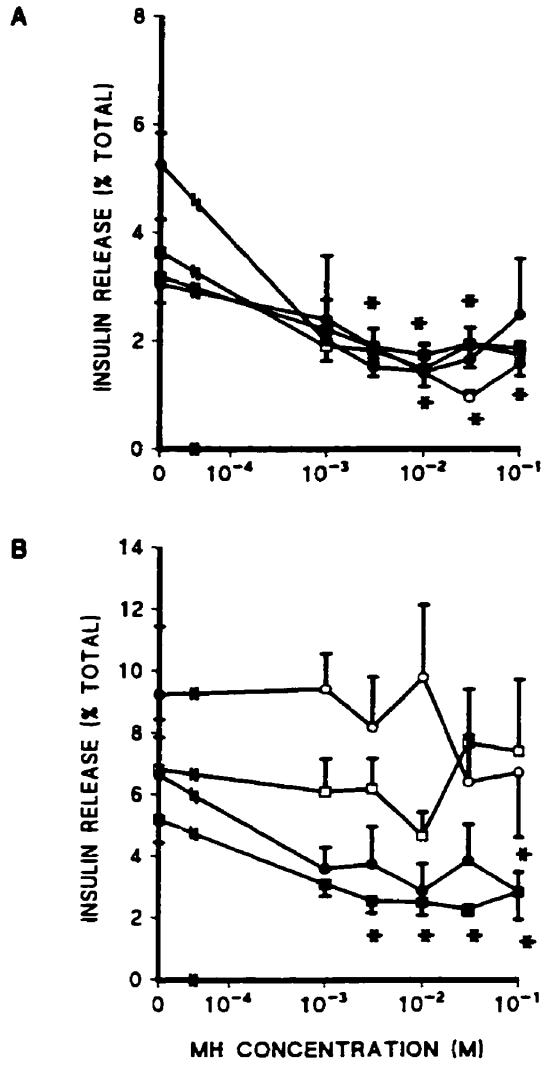

Figure 14. Effects of ADX and high fat diet on sensitivity to MH inhibition in isolated islet from Zucker (A) lean and fa/fa (B) rats. Insulin secretion was measured in the presence of 16.5 mM glucose in the absence (0) or presence of graded MH concentrations as described in the Methods section. The percentage of insulin released in the presence of MH is compared to the control for each rat group. Values are means ± SEM for lean rats: n=6, ADX.LF ■; n= 9, SH.LF □; n=6, ADX.HF ●; and n=6, SH.HF ○ rats and fa/fa rats: n=4, ADX.LF ■; n=5, SH.LF □; n=6, ADX.HF ●; n=6, SH.HF ○ rats. * $p < 0.05$ compared to GSIS (16.5 mM) without MH for each group within phenotype, using SNK after ANOVA.

In the ADX.HF isolated islets, a MH concentration of 10 mM lowered 16.5 mM GSIS by 56% ($p<0.05$). Although MH concentration of 100 mM significantly reduced GSIS ($p < 0.05$), this effect may not reflect MH effect on glucokinase in normal islets since maximum glucokinase inhibition is obtained by MH concentration of 20 mM (Sweet *et al.*, 1996). However, in some tumour cell lines with high glycolysis rate, a higher MH concentration of 30 mM was required to inhibit glucose uptake (Board *et al.*, 1995). Therefore feeding a high fat diet to ADX fa/fa rats did not fully abolish the effects of ADX on MH response.

3.3.2.3. Summary of insulin secretion in fa/fa rats

In vivo, ADX improved glucose tolerance without appreciable effects on glucose stimulated insulin secretion, indicating only partial amelioration of tissue resistance and continued stress on pancreatic B-cells. High fat diet tended to reduce but did not entirely erase the beneficial effects of ADX. The *in vivo* results were accompanied *in vitro* by an ADX-related reduction in islet insulin output at all glucose concentrations. However a high fat diet induced a left-shift in glucose sensitivity (lowering EC_{50}) which persisted in ADX fa/fa islets and MH sensitivity was partially restored in high fat fed fa/fa rats.

3.3.2.4. Glucokinase and hexokinase activity in isolated islets

3.3.2.4.1. Hexokinase

Glucose phosphorylating activity was measured in disrupted islet

preparations 5 wk after ADX or sham operation and the results are presented in Table 7 and Figures 15 and 16. Hexokinase activity was not different among lean rats ($p > 0.05$) even though, ADX.HF rats had a significantly higher hexokinase V_{max} than SH.HF rats ($p < 0.05$). Hexokinase glucose sensitivity (K_m) was not influenced by diet or ADX in lean rats. Also pancreatic islets from sham lean rats exhibited lower V_{max} than those from sham fa/fa rats regardless of the diet composition, but only SH.LF lean and fa/fa rats had a significant difference between the hexokinase K_m, with islets of fa/fa rats exhibiting a higher K_m than that exhibited by the islets of lean rats ($p < 0.05$, Table 7). In fa/fa rats, hexokinase activity was higher in SH.LF and this was significantly reduced by feeding rats a high fat diet ($p<0.05$). No effect of ADX was observed in low fat diet fed fa/fa rats. In sham rats, increasing dietary fat content significantly reduced both hexokinase K_m and V_{max} ($p < 0.05$, Table 7). However the overall hexokinase activity in fa/fa rats was not affected by either ADX or a high fat diet (Figure 15 B).

3.3.2.4.2. Glucokinase

In lean rats glucokinase (GK) activity was not significantly affected by either ADX or diet composition (Figure 16 A). In fa/fa rats both ADX and diet composition had significant effects on glucokinase activity. ADX significantly reduced glucose phosphorylation by glucokinase in islets of ADX.LF rats compared to islets from SH.LF rats ($p<0.05$). A high dietary fat content antagonized the effects of ADX , such that islets from ADX.HF rats exhibited a significantly increased glucokinase

phosphorylating rate that was not different from that of sham rats ($p > 0.05$) but significantly higher than that of islets from ADX.LF fa/fa rats ($p<0.05$) (Table 7). There was no diet effect on glucokinase V_{max} in sham fa/fa rats. Adrenalectomy significantly reduced glucokinase glucose sensitivity (K_m) in low fat diet fed rats ($p <0.05$) and this was not modified by feeding a high fat diet ($p>0.05$) (Table 7). A combination of low V_{max} and increased K_m in ADX.LF resulted in overall lower glucokinase activity compared to SH.LF rats ($p< 0.05$, Figure 16 B). Increasing dietary fat induced a significant reduction in the glucokinase glucose sensitivity in islets from SH.HF rats compared to those of SH.LF fa/fa rats ($p<0.05$).

3.3.2.5. FFA oxidation in isolated islets

Increasing dietary fat may alter the balance between glucose and FFA catabolism in islets. FFA oxidizing capacity can be assessed by measuring carbon dioxide (CO_2) production from radiolabelled [1- ^{14}C]- palmitic acid. This method can also be used to indirectly assess the activity of carnitine palmitoyltransferase-1 (CPT-1) in islets since this rate-limiting enzyme is responsible for transporting long chain FFA to the inner mitochondrial membrane where FFA ß-oxidation takes place (Chen *et al.*, 1994d; Zhou *et al*, 1996b). In this study we investigated whether ADX and/or chronic feeding of a high fat diet to Zucker rats would enhance FFA oxidation in isolated islets. Oxidation of 0.5 mM of [1-^{14}C] palmitic acid by isolated islets from Zucker rats was measured in the presence of low (3 mM) or high (25 mM) glucose concentrations. Results are shown in Table 8.

Table 7

Effects of ADX and a high fat diet on hexokinase and glucokinase kinetics V_{max} (pmol/µg protein/h) and K_m (mM) in pancreatic islets from Zucker rats.

Animals	N	Hexokinase		Glucokinase	
Lean rats		V_{max}	K_m	V_{max}	K_m
ADX.LF	3	184 ± 43	0.432 ± 0.142	1406 ± 40	14.6 ± 0.8
ADX.HF	5	225 ± 43.1	0.446 ± 0.127	2032 ± 624	10.2 ± 6.0
SH.LF	5	147 ± 33.5 [a]	0.438 ± 0.149 [a]	1538 ± 175	21.1 ± 3.6
SH.HF	5	84.1 ± 12.1 [c a]	0.380 ± 0.073	911 ± 414	7.5 ± 8.2
Obese rats					
ADX.LF	5	304 ± 100	0.667 ± 0.300	489 ± 102 [cd]	6.85 ± 3.5
ADX.HF	5	197 ± 40	0.457 ± 0.124	1314 ± 417	8.53 ± 6.3
SH.LF	5	522 ± 55	1.34 ± 0.164	1114 ± 100	2.78 ± 1.1 [c]
SH.HF	5	170 ± 24 [d]	0.327 ± 0.075 [d]	1327 ± 168	10.7 ± 2.5 [d]

V_{max} and K_m values were calculated using Eadie-Hofstee analysis (V vs V/S) using glucose concentrations of 0.05-0.5 mM for hexokinase and 6-16 mM for glucokinase after correction for hexokinase activity at a glucose concentration of 0.5 mM. Data are means ± SEM, N=animals in each group. [a] $p < 0.05$ lean vs fa/fa rats, [c] $p < 0.05$ sham vs ADX, [d] $p < 0.05$ high fat vs low fat using SNK after ANOVA.

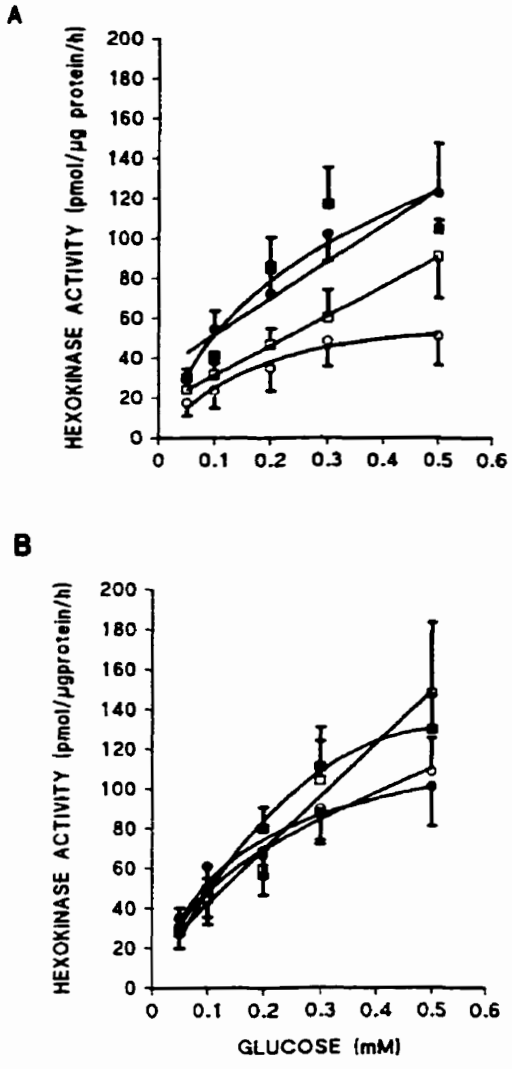

Figure 15. Effects of ADX and a high fat diet on pancreatic B-cell hexokinase activity (pmol/µg protein/h) in Zucker lean (A) and fa/fa (B) rats. Hexokinase activity was measured in disrupted islets and kinetic values, V_{max} and K_m (Table 7) were calculated by Eadie-Hofstee analysis and $V_{max} = (V/(1+K_m/S))$ using glucose concentrations of 0.05-0.5 mM. Values are means ± SEM of number of rats reported in Table 7 (ADX.LF ■, SH.LF □, ADX.HF ●, SH.HF ○ rats).

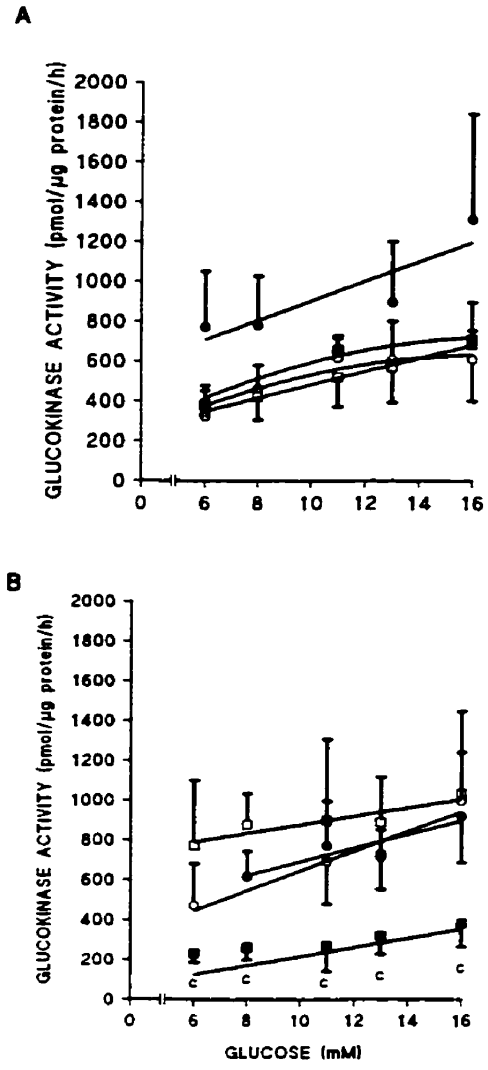

Figure 16. Effects of ADX and high fat diet on pancreatic B-cell glucokinase activity (pmol/µgprotein/h) in Zucker lean (A) and fa/fa (B) rats. Kinetic values (V_{max} and K_m) were calculated by Eadie-Hofstee analysis, V_{max}= (V/(1+K_m/S)) using glucose concentrations of 6-16 mM after correction for hexokinase activity at 0.5 mM glucose. Values are means ± SEM of animals reported in Table 7; ADX.LF ■, SH.LF □, ADX.HF ●, SH.HF ○ rats. ^{c} $p < 0.05$ ADX.LF compared to SH.LF fa/fa rats using SNK after ANOVA.

Analysis of the data by ANOVA, showed that islet production of CO_2 from palmitic acid was significantly influenced by diet composition, phenotype and ADX (all $p< 0.0001$). Glucose concentration had no effect on palmitic acid oxidation in islets from both phenotypes. In lean rats there was no difference among the groups. Neither ADX nor increasing dietary fat influenced islet FFA ß-oxidation in these rats.

In obese rats, CO_2 production from palmitic acid was significantly higher in SH.LF rat islets which was reduced by ADX ($p <0.05$). Feeding a high fat did not have a significant effect in islet FFA ß- oxidation in ADX rats in the presence of low or high glucose concentrations. In sham rats feeding high fat diet significantly reduced FFA oxidation. CO_2 production was more than 2-fold higher in SH.LF than in SH.HF rats at both low and high glucose concentrations ($p<0.05$) (Table 8).

3.3.2.6. Pancreatic islet TG

Elevation of pancreatic islet TG is reported to precede the loss of GSIS in animal models of diabetes including the Zucker diabetic fatty (ZDF-drt) rat (Lee *et al.*, 1997; Unger, 1995) and the obese diabetic (db/db) mouse (Zhou *et al.*, 1996a) and in OLETF rats (Man *et al.*, 1997) . The increase in islet TG concentrations mirrors the high plasma FFA concentrations that are present in these animals. Glucocorticoid replacement after ADX and feeding high fat diets leads to a rise in plasma FFA, while ADX reduces plasma FFA concentrations. In light of this we measured islet TG content in sham and ADX Zucker rats after feeding a high fat diet for 4 wk.

There were no significant differences between the islet TG content of the lean

rat groups ($p > 0.05$), (Table 8). Correlation between islet TG content and palmitic acid oxidation was found to be negative with 3 mM glucose ($r = -0.83$), while no correlation was found with 25 mM glucose. This indicated that in the presence of low glucose concentrations, FFA (palmitic acid) oxidation was increased in lean rats as expected. When the glucose concentration was high the islets oxidized glucose instead of FFA (Lee *et al.*, 1994), and a similar observation was reported in human studies (Sidossis and Wolfe, 1996). The islet TG content in lean rats was 50% lower than that of the fa/fa rats except for the lean ADX.HF in which TG content was actually 3-fold higher than ADX.HF fa/fa rats. Both ADX and high fat diet did not induce any significant changes in islet TG content of lean rats (Table 8).

In the obese rats, TG content of islets isolated from SH.LF and ADX.LF rats was not different. However, feeding rats a high fat diet significantly lowered islet TG content in both ADX and sham rats ($p < 0.05$). High dietary fat induced an approximately 33% reduction in islet TG in SH.HF compared to SH.LF and 85% reduction in ADX.HF islets compared to ADX.LF rats ($p < 0.05$). Lipid oxidation in fa/fa rat islets was not affected by increasing glucose concentrations. Feeding a high fat diet to Zucker rats for 4 wk did not result in higher islet TG but did produce similar metabolic changes in pancreatic islets as those documented for *in vitro* islet exposure to high FFA concentrations (Milburn *et al.*, 1995).

Table 8.

The effect ADX and diet composition on palmitic acid oxidation and TG content in isolated islets in Zucker rats.

Animals	N	TG (μg/islet)	N	CO_2 production (fmol/islet/h)	
				3 mM glucose	25 mM glucose
Lean rats					
ADX.LF	4	0.90 ± 0.70 [a]	8	4.96 ± 1.12	3.96 ± 0.10
ADX.HF	5	0.89 ± 0.28 [a]	9	5.02 ± 1.45	3.65 ± 0.50
SH.LF	10	1.17 ± 0.20 [a]	9	3.83 ± 0.30 [a]	3.62 ± 0.30 [a]
SH.HF	9	0.99 ± 0.20	7	3.67 ± 0.80	2.52 ± 0.60
obese rats					
ADX.LF	4	1.96 ± 0.07	6	7.60 ± 2.37	6.65 ± 3.50
ADX.HF	5	0.29 ± 0.05 [d]	4	4.51 ± 1.10	4.83 ± 0.62
SH.LF	4	2.14 ± 0.13	9	14.50 ± 3.31 [c]	15.10 ± 4.58 [c]
SH.HF	7	1.44 ± 0.10 [c d]	7	5.83 ± 0.93 [d]	4.19 ± 0.32 [d]

Values are means ± SEM, N = animal donors, [a] $p < 0.05$ lean vs fa/fa rats. [c] $p < 0.05$ sham vs ADX , [d] $p < 0.05$ high fat vs low fat diet using SNK after ANOVA.

Table 9.

Summary of the *in vitro* results of ADX and high fat diet compared to the SH.LF rats values in each phenotype.

Animals	Insulin release (% Total)		EC_{50}	Insulin content	Hexokinase		Glucokinase		FFA Oxidation	Islet-TG	MH
Lean	(2.8 mM)	(25 mM)			V_{max}	K_m	V_{max}	K_m			
ADX.LF	0.97 ± 0.27	5.96 ± 0.90	–	–	–	–	–	–	–	–	–
SH.LF	1.35 ± 0.22	3.40 ± 0.64									
ADX.HF	1.00 ± 0.98	3.10 ± 0.20	–	–	–	–	–	–	–	–	–
SH.HF	2.14 ± 0.43	3.00 ± 0.19	–	–	–	–	↓	–	–	–	–
Obese											
ADX.LF	1.86 ± 0.25	5.22 ± 0.5	–	–	–	↑	–	–	↓	–	↑
SH.LF	3.34 ± 0.4	5.85 ± 1.48									
ADX.HF	1.31 ± 0.36	3.85 ± 0.32	↓	–	↓	↑	↓	↑	↓	↓	↑
SH.HF	5.27 ± 0.82	6.89 ± 1.01	↓	–	↓	↑	–	–	↓	↓	–

The values of other rats are compared to those of SH.LF rats within each phenotype _ no change , ↓ decreased and ↑ increased.

3.3.3. Summary of *in vitro* results

Effects of ADX and high fat diet in isolated islets are summarized in Table 9. Pancreatic islet B-cell function in fa/fa rats was influenced by both ADX and diet composition. ADX restored MH inhibitory actions, and reduced glucokinase activity in low fat fed fa/fa rats without affecting pancreatic islet glucose sensitivity or insulin content. ADX reduced FFA ß-oxidation without changing islet TG content in low fat fed fa/fa rats. Feeding a high fat diet to ADX rats, reduced the effects of ADX on restoration of MH inhibitory actions, whether this effect was related to the restoration of glucokinase V_{max} to that of the control rats is not clear, since the K_m of ADX.LF and ADX.HF were not different. In addition, high fat diet increased islet glucose sensitivity in ADX rats. In contrast, high fat diet augmented ADX effects by further lowering FFA ß-oxidation in islets. In sham fa/fa rats a high fat diet increased pancreatic islet glucose sensitivity, reduced hexokinase V_{max} and K_m, increased glucokinase K_m, islet TG content and FFA ß-oxidation but had no effect of MH action. In lean rats ADX and high fat diet did not have significant effects on pancreatic functions.

3.4. Discussion

The purpose of this study was to investigate the possible effects of dietary fat on the beneficial effects produced by ADX on pancreatic islet function in fa/fa rats, specifically the normalization of islet sensitivity to glucose and the restoration of inhibitory action of mannoheptulose on GSIS (Kibenge and Chan, 1996). Our

hypothesis was that feeding rats for 4 wk a diet with 4-fold higher fat content to ADX fa/fa rats would negate all the beneficial effects observed in ADX fa/fa rats. Overall, the data did not fully support the hypothesis because ADX retained beneficial effects on B-cell function despite an increase in the dietary fat content that evoked measurable changes in *in vivo* glucose metabolism. Partial negation of some of the benefits observed may be due to continued stress on the islets invoked by insulin resistance induced by high fat/low fiber diet (Fairchild *et al.*, 1996; Storlein *et al.*, 1996). Signs of insulin resistance were observed in this current study. A relatively long period of recovery after surgery resulted in normalization of weight gain patterns of fa/fa rats to those of sham lean rats fed similar diets compared to a reduction of only 11% in weight gain observed in ADX fa/fa rats maintained on rat chow diet for 2 wk post surgery (Kibenge and Chan, 1996). ADX.LF rats gained 45% less weight than SH.LF fa/fa rats in this study and ADX reduced weight in lean rats as well.

3.4.1. *In vitro* responses to ADX and dietary fat

Studies of isolated islets were undertaken to assess the effects of ADX and diet on insulin secretion in the absence of internal environmental influences. The necessity for this is illustrated by comparing the *in vivo* and *in vitro* insulin secretion of ADX.LF rats, which clearly shows that low insulin secretion during OGTT is not due to impaired sensitivity of islets to glucose.

3.4.1.1. Insulin release and glucose phosphorylating activity

The results of our previous study (Kibenge and Chan, 1996) were only partially reproduced here; islets from fa/fa rats did demonstrate insensitivity to MH, however, pancreatic islet glucose sensitivity (EC_{50}) of fa/fa rats was similar to that of islets from lean rats. Sham obese rats did tend to have higher percent insulin release in response to low glucose than did sham lean rats, also shown previously (Kibenge and Chan, 1996). Adrenalectomy decreased both basal insulin secretion and GSIS in obese ADX.LF rat islets compared to the sham rats. The current data also confirmed the ability of ADX to restore MH sensitivity of B-cells from fa/fa rats, indicating that glucokinase plays some role in B-cell functional changes. Consistent with this observation were data showing that total glucose phosphorylation was decreased in ADX.LF obese rat islets, apparently due to a decrease in glucokinase V_{max} in this group. That glucokinase kinetic changes 2 wk after ADX were attributed to increased K_m but after 5 wk were due to decreased maximal velocity (V_{max}) and increased K_m might reflect sequential adaptation, first in the activity of the existing enzyme or its regulatory protein and second in the expression of glucokinase protein in the B-cells. This hypothesis needs to be confirmed.

Glucokinase activity and protein concentrations in B-cells are regulated by glucose. Both are increased in the presence of high glucose concentration but are decreased in presence of low glucose concentrations (Liang *et al.*, 1992; Chen *et al.*, 1994 b). Tiedge *et al.*, (1999) showed that glucokinase enzyme in islets exist in two fractions, a diffusible glucokinase fraction with high enzyme activity which could

be distinguished from an intracellularly bound fraction with low activity which is bound to a protein character that may have similar function as glucokinase regulatory protein in the liver (Reitz and Pagliaro, 1997). Glucose increases the active glucokinase fraction by releasing it from an intracellular binding site of protein character (Tiedge *et al.*, 1999). In turn glucokinase controls B-cell glucose usage and GSIS because inhibition of glucokinase activity by MH results in reduced islet glucose metabolism and GSIS (Sweet *et al.*, 1996). Pancreatic islets isolated from ADX rats have been reported to exhibit reduced glucose metabolism and this was accompanied by reduced insulin secretion as well insulin content when compared to sham controls (Borelli *et al.*, 1982). In this study pancreatic islet glucose usage was not measured, however, *in vivo* GSIS during OGTT and pancreatic islet insulin content tended to be lower in islets isolated from ADX than sham rats. An assumption might be made that ADX reduced islet glucose usage because of overall reduction plasma glucose concentration, thus resulting in adaptive changes in glucokinase kinetics leading to reduced GSIS in ADX rat islets. The increased islet glucose sensitivity by 60% in islets of sham fa/fa rats fed a high fat/low fiber diet compared to that of rats fed rat chow also reflects the adaptation of the islet B-cells to meet increased insulin demand as rats gain more weight and become more insulin resistant (McGarry, 1992). The idea that glucokinase is not a major contributor to the effect of dietary fat is supported by the MH sensitivity data, which essentially demonstrated parallelism between ADX.LF and ADX.HF obese islets even though a high fat diet inhibited the ADX-induced reduction on glucokinase V_{max}.

High fat feeding has been shown to impair glucose signal transduction mechanisms in pancreatic B-cells of normal rats by reducing gene expression of glucokinase and GLUT2 mRNA (Kim *et al.*, 1995), while in mouse pancreatic islets, high fat feeding reduced insulin content and proinsulin mRNA (Capito *et al.*, 1992). In both rat and mouse B-cells, there was a 50% reduction in GSIS from the islets of high fat fed rodents, compared to GSIS from islets of those fed a high carbohydrate diet (Capito *et al.*, 1992; Kim *et al.*, 1995; Capito *et al.*, 1999). Similar results on GSIS and proinsulin biosynthesis were obtained by prolonged *in vitro* exposure of islets from normal rats to a high concentration of FFA (Zhou and Grill, 1994). Thus the 16% fat diet used in this study induced similar changes in lean rat B-cell function as reported elsewhere, except that glucokinase function and islet insulin content were not affected, which might be a consequence of the less extreme fat content. In fa/fa rats, feeding of high fat diet failed to reduce insulin secretion or pancreatic insulin content even though the animals became severely insulin resistant, similar to previous results (Fürnsinn *et al.*, 1991). This may be due to the high endogenous plasma TG concentrations having already induced adaptive responses in B-cells in fa/fa rats.

Feeding ADX fa/fa rats a high fat diet reduced MH inhibitory actions on GSIS.. High dietary fat restored the ADX-reduced glucokinase V_{max} to those of sham rat values without a significant change on glucokinase K_m. A combination of high glucokinase phosphorylating rate and a lower islet EC_{50} was associated with a reduced MH inhibitory action on GSIS in islets from ADX.HF fa/fa rats. Although

it is suggested that increased plasma FFA concentrations modulates islet function, FFA concentrations in these rats were not different from those in ADX.LF fa/fa or from lean rats groups.

Because long chain FFA CoA competitively inhibit liver glucokinase Tippett and Neet, 1982), which is closely related to islet glucokinase, one would expect that feeding a high fat diet would have similar effect on B-cell glucokinase activity. Even though we did not analyze the FFA composition of our diet, it was a mixture of long chain unsaturated (from corn oil) and both long and short chain saturated FFA (from milk) and they would have different effects on the islet function (Storlien *et al.*, 1991). The mechanism of interaction between MH and FFA on pancreatic B-cell glucokinase remains to be elucidated.

3.4.1.2. Pancreatic TG content and FFA oxidation

Pancreatic islets from normal animals exposed to high FFA both *in vivo* and in vitro exhibit similar characteristics to those observed in obese animals, including islet hyperplasia, and enhanced low K_m glucose metabolism (Sako and Grill, 1990; Milburn *et al.*,1995). These changes are attributed to increased lipid metabolism in pancreatic islet B-cells (Hirose *et al.*, 1996; Zhou *et al.*, 1996a; Lee *et al.*, 1994) because inhibition of CPT-1 activity by etomoxir and acipimox *in vivo* (Paolisso *et al.*, 1998) improves fasting glucose and insulin sensitivity and, *in vitro,* etomoxir (Zhou and Grill, 1994) and α-bromostearate (Elks, 1993) abolish the inhibitory effect of FFA on glucose oxidation and GSIS. Therefore we also measured islet TG

content and FFA oxidation in lean and obese islets to see if these parameters could account for altered GSIS. Overall, no phenotype-related changes in islet TG content were detected, but in obese ADX.HF rats there was a marked reduction in TG content compared that in SH.HF or SH.LF obese islets. Recalling however that GSIS was similar in magnitude in ADX.LF and ADX.HF obese rats, it seems unlikely that altered islet TG or FFA oxidation can account for the dampening effects on GSIS in ADX compared with sham obese rats. Further, no differences in TG content or FFA oxidation were detected in lean rat islets from different treatments, even though GSIS was altered by both ADX and diet. However since we did not control for dilution effect due endogenous islet FFA concentration, the our values ß- FFA oxidation might be underestimated. Nonetheless, our studies have demonstrated phenotype-related alterations in islet lipid metabolism. In SH.LF obese islets FFA oxidation was 3-fold higher than in SH.LF lean islets but islet TG was not significantly different, suggesting a different set point for regulation or an alternative regulator. These data indicate that FFA oxidation in fa/fa rat islets is less well regulated by the changing balance between glucose and lipids under normal metabolic conditions, and may contribute to the inappropriate insulin secretion observed at low glucose concentrations.

3.4.1.3. Mode of FFA action in B-cells

Free fatty acids reduce glucose usage in pancreatic islets by inhibiting the action of several enzymes, receptors or membrane channels that are crucial to

glucose metabolism. Prolonged exposure to high concentrations of long chain FFA were shown to inhibit GSIS by stimulating K_{ATP} channels in pancreatic *B*-cells from ob/ob mice and in clonal pancreatic B-cells (Larsson *et al.*, 1996; Fernandez and Valdeolmillos, 1998), thus preventing the normal closure in the presence of high glucose concentrations. Free fatty acids also inhibit the pyruvate dehydrogenase complex in db/db mouse islets (Zhou *et al.*, 1996a) preventing further metabolism of the glycolytic product (pyruvate) by oxidation in Krebs cycle. By inhibiting acetyl-CoA carboxylase synthesis (Brun *et al.*,1997), FFA prevent the synthesis of malonyl-CoA normally formed in the presence of high glucose. Malonyl-CoA acts as an inhibitor to CPT-1 (Chen *et al.*, 1994d), and thus as an inhibitor of FFA acid ß-oxidation. This would result in FFA synthesis, and an increase in the cytosolic concentration of long chain acyl-CoA (LC-CoAs) which then act as a signaling molecule for insulin secretion (Assimacopoulos-Jeannet *et al.*, 1997; McGarry and Dobbins, 1999). The actual mechanisms by which FFA regulate insulin secretion are not clear. However, cytosolic LC-CoAs may participate in events leading to insulin exocytosis, such as an increase in the diacylglycerol pool which activates protein kinase C (Wollheim and Regazzi, 1990), and subsequent facilitation of insulin vesicle trafficking (Pfanner *et al.*, 1989) and/or by modulating the ion channel activity (Larsson *et al.*, 1996).

The inhibitory effects of high FFA concentrations on pancreatic B-cell GLUT2, glucokinase and insulin mRNA expression are suggested to occur through the transcription factor IDX-1 (Gremlich *et al.*, 1997). In this way increased FFA

oxidation negatively modulates pancreatic B-cell function. However, a report showing that insulin, glucokinase and islet amyloid polypeptide gene transcription were not reduced in a beta cell-derived MIN6 cells where IDX-1 gene expression was suppressed using antisense oligodeoxynucleotide (Kajimoto *et al.*, 1997) might indicate that FFA affects more than the transcription factor in B -cells or insulin-secreting cell lines. However, some of the studies mentioned above were conducted using clonal B-cells and need to be verified using normal islets. Furthermore, the effects of FFA on modifying enzyme action and synthesis have also been shown to occur in other tissues such as the liver, muscle and kidneys (Chatelain *et al.*, 1996; Oakes *et al.*, 1997; Jump *et al.*, 1999), thus pancreatic B-cells might also show similar responses to high FFA.

3.4.1.4. Interaction of neurohormones and islet B-cell function in fa/fa rats

The obesity syndrome of fa/fa rats is caused by the lack of functional receptors for leptin, a hormone secreted by white adipose tissues (Chua *et al.*, 1996b) that communicates information about peripheral body energy storage levels to the CNS. In fa/fa rats, leptin resistance may contribute to increased insulin secretion directly (Kieffer *et al.*, 1997) and through decreased activation of the sympathetic nervous system (Mizuno *et al.*, 1998). Inducing expression of normal leptin receptors reverses hyperinsulinemia in diabetic Zucker fatty rats (Wang *et al.*, 1998). Thus the question is how ADX, whose immediate effect is on plasma glucocorticoid concentrations, can also ameliorate insulin secretion abnormalities

even in the presence of leptin resistance.

In the CNS, NPY is a key regulator of autonomic nervous system outflow and leptin is shown to inhibit its synthesis in the hypothalamus (Uehara *et al.*, 1998), thereby increasing SNS activity in peripheral tissues. Intracerebroventricular injection of a dose of CRH insufficient to stimulate the HPA axis normalized both basal insulin secretion and GSIS in fa/fa rats, an action attributed to stimulation of the SNS (Rohner-Jeanrenaud *et al.*, 1989; Rohner-Jeanrenaud and Jeanrenaud, 1992). Most genetically obese animals including fa/fa rats exhibit lower concentrations of CRH in the hypophyseal-portal circulation than concentrations found in lean rats, and this deficiency is normalized after ADX (Plotsky *et al.*, 1992).

Thus, the current model of energy balance regulation which includes centrally-mediated effects on insulin secretion suggests the existence of two parallel regulators of NPY expression, CRH, and peripheral autonomic nervous activity. Adrenalectomy of obese animals produces similar effects to those induced by centrally injected CRH or leptin, indicating that a common efferent pathway is utilized to normalize energy balance and peripheral endocrine dysfunction. Therefore, in fa/fa rats, even without fully functional leptin receptors, by correcting the imbalance in the HPA axis ADX is able to mimic the actions of leptin. However, complete restoration of metabolic health is unlikely because there are several levels of interaction between the parallel systems. For example, leptin infused icv increased hypothalamic CRH concentrations, thereby potentiating its own direct effects on NPY (Uehara *et al.*, 1998). In addition, in the periphery leptin can inhibit

corticosterone secretion (Pralong *et al.*, 1998), while glucocorticoids increase leptin expression in adipocytes (Halleux *et al.*, 1998) but inhibit leptin's central anorectic effects (Zakrzewska *et al.*, 1997).

In fa/fa rats, reduced sympathetic and increased parasympathetic nervous system activity in the periphery is evident as early as 2 days post-natally (Moore *et al.*, 1985; Atef *et al.*, 1991). By 17 days of age these differences between lean and preobese rats are more pronounced as fa/fa rats exhibit reduced BAT thermogenesis in cold conditions (Greco *et al.*, 1987), and increased insulin secretion in response to parasympathetic stimulation (Jeanrenaud, 1985). Abnormal corticosterone secretion is observed at the onset of overt obesity (Fletcher *et al.*, 1986). It may be presumed that these developmental aspects of leptin resistance are secondary because neonatal hyperleptinemia in fa/fa pups is seen in 7 day old pups (Horwitz *et al.*, 1998).

3.4.2. *In vivo* response to ADX and dietary fat

3.4.2.1. Caloric intake and weight gain

The observation that ADX prevents the development of genetic obesity by reducing caloric intake was demonstrated in this study. Caloric intake of ADX.LF was normalized to that of the lean rats. It was also shown elsewhere that the reduced weight gain is partly due to the prevention of depositing more fat and reducing fat cell size in the retroperitoneal and gonadal fat pads because of reduced lipoprotein lipase in these cells of the ADX rats (Castonguay *et al.*, 1986; Bray *et al.*,

1992). Neither fat pad weights nor enzyme activities were measured in our study, however from visual inspection, there was less abdominal fat in ADX rats than in sham rats.

The diet used in this study (Triscari *et al.*, 1985) had only a 4-fold increase in fat whereas many other investigators use diets with 40-60% fat (Bray *et al.*, 1992). Nonetheless, this diet did induce several metabolic perturbations, including hyperphagia, weight gain, and glucose intolerance, indicating that for rats. 16% dietary fat is detrimental to health and that this diet in rats serves as a suitable model for human studies, where a 2-fold increase over the optimal dietary fat content induces metabolic pathologies (Stubbs *et al.*, 1995a; Prentice 1998). This diet also lacked adequate fiber, vitamins and minerals which could have played a significant role in animals response to the diet resulting in the metabolic changes observed (Bakker *et al.*, 1998). However, to evaluate the effect of a diet with 16% fat content it may be desirable to feed animals this diet but supplement it with adequate micro nutrients and fiber for a longer period to determine if certain trends noted in the results section might become significant alterations. The hyperphagia and consequent weight gain induced in both phenotypes was more evident in fa/fa rats, an observation also documented by others (Castonguay *et al.*, 1986; Bray *et al.*, 1992).

The interaction between ADX and dietary fat in adult fa/fa rats was investigated by Bray *et al.* (1992) where it was found that increasing dietary fat tended to reduce ADX benefits in both lean and fa/fa rats (body weight and

composition and adipocyte characteristics). However, that study used a diet containing 40% fat content and mature animals. Another study performed in 18 d old Zucker rat pups showed that ADX prevented development of obesity and hyperinsulinemia in rats fed both low and high fat diets (8g /kg vs178 g/kg) (Fletcher and MacKenzie, 1988 b). In both studies, ADX fa/fa rats fed a high fat diet had high body lipid content 32 d post surgery although in the Fletcher and MacKenzie study, there was no difference in the body weight between lean and fa/fa rats fed the two diets. Our data show that even though the weight gain patterns of fa/fa rats were normalized to those of sham lean rats, ADX fa/fa rats on either diet still weighed more than the ADX and sham lean rats probably due to the previously accumulated fat. ADX in adult fa/fa rats was shown to reduced retroperitoneal and inguinal, and subcutaneous fat pads but not the epididymal fat pad (Turkenkopf *et al.*, 1991; Bray *et al.*, 1992). However, benefits of ADX on wt gain were pronounced in this study compared to our previous one (Kibenge and Chan, 1996). The ADX.LF gained 45% less weight than the SH.LF in this study compared to 11% observed after 2 wk post surgery.

3.4.2.2. Insulin and glucose responses.

A positive correlation between high body weight and plasma insulin in humans (Peiris *et al.*,1986; Manolio *et al.*, 1992) and animals is well documented (Fletcher and McKenzie, 1988a, Bray *et al.*,1992). In this study fa/fa rats on both diets exhibited hyperinsulinemia, which was reduced but not normalized to the lean

concentrations by ADX. Failure of ADX to normalize plasma insulin concentrations of fa/fa rats to those of the lean rats could be attributed to the fact that fa/fa rats were still heavier than lean rats (Table 3). Dietary fat induced mild insulin resistance in lean rats while it worsened the existing glucose intolerance of fa/fa rats, as evidenced by the high glucose concentrations during OGTT. High fat diet can reduce glucose metabolism in all insulin sensitive tissues resulting in hyperglycemia and hyperinsulinemia (McGarry, 1992; Storlein *et al.*, 1996). Also, our results from lean rats show that *in vivo* insulin output was increased in order to maintain a normal plasma glucose concentration, an indication that this diet induced a compensatory response in pancreatic B-cells. However in order to make sure that the pancreatic response was increased, plasma C-peptide levels should have been measured because C-peptide is cosecreted in equal molar concentrations with insulin but has a longer half life than insulin and is used to evaluate the pancreatic B-cell insulin secretion capacity (Escobar *et al.*, 1999; Makuyana *et al.*, 1999). Thus, ADX did reduce insulin secretion in fa/fa rats *in vivo;* however these effects were accompanied by small *in vitro* changes in pancreatic B-cells. Furthermore, consumption of a high fat diet partially blocked the effects of ADX but this is likely at least partially due to secondary effects on tissue insulin sensitivity. In fa/fa rats ADX exerted its effects on insulin secretion and tissue sensitivity concurrently while also lowering plasma TG but not FFA concentrations. However, in this study we measured fasting FFA concentrations, so improvement in the fed state cannot be ruled out.

3.5. Conclusion

The data also showed that the benefits of ADX remained dominant, indicating that the presence of an intact HPA axis is essential for a high fat diet to have detrimental effect on pancreatic islet B-cell function and energy balance. The effects of ADX largely, although not entirely, superseded the dietary effects, demonstrating the importance of central regulation of metabolism as alluded to by others (Mizuno *et al.*, 1998). Results *in vitro* confirmed the dominant effect of ADX on several aspects of B-cell function, including the magnitude of GSIS and MH sensitivity. However, the high fat diet did increase sensitivity to low glucose even in ADX fa/fa rats, and this was accompanied by reduced MH inhibitory action. Thus, ADX and dietary fat have distinct actions on B-cell function and insulin secretion and the best way to normalize insulin secretion after ADX is achieved by maintaining animals on a low fat diet. The data also showed that lean and fa/fa rats responded similarly to increased dietary fat in most of the parameters measured *in vivo*, indicating that high fat diet can independently derange energy balance in both genetically susceptible and normal animals. We have shown that even a modest increase in dietary fat content induced metabolic changes *in vivo,* including weight gain and glucose intolerance.

Chapter 4

THE EFFECTS OF EXERCISE AND HIGH FAT DIET ON PANCREATIC ISLET B-CELL LESIONS IN ZUCKER fa/fa RATS.

4.1. Introduction

4.1.1. Hyperinsulinemia in obesity

Obesity of both humans and experimental animals is associated with hyperinsulinemia, insulin resistance, hypertriglyceridemia, and impaired glucose tolerance but also with normal or slightly elevated fasting glucose levels (Jeanrenaud 1985; Manolio *et al.*, 1992). The degree of hyperinsulinemia in humans is positively correlated with body mass index (BMI, kg/m^2) in both adults and children (Bruce *et al.*, 1994; Jiang *et al.*, 1996).

The causes of hyperinsulinemia in obesity are still not clearly understood but an increased pancreatic B-cell response to stimulation plays a significant role. Several lesions have been identified in islets from genetically obese rodents. In ob/ob mice, dysfunction of calcium and ATP-sensitive potassium (K_{ATP}) channels (Fournier *et al.*, 1990), abnormal regulation of cAMP (Black *et al.*, 1988) and increased glucose 6-phosphatase activity (Khan *et al.*, 1995) have been described. In fa/fa rats, reduced adrenaline-mediated inhibition of GSIS was evident under some experimental conditions (Cawthorn and Chan, 1991); however, K_{ATP} channel function was not different between fa/fa and lean Zucker rats (Chan and MacPhail, 1996). In genetically obese rodents such as Zucker diabetic fatty (ZDF) rats (Lee *et al.*, 1997) and diabetic (db/db) mice (Unger *et al.*, 1995), which eventually

develop non-insulin dependent mellitus diabetes, islets showed increased lipogenic capacity and loss of the glucose transporter GLUT2 (Johnson *et al.*, 1990).

Pancreatic islets or pancreases isolated from genetically obese animals such as cp/cp rats (Timmers *et al.*, 1992; Chan *et al.*, 1995), fa/fa rats (Curry and Stern, 1985; Chan *et al.*, 1993) and ob/ob mice (Chen *et al.*, 1993) exhibit increased sensitivity to low glucose concentrations. Also, these animals possess larger pancreatic islets on average than do normal rodents (Chan *et al.*, 1985; Starich *et* al., 1991; Timmers *et al.*, 1992; Tomita *et al.*, 1992) however, the hypertrophy and hyperplasia are said not to explain the magnitude of excess insulin secretion observed in the obese rodents. Increased parasympathetic nervous system drive to pancreatic B-cells has been shown to greatly enhances GSIS in obese animals and may contributes to the early onset of hyperinsulinemia (Rohner-Jeanrenaud *et al.*, 1983; Atef *et al.*, 1991; Stubbs and York, 1991).

4.1.2. Exercise effects on metabolism

The benefits of aerobic exercise include prevention of weight gain, reduction of plasma insulin levels in both normal and NIDDM individuals, and normalization of glucose tolerance (Giacca *et al.*, 1998; Ayre *et al.*, 1998) by increasing insulin sensitivity in many tissues (King *et al.*, 1987; Torgan *et al.*, 1993). Exercise normalizes energy and hormonal imbalances through its potential to increase sympathetic nervous system activity in many tissues (Howlett *et al.*, 1999), an effect that seems to be mediated by CRH. Exercise being a form of stress stimulates

the137 activity of the HPA axis since both ACTH and cortisol concentrations are increased during exercise compared to controls (Tabata *et al.*, 1991; Duclos *et al.*, 1998). However, the anorexic effect of exercise is attributed to CRH, which is reported to be elevated in the periphery in humans (Luger *et al.*, 1991; Inder *et al.*, 1998) and in rats (Watanabe *et al.*, 1991) during exercise. The anorexic effect of exercise is abolished by a CRH antagonist (α-helical CRH_{9-41}) given to animals before exercise (Rivest and Richard, 1990; Richard, 1995). The effects of administration of CRH on regulation of food intake and glucose tolerance have been demonstrated in genetically obese Zucker fa/fa rats (Arase *et al.*, 1989; Rohner-Jeanrenaud and Jeanrenaud, 1992). The reduction of food intake induced by CRH administration only lasts for 6 h then the animals resume their normal eating behavior. This short-lived effect was shown to be similar to that induced by exercise (Luger *et al.*, 1991; Cabanac and Morrissette, 1992). Also exercise increases energy expenditure by increasing thermogenesis in BAT. Thermogenesis in BAT is also increased by ADX and icv injection of CRH (Holt and York, 1989). Corticotropin releasing hormone has also recently been shown to mediate the anorexigenic effects of leptin within the hypothalamus (Uehara *et al.*, 1998).

4.1.2.1. Exercise and tissue gene expression

Exercise regulation of gene expression of proteins involved in lipid and glucose metabolism is well documented. In the liver of exercised animals different effects are seen depending on the diet fed; in cornstarch-fed trained rats, enzymes

involved in lipid metabolism were affected, including reduction of fatty acid synthase and reduced expression of acetyl CoA-carboxylase mRNA, while in sucrose- fed trained rats only L-pyruvate kinase activity was reduced (Fiebig *et al.*, 1997). In skeletal muscle and adipose tissue, GLUT4 mRNA expression and protein translation (Neufer *et al.*, 1992; Dela *et al.*, 1994) and its translocation into the plasma membranes were increased (Hansen *et al.*, 1998; Kennedy *et al.*, 1999). Exercise also increased skeletal muscle hexokinase II (Koval *et al.*, 1998), citrate synthase (Banks *et al* ., 1992) and lipoprotein lipase (Ladu *et al.*, 1991; Seip *et al.*, 1995). Exercise reduced pyruvate dehydrogenase complex activity (Denyer *et al.*, 1991) and malonyl CoA (Elayan and Winder, 1991). Increased skeletal muscle GLUT4 synthesis and activity is induced by both low and high intensity activity in fa/fa rats (Banks *et al.*, 1992).

4.1.2.2. Pancreatic B-cell response to exercise

The beneficial effects of aerobic exercise on the pancreas are demonstrated in NIDDM prone OLETF rats, where aerobic exercise (cage wheel-running) prevents the pancreatic degenerative lesions (eg; enlargement, fibrosis development and deposition of TG droplets) associated with NIDDM (Shima *et al.*, 1996; 1997; Man *et al.*, 1997). Exercise also increases SNS activity in the pancreas resulting in inhibition of insulin secretion by adrenaline (Wasserman *et al.*, 1995; Houwing *et al.*, 1995). Pancreatic islet B-cells isolated from aerobically exercised rats exhibit reduced insulin secretion (Farrell *et al.*, 1992) and have

reduced expression of proinsulin mRNA and glucokinase mRNA but normal GLUT2 mRNA (Koranyi *et al.*, 1991). Zawalich and colleagues reported that insulin secretion in response to low glucose concentrations was not affected by exercise while that in the presence of 10 mM glucose was reduced by 40-50% (Zawalich *et al.*, 1982). However, due to lack of changes in pancreatic islet glucose utilization it was concluded that exercise reduced pancreatic glucose sensitivity independent of islet cell glucose utilization. Although pancreatic glucose phosphorylation rate was not measured, hepatic glucose phosphorylation rate was shown to be reduced and hexokinase and glucokinase activities were reduced by 15% and 40%, respectively. The reduced liver hexokinase and glucokinase activities were attributed to hypoinsulinemia induced by exercise (Zawalich *et al.*, 1982). Also Kahn *et al.* (1990), King *et al.* (1990) and Engdahl *et al.* (1995) reported that aerobic exercise reduced B-cell function while increasing insulin sensitivity in target tissues in exercised men (Kahn *et al.* 1990; King *et al.* 1990; Engdahl *et al.*, 1995). However, not all types of exercise reduce insulin secretion since resistance exercise has been shown to increase insulin secretion in response to arginine and glucose in Sprague-Dawley rats (Fluckey *et al.*, 1995).

Although exercise was shown to reduce glucokinase gene expression in B-cells (Koranyi *et al.*,1991) and reduce its activity in liver (Zawalich *et al.*, 1982), the effect of exercise on B-cell glucokinase activity in normal animals versus obesity syndromes has not been studied. Therefore in this study we investigated the effects of exercise on pancreatic hexokinase and glucokinase phosphoryating activities in

rat chow fed and high fat fed Zucker rats. Having recently shown that ADX reduced glucokinase activity in islets from fa/fa rats (Kibenge and Chan, 1996), we also wanted to see if these effects could be duplicated by exercise. Since Both ADX (Arase *et al.*, 1989) and exercise increase CRH (Inder *et al.*, 1998) and result in increase SNS activity in peripheral tissue including the pancreas (Houwing *et al.*, 1995; Wasserman *et al.*, 1995).

4.1.3. Hypothesis, objectives of the study and choice of exercise program

As outlined above, basal insulin and GSIS from pancreatic islets isolated from genetically obese Zucker rats are relatively high compared to that from lean rat islets and the GSIS fails to respond appropriately to inhibitors of glucose metabolism, such as starvation and mannoheptulose (Chan *et al.*, 1993; Kibenge and Chan 1995), these defects that were normalized by ADX. ADX (Arase *et al.*, 1989) and aerobic exercise (Houwing *et al.*, 1995; Wasserman *et al.*, 1995, Houwing *et al.*, 1997) increase SNS activity in the peripheral tissues including the endocrine pancreas, and because of the report by Koranyi *et al.* (1991) that the reduced GSIS in islets from exercised rats was due to the ability of exercise to inhibit glucokinase gene expression and glucokinase activity.

The effects of ADX such as changes in body weight, food intake (Castonguay *et al.*, 1986) and in islet B-cell glucokinase (Kibenge and Chan, 1996) are observed within 2 wk, while others like increase in insulin sensitivity (Chavez *et al.*, 1997) and hepatic enzyme activity (Langley and York, 1992) and leptin (Spinedi and Gaillard,

1998) are modified with in 7 d after ADX. However, greater weight loss and change in metabolic and hormonal changes are observed after a longer period after ADX (Bray *et al.*, 1992). Acute exercised is reported to lower body set point in rats (Cabanac and Morrissette, 1992), an effect shared by ADX (Gosselin and Cabanac, 1997) and acute icv CRH infusion (Cabanac and Richard, 1995). Also acute exercise and increased insulin sensitivity in peripheral tissue (Burstein *et al.*, 1992). The long term effects of exercise by swimming in warm water include reduction weight gain and food intake (Jen *et al.*, 1992), reduction in adipocyte size as well improvement of glucose metabolism (Craig and Foley, 1984). The reported exercise effects of exercise on islet B-cell glucokinase activity and GSIS were produced by treadmill running 90 min /d for 6 days/ wk for 3 wk (Koranyi *et al.*, 1991). Adrenalectomy (Arase *et al.*, 1989) and exercise regulates insulin secretion through activating SNS (Houwing *et al.*, 1995), and high fat diets reduce sympathetic nervous system (Lu *et al.*, 1998) activity we made the following hypothesis

4.1.3.1. Choice of exercise program

In choosing the exercise program we considered the cost, ease of administration and comfort of the rats. A swimming exercise program was chosen because it has been observed to acutely induce measurable changes in insulin sensitivity in obese rats (Burstein *et al.*, 1992) and is also less stressful to obese humans with conditions like hypertension, asthma and osteoarthritis who are unable

to engage in more strenuous exercise programs such as jogging (Tanaka *et al.*, 1997). Although most swimming protocols in rodents call for adding weights on the rats' tails or backs to reduce buoyancy of obese animals, this practice also increases mortality rate during swimming if they are not carefully watched during exercise (Walberg *et al*,. 1984). Therefore in our protocol we did not use extra weight and the rats were allowed to swim freely. Water and room temperature was maintained at 34-35°C (Craig and Foley, 1981), which is near the rats' body temperature of 37-38°C to minimize stress induced by cold water temperature exposure (Leitner, 1989).

Treadmill running was another exercise that could have been used in this study. However, it was eliminated because in order toinduce the rats to run they are subjected to a shock treatment (Ardevol *et al.*, 1995). The shock treatment might have induced physiological adaptations either not related or additive to those induced by exercise.

4.1.3.2. Hypothesis:

The hypothesis of this study was that swimming exercise for 1h/d, 5 d/wk for 4 wk would produce pancreatic islet B-cell benefits similar to those of ADX in fa/fa rat islets including reduction of pancreatic islet glucose sensitivity, restoration of MH inhibitory action on GSIS and reduction of glucokinase activity in rat chow fed fa/fa rats. Also that increasing dietary fat would negate exercise actions on insulin secretion in fa/fa rats.

4.1.3.3. Objectives

The objectives were:

1). To compare both the *in vivo* and *in vitro* insulin responses to glucose stimulation in exercised fa/fa rats fed low or high fat diet.

2). To investigate any interactions between exercise and high fat diet on pancreatic islet glucose sensitivity, B-cell TG content and FFA metabolism and glucokinase activity.

3). To compare the effectiveness of exercise and ADX in improving B cell function in fa/fa rats. Identical experiments were carried out in age-matched lean rats so that the effects of phenotype could also be assessed.

4.2. Materials and methods

4.2.1. Animals and diet

Sixty-four, 5 wk old Zucker (32 lean and 32 obese) rats were purchased from Charles River and were fed regular rat chow for one week. At 6 wk of age the animals were separated and housed individually in plastic cages. Half the rats of each phenotype were fed rat chow (LF) while the other half fed a high fat diet (HF) *ad libitum* for 4 wk. The animals were further subdivided into exercise (LF.EX or HF.EX) or sedentary control (LF.SED or HF.SED) groups for both phenotypes. The high fat diet (condensed sweetened milk diet) was made according to Triscari *et al.* (1985) as previously described (Chapter 2: General Methods).

4.2.2. Exercise

At 6 wk of age eight Zucker rats consuming HF and LF diets of both phenotypes swam daily (Monday to Friday) from 0900-1000 h in a 150 cm diameter pool with enough room to exercise 8 rats at the same time. The water depth of > 30 cm was used to prevented the animals from resting at the bottom of the pool. Water and room temperatures were maintained at 34° - 35°C (Craig *et al.*, 1981) to eliminate cold induced stress (Leitner, 1989). The protocol started with the animals swimming 20 min on the first day and this was gradually increased so that by the end of the week they could swim for 60 min. After swimming the animals were taken out of the water, towel dried and returned to their cages in the animal quarters.

4.2.3. Assessment of exercise and high fat induced stress, caloric intake and weight gain

In order to make sure that the animals were adapting to the exercise routine without excessive stress, blood samples were collected from the tail vein on Saturdays between 0900-1000 h starting at 5 weeks of age, before the animals started swimming and weekly after that for measurement of glucose and corticosterone. Glucose and corticosterone were also measured at the end of 4 weeks of exercise and diet. Food intake was measured on three consecutive days of each week and then averaged to calculate daily food intake while animals were weighed weekly. Total weight gain was determined as the difference between the

weight of the rats at 5 wk and 10 wk of age.

4.2.4. *In vivo* metabolic assessment and *in vitro* B-cell function measurements

Fasting plasma corticosterone, glucose, insulin, TG, FFA measurements and oral glucose tolerance tests were carried out as described in the General Methods (Chapter 2). Pancreatic islet isolation and culture, and measurement of GSIS, glucose phosphorylating activity and pancreatic FFA oxidation were all also carried out as described in Chapter 2.

4.2.5. Statistical analysis

Data are expressed as means ± SEM. They were analyzed using repeated measure ANOVA using SAS statistical package, however differences between means were assessed using SNK. In the Results section, "treatment" means exercise or sedentary and the significant effect of exercise is denoted by $^{c.}$ whereby, LF.EX are compared with LF.SED and HF.EX are compared with HF.SED. The significant effect of diet is noted by ^{d} , whereby the LF.EX was compared with HF.EX rats and similar comparisons were carried out for the sedentary group. All results were considered significant at $p \leq 0.05$.

4.3. RESULTS

4.3.1. *IN VIVO* RESPONSES

4.3.1.1. Caloric intake

Analysis of the data with repeated measure ANOVA showed that caloric intake was significantly influenced by phenotype diet, age (all, $p<0.001$), exercise ($P<0.05$). Caloric intake was significantly influenced by 3-way interaction between phenotype and age ($p<0.001$), and 4 -way interaction of phenotype, exercise and age ($P<0.05$).

Caloric intake in fa/fa rats was approximately 1.5-2-fold higher than that of lean rats regardless of the diet composition (Figure 17). Exercise prevented the increased caloric intake in low fat fed rats that is normally associated with age. Introducing a high fat diet at 6 wk significantly increased food intake of both HF.EX and HF.SED fa/fa rats by approximately 15% compared to intake when they were fed rat chow during 5 - 6 wk of age ($p <0.05$). During the 6-7 wk age period HF.SED consumed 13% more calories than LF.SED rats ($p< 0.05$), while the HF.EX consumed 35% more calories than LF.EX fa/fa rats ($p< 0.005$). Age also affected food intake in high fat fed rats and in LF.SED but not in LF.EX rats. Caloric intake at 10 wk was 11%, 20% and 14.5% less than calories consumed at 6 wk for HF.SED, HF.EX and LF.SED fa/fa rats respectively, ($p< 0.05$). At 10 wk of age, HF.EX caloric intake was similar to that of the LF.EX rats, while HF.SED continued to eat more than LF.SED rats by 18% ($p< 0.05$). Overall, caloric intake in the fa/fa rats was decreased by exercise and age but increased in response to a high dietary

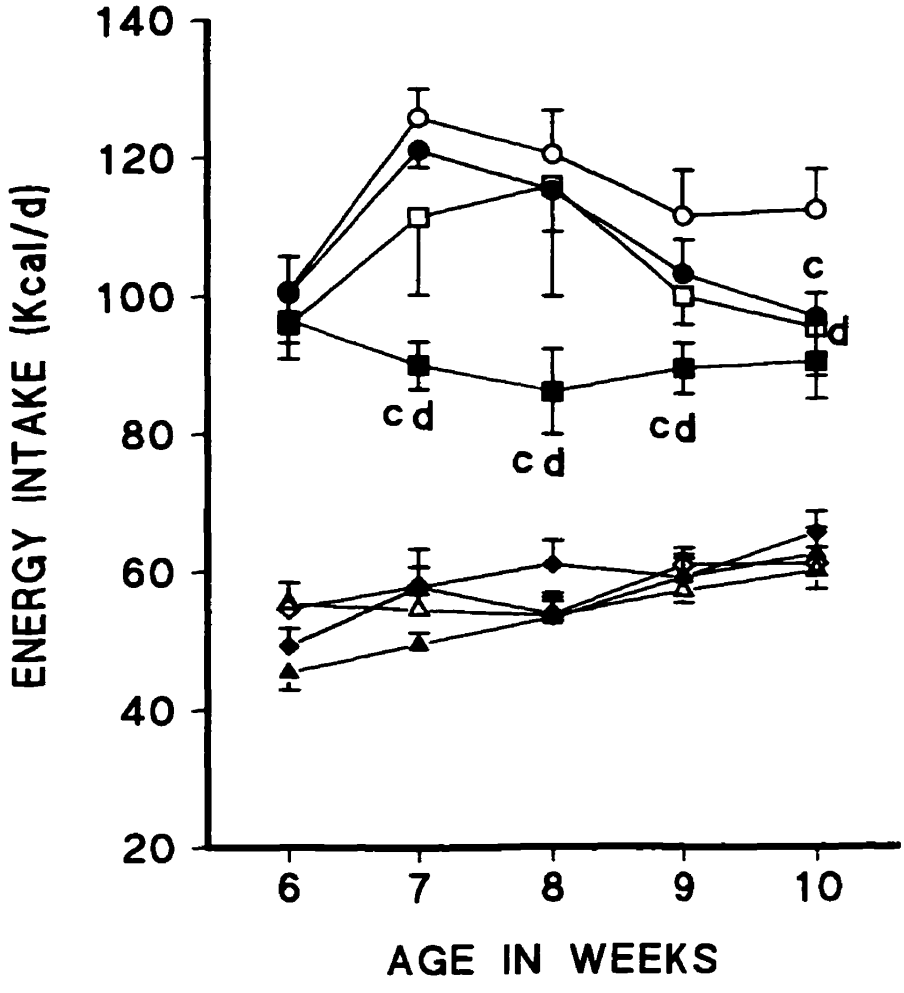

Figure 17. The effects of exercise and high fat diet on caloric intake in Zucker rats. Caloric intake was measured as described in the General Methods section. Data are expressed as means ± SEM of 8 rats per group. Lean rats: LF.EX ▲, LF.SED △, HF.EX ◆, HF.SED ◇ and fa/fa rats: LF.EX ■, LF.SED □, HF.EX ● and HF.SED ○ rats. ^{c} $p < 0.05$ exercised rats compared to sedentary rats fed similar diet, $^{d}p < 0.05$ low fat compared to high fat fed rats with in each phenotype using SNK after ANOVA.

fat content ($p < 0.005$) as shown in Figure 17.

In lean rats, neither increasing dietary fat content nor exercise had a significant influence on caloric intake (Figure 17).

4.3.1.2. Body weight

Diets with very high fat content (30%-60%) have been shown to significantly increase weight gain in obesity-prone lean rats and Zucker fa/fa rats (Hill *et al.*, 1992; Bray *et al.*, 1992; Castonguay *et al.*, 1986). In this study, the effects on body weight of a 4-fold increase of fat content over the regular rat chow in exercised and sedentary Zucker lean and fa/fa rats were evaluated. The animals were weighed weekly from 5 wk to 10 wk of age and the results are shown in Figure 18 A and B and Table 10. Analysis of variance revealed that body weight change was significantly influenced by phenotype, diet and age (all $p < 0.0001$). Two-way interactions between phenotype and diet, and phenotype and age significantly influenced body weight changes in Zucker rats ($p < 0.0001$).

In obese rats (Figure 18 B) there was no difference in weight among the rat groups at 5 wk. However, by the end of 10 wk there was a significant difference in weight between the low fat and the high fat diet rats. High fat diet significantly increased weight gain in both exercised and sedentary fa/fa rats. The HF.SED fa/fa rats gained approximately 45% more weight than the LF.SED fa/fa rats, while the HF.EX gained 37% and 85% more weight than LF.SED and LF.EX rats, respectively (Table 10). Exercise significantly reduced weight gain in the rat chow fed fa/fa rats,

such that at the end of the study, the LF.EX rats had gained 26% less weight than the LF.SED fa/fa rats ($p < 0.05$), and high fat diet blocked the effects of exercise on preventing weight gain as the final weight of HF.EX rats was significantly higher than that of LF.EX and LF.SED rats ($p< 0.05$) but similar to that of the HF.SED rats Similarly, high fat diet significantly increased weight gain in sedentary fa/fa rats ($p< 0.05$).

In lean rats (Figure 18 A), exercise had no effect on weight gain in low fat fed rats. High fat exercise lean rats gained approximately 15% more weight than the LF.EX and HF.SED lean rats (Table 10) ($p<0.05$).

Phenotype significantly affected body weight independent of diet or exercise regime. Obese rats' weight was 1.5-fold higher than the weight of the lean rats at 5 wk of age and this difference was increased to 1.8 fold in the LF.SED rats by 10 wk of age. Feeding a high fat diet significantly increased weight gain in fa/fa rats regardless of physical activity ($p<0.05$). The HF.EX fa/fa rats gained 110% more weight than the HF.EX lean rats, while the HF.SED fa/fa rats gained 126% more weight than the HF.SED lean rats ($p<0.05$). Exercise reduced but did not normalize the rate of body weight gain of fa/fa rats compared to the lean rats since the weight difference between LF.EX fa/fa rats was still 68% and 60% higher than the final weight of LF.EX and LF.SED lean rats, respectively. Increased dietary fat induced weight gain in exercised lean rats but reduced weight gain in control lean rats, while this had an opposite effect in the fa/fa rats (Table 10).

Table 10.
The effect of exercise and dietary fat on body weight changes in Zucker rats

Animals	N	Initial Wt (5 wk) g	Final weight (10 wk) g	Total wt gained g	% wt change vs LF.SH
Lean rats					
LF.EX	8	100.3 ± 3.4 [ab]	168.8 ± 6.9 [a]	68.5 ± 4.2 [a d]	-9.6
LF.SED	8	102.4 ± 3.4 [ab]	178.1 ± 6.9 [a]	75.8 ± 5.0 [a]	
HF.EX	8	106.7 ± 3.3 [ab]	186.0 ± 6.2 [a]	79.3 ± 3.8 [a c]	+ 4.6
HF.SED	8	104.0 ± 3.7 [ab]	173.0 ± 5.3 [a]	69.0 ± 2.4 [a]	- 8.7
Obese rats					
LF.EX	8	169.6 ± 11.9 [b]	284.4 ± 8.3 [c b]	114.8 ± 4.2 [c b d]	- 25.7
LF.SED	8	153.6 ± 9.3 [b]	308.2 ± 3.8 [c]	154.6 ± 11.6 [c d]	
HF.EX	8	179.8 ± 8.2 [b]	391.9 ± 13.2	212.1 ± 10.1	+37.2
HF.SED	8	167.7 ± 11.0 [b]	391.3 ± 13.3	223.6 ± 11.8	+44.6

Values are means ± SEM, N= animals in each group. [a] $p < 0.005$ lean compared to fa/fa rats, [b] $p < 0.005$ for age effect, [c] $p < 0.05$ exercised rats compared to sedentary rats fed a similar diet, [d] $p < 0.05$ low fat compared to high fat diet using ANOVA followed by SNK.

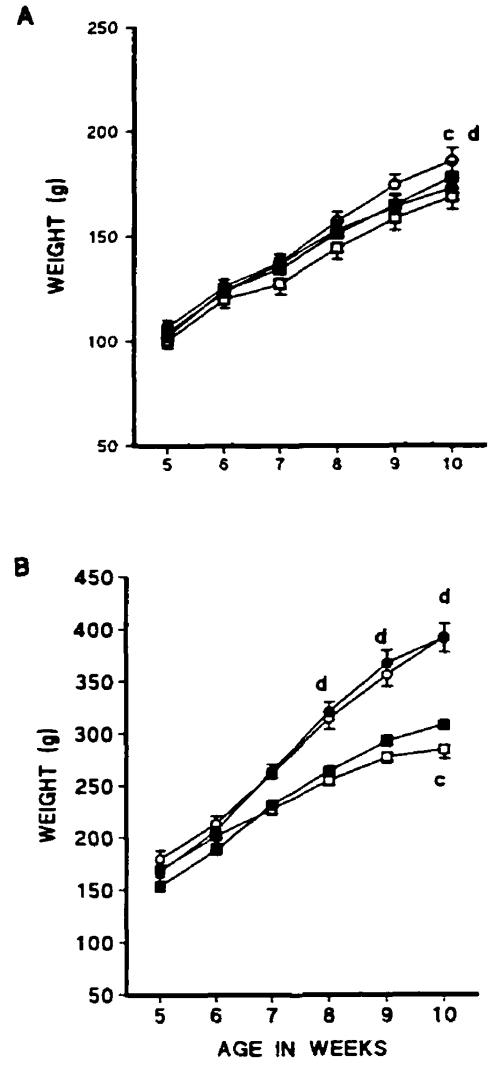

Figure 18. Effects of exercise and high fat diet on weight gain in Zucker (A) lean and (B) fa/fa rats. Rat were weighed weekly as described in General Methods. Data are expressed as means ± SEM of 8 rats per group: LF.SED ■, LF.EX □, HF.SED ● and HF.EX ○ rats. ^{c} $p < 0.05$ exercised rats compared to sedentary rats fed a similar diet, ^{d} $p < 0.05$ high fat diet fed rats compared to low fat diet fed rats that received similar treatment within phenotype using SNK.

4.3.1.3. Plasma corticosterone glucose, insulin, TG and FFA

4.3.1.3.1. Corticosterone

During the experimental period blood samples were collected from the animals in the fed and rested state (24 h after exercising), before and one week after introducing them to high fat diet and/or exercise. Plasma corticosterone and glucose levels at 6 and 7 wk were determined. Plasma corticosterone was not different in Zucker rats before and after one week after the introduction of exercise (Table 11). At the end of the study fasting plasma corticosterone levels were measured and there was no difference between the lean and fa/fa rat corticosterone levels (Table 12). However, in both phenotypes, the fasting corticosterone levels were significantly higher ($p < 0.05$) than before the animals were introduced to exercise and diet at 6 wk and one week into the diet and exercise regimen at 7 wk of age in the fed state (Table 11 and 12)

4.3.1.3.2. Plasma insulin concentrations

Fasting hyperinsulinemia was evident in fa/fa rats and this was increased by feeding a high fat diet (Table 12). Plasma insulin concentrations were approximately 5-fold higher in chow fed fa/fa rats than in lean rat and exercise did not induce any changes. However, a high fat diet significantly increased fasting insulin concentrations by 4-fold and 2-fold in HF.EX and HF.SED rats compared to the low fat fed rats ($p < 0.05$). During OGTT, there was no significant difference in the insulin response in LF.EX and LF.SED rats. There was an increase of 190% and

Table 11.

Plasma corticosterone concentrations in Zucker rats before (6 wk) and during (7 wk) exercise.

Animals	N	Corticosterone (ng/ml)	
		6 wk	7 wk
Lean rats			
LF.EX	8	381 ± 82	328 ± 61
LF.SED	8	483 ± 72	489 ± 74
HF.EX	8	408 ± 60	371 ± 70
HF.SED	8	408 ± 82	445 ± 91
Obese rats			
LF.EX	8	446 ± 63	252 ± 74
LF.SED	8	394 ± 47	479 ± 76
HF.EX	8	425 ± 42	393 ± 48
HF.SED	8	414 ± 75	324 ± 34

Corticosterone concentrations in plasma collected in 6 wk and 7 wk old rats were measured as described in Chapter 2 in the General Methods section.

Data are expressed as means ± SEM, N= animals per group.

177% in insulin concentration within the first 10 min of glucose administration in LF.EX and LF.SED rats, respectively. Exercise also reduced insulin resistance in rat chow fed fa/fa rats as indicated by 43% lower plasma insulin concentrations in LF.EX than LF.SED rats at the end of 60 min ($p<0.05$). Increasing dietary fat content significantly reduced the insulin response in both exercised and sedentary rats, where an increase of only 50% for HF.EX and 60% for HF.SED rats' insulin concentrations was observed 10 min post glucose gavage. The high fat diet fed rats maintained significantly higher plasma insulin concentrations than those of low fat fed rats throughout the 60 min of OGTT ($p <0.05$, Figure 19). Sixty minutes post glucose gavage, there was no difference between plasma insulin concentrations of HF.EX rats and HF.SED rats. Increased dietary fat content exacerbated insulin resistance and reduced the effects of exercise in fa/fa rats (Table 12 and Figure 19 B).

In lean rats there were no significant differences in the fasting insulin concentrations. Exercise reduced the insulin response during OGTT, as shown by an increase of only 220% of plasma insulin concentrations in the first 10 min after glucose administration compared to an increase of 345% in LF.SED rats in the same period. The significantly reduced insulin response in LF.EX rats to that of compared to LF.SED rats was maintained throughout the 60 min post glucose gavage ($p<0.05$). Feeding a high fat diet significantly increased the insulin response to glucose stimulation within the first 10 min of glucose administration in both exercised and sedentary lean rats ($p< 0.05$). Plasma insulin concentrations

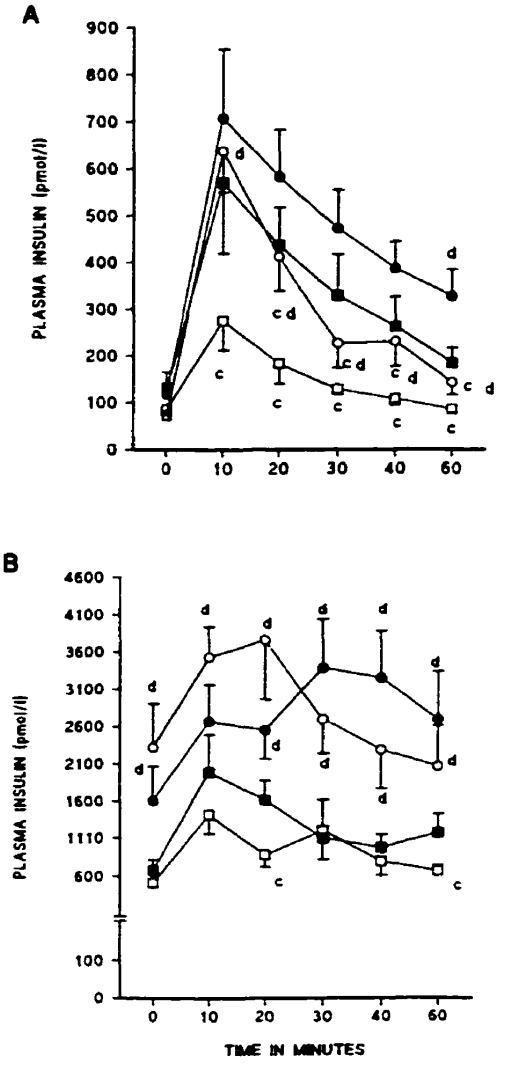

Figure 19. Effects of exercise and high fat diet on plasma insulin concentrations in Zucker (A) lean and (B) fa/fa rats. Plasma insulinconcentrations were measured during OGTT of overnight fasted animals as described in General Methods section. Data are expressed as means ± SEM of 8 rats per group: LF.SED ■, LF.EX □, HF.SED ● and HF.EX ○ rats. $^{c}p< 0.05$ exercised rats compared with sedentary rats fed a similar diet, ^{d} high fat fed rats compared to low fat diet fed rats that received similar treatment using SNK after ANOVA.

Table 12.

The effects of exercise and dietary fat on fasting plasma corticosterone, glucose, insulin, TG and FFA concentrations in Zucker rats at age 10 wk.

Animals	Corticosterone (ng/ml)	Insulin (pM)	Glucose (mM)	TG (mg/dl)	FFA (mM)	
Lean rats					Fasted	Fed
LF.EX	543 ± 80	86 ± 13 [a]	7.24 ± 0.32	1.45 ± 0.38 [a]	0.51 ± 0.15 [af]	1.18 ±0.11
LF.SED	782 ± 122	128 ± 38 [a]	7.49 ± 0.42	2.22 ± 0.98 [a]	0.30 ± 0.09 [af]	1.33 ±0.81
HF.EX	671 ± 72	73 ± 9.0 [a]	6.35 ± 0.24 [a]	1.01 ± 0.13 [ac]	0.23± 0.07 [af]	0.86 ±0.91
HF.SED	810 ± 105	118 ± 24 [a]	7.33 ± 0.30 [a]	2.85 ± 0.70 [a]	0.68 ± 0.24 [af]	1.26 ±0.11
Obese rats						
LF.EX	798 ± 68	513 ± 149 [d]	7.77 ± 0.51	20.83 ± 3.11 [c]	1.30 ± 0.23 [f]	2.05 ±0.12
LF.SED	712 ± 48	683 ± 139 [d]	8.60 ± 0.37	39.05 ± 5.15 [d]	1.28 ± 0.24	1.42 ±0.22
HF.EX	837 ±76	2319 ± 590	8.53 ± 0.47	34.66 ± 8.21	1.45 ± 0.27	1.15 ±0.23
HF.SED	640 ± 76	1607 ± 462	9.38 ± 0.49	50.97 ±13.5	1.95 ± 0.43	1.15 ±0.45

Data are expressed as means ± SEM, N= 8 animals in each group except for FFA (fed) where n=7.

[a] $p< 0.05$ lean compared to fa/fa rats, [c] $p< 0.05$ exercise compared with sedentary rats fed similar diet, [d] $p< 0.05$ high fat compared to high fat diet within each treatment and [f] $p< 0.05$ fasted rats vs fed rats within each rat group.

increased by 770% in HF.EX and 520% in HF.SED rats within ten minutes of glucose administration. High fat diet also induced some insulin resistance in the lean sedentary rats because the plasma insulin concentrations were still elevated 60 minutes post-glucose gavage. Exercise improved glucose tolerance in both low fat and high fat fed rats (Figure 19 A).

The integrated insulin response was significantly influenced by phenotype ($p < 0.05$). The integrated insulin response was significantly higher in obese HF.SED rats than in any low fat diet fed fa/fa rat groups ($p<0.05$). Exercise did not have any effect on integrated insulin response in low fat diet rats.

In lean rats, LF.EX rats had the lowest integrated insulin response compared to the other groups ($p< 0.05$), and increasing dietary fat content opposed the exercise effect by significantly increasing integrated insulin response in HF.EX rats compared to that of LF.EX rats ($p<0.05$) but remained significantly lower than HF.SED rats ($p<0.05$) (Figure 20).

4.3.1.3.3. Plasma glucose concentrations

Plasma glucose concentrations in the fed state were measured to assess whether exercise induced stress in Zucker rats as they adapt to swimming exercise. Analysis of the data by repeated measure ANOVA revealed that diet and age and 2-way interaction between age and phenotype had a significant effect on plasma ambient glucose concentration (all; $p<0.005$). Ambient glucose concentrations in low fat fed lean and fa/fa rats remained relatively stable throughout the study.

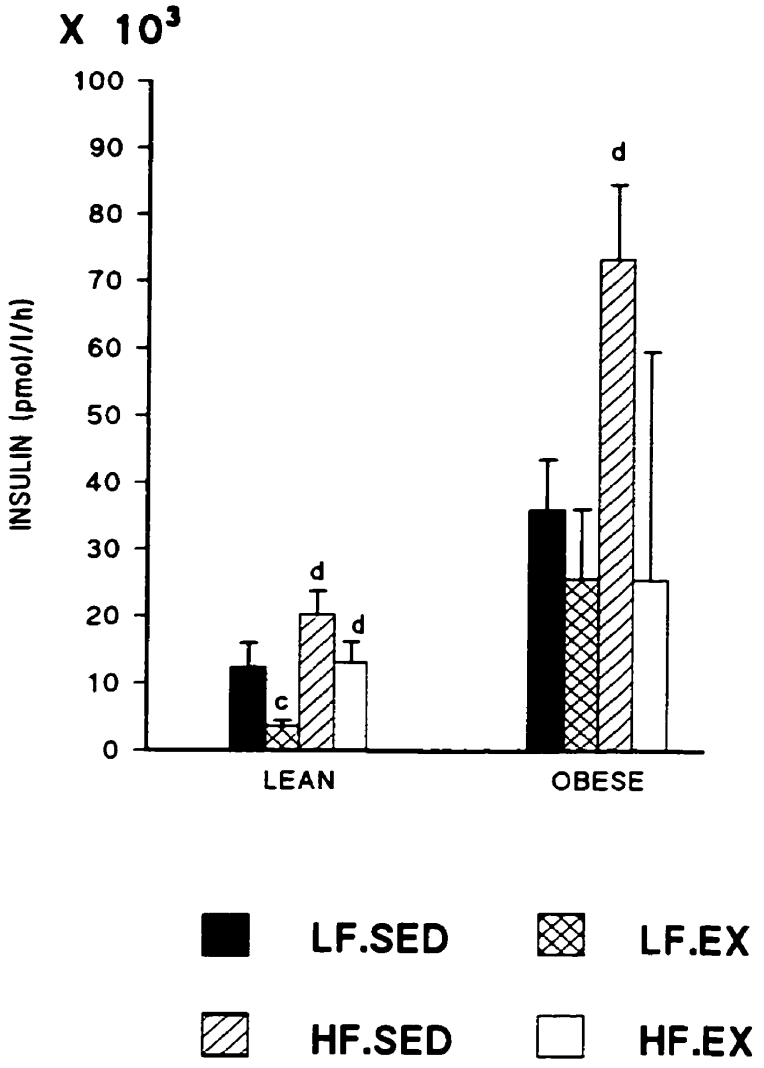

Figure 20. Effects of exercise and high fat diet on integrated insulin during OGTT in Zucker rats. Integrated insulin response was calculated as described in the the Chapter 2. Data are expressed as means ± SEM of 8 rats in each group. ^{c} $p < 0.05$ exercised rats compared to sedentary rats fed similar diet, ^{d} $p < 0.05$ high fat diet compared to low fat diet in rats that were treated simiarly in each phenotype using SNK.

In the high fat fed rats of both phenotypes plasma glucose levels tended to increase with age of the rats . Glucose concentrations of 10 wk old HF.EX and HF.SED fa/fa rats were 16% and 18% higher than in 5 wk old rats, while this difference between 10 wk and 5 wk lean rats was 22% and 18% in HF.EX and HF.SED lean rats, ($p<0.05$) respectively (Table 13).

Plasma glucose levels were measured in 10 wk old Zucker lean and fa/fa rats after an overnight fast prior to OGTT. Fasting glucose concentrations were not different among chow fed fa/fa or lean rats (Table 12). However, fasting plasma glucose concentrations of HF.EX and HF.SED fa/fa rats were significantly higher than those of HF.EX and HF.SED lean rats ($p <0.05$).

Analysis of the data by repeated measure ANOVA revealed that plasma glucose concentrations after an oral glucose load were significantly influenced by phenotype, diet, exercise and time (all, $p< 0.0001$). Two-way interactions between phenotype and diet ($p< 0.0001$), phenotype and exercise ($p< 0.001$), phenotype and time ($p< 0.001$), and between diet and exercise ($p< 0.005$) and time ($p< 0.0001$) were observed. During the OGTT, LF.EX and LF.SED rats maintained significantly lower plasma glucose concentrations compared to those in the HF.EX and HF.SED fa/fa rats (Figure 21 B). However, there was little or no change in glucose concentration from 20 min to 60 min post-glucose administration within each group, a strong indication of abnormal glucose metabolism in fa/fa rats. Increased dietary fat worsened glucose intolerance already present in these rats such that glucose concentrations 60 min post-glucose gavage in HF.EX and HF.SED were 43% and

Table 13.

Effect of exercise and dietary fat on ambient plasma glucose concentrations in Zucker rats age 5-10 wk.

Animals	N	Glucose concentration (mM ± SEM)					
		5 wk	6 wk	7 wk	8 wk	9 wk	10 wk
Lean rats							
LF.EX	8	7.01 ± 0.81	7.66 ± 0.23	8.31 ± 0.22	7.84 ± 0.20	7.82 ± 0.25	7.97 ± 0.33
LF.SED	8	6.94 ± 0.52	7.85 ± 0.27	7.88 ± 0.21	7.74 ± 0.31	7.88 ± 0.28	7.69± 0.29
HF.EX	8	6.98 ± 0.85	8.08 ± 0.27	8.57 ± 0.24	7.73 ± 0.22	8.13 ± 0.26	8.25± 0.31 [b]
HF.SED	8	6.94 ± 0.53	7.62 ± 0.47	8.45 ± 0.34	8.45 ± 0.34	8.75 ± 0.37	8.30 ± 0.35 [b]
Obese rats							
LF.EX	8	8.08 ± 0.26	7.17 ± 0.30	7.21 ± 0.33	7.33 ± 0.23	7.91 ± 0.09	7.74 ± 0.22
LF.SED	8	8.04 ± 0.22	7.41± 0.43	7.22 ± 0.37	7.76 ± 0.34	8.17 ± 0.11	8.61 ± 0.38
HF.EX	8	7.88 ± 0.32	8.35 ± 0.45	8.53 ± 0.29	7.87 ± 0.21	8.21 ± 0.32	8.91 ± 0.24 [b d]
HF.SED	8	8.01 ± 0.23	7.55 ± 0.25	8.00 ± 0.20	8.36 ± 0.16	8.45 ± 0.19	8.77 ± 0.28 [b]

Data are means ± SEM, N=number of animals per group.

[b] $p < 0.05$, 10 wk compared to 5 wk old rats, [d] $p < 0.05$ high fat diet compared to low fat diet using un paired test.

44% greater than those of LF.EX and LF.SED ($p<0.05$) (Figure 21 B).

In the lean group, regardless of the diet composition, exercised rats had lower plasma glucose concentrations than their controls throughout the 60 min OGTT. By 60 min post-glucose gavage, there was no significant difference between glucose concentration of LF.EX, LF.SED or HF.EX rats. Glucose concentrations of HF.SED rats were significantly higher than in the HF.EX, LF.EX and LF.SED rats from 20 min to 60 min and remained 49% and 39% higher than that of HF.EX and LF.SED lean rats ($p< 0.05$) (Figure 21 A).

The integrated glucose responses was significantly affected by phenotype ($p< 0.005$), diet, and exercise (all $p< 0.0001$) and an interaction between phenotype and exercise ($p< 0.02$). In fa/fa rats the integrated glucose response was significantly higher in the high fat fed fa/fa rats than chow fed rats regardless of the activity level ($p< 0.05$) (Figure 22). Exercise did not have any effect on overall glucose integrated responses in low fat nor in high fat fed fa/fa rats. High fat diet significantly increased integrated glucose responses in exercised and sedentary rats ($p< 0.05$). This emphasized the over-riding effect of diet on glucose metabolism in fa/fa rats.

In lean rats the integrated glucose response was significantly increased by feeding a high fat diet. Lean HF.SED rats showed greater glucose response than lean LF.SED rats ($p< 0.05$). Exercise reduced the glucose response in low fat fed rats($p< 0.05$), and increasing dietary fat abolished this effect in exercise rats. ($p<0.05$, Figure 22).

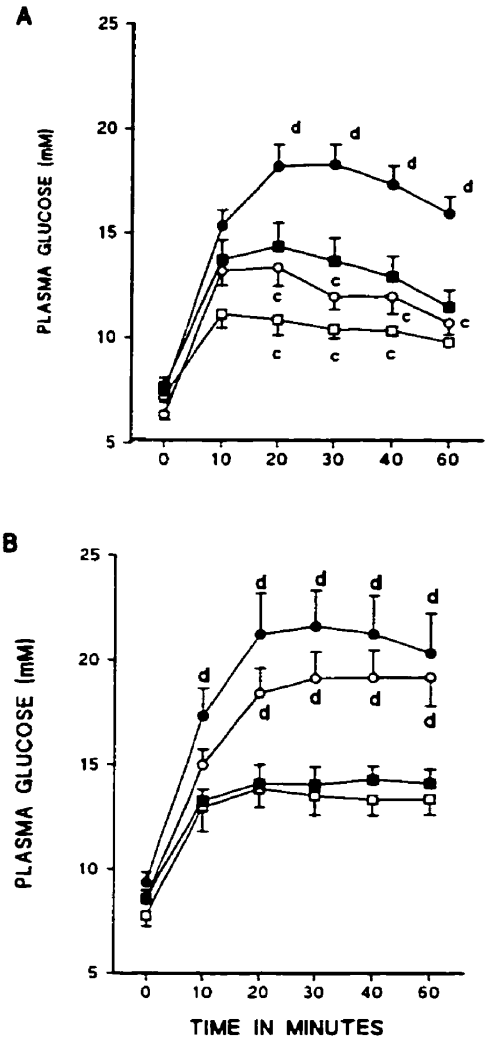

Figure 21. Effects of exercise and high fat diet on plasma glucose concentration in Zucker (A) lean and (B) fa/fa rats. Plasma glucose concentrations were measured in overnight fasted rats during OGTT as described in General Methods section. Data are expressed as means ± SEM of 8 animals in each group: LF.SED ■, LF.EX □, HF.SED ● and HF.EX ○ rats. ^{c} $p< 0.05$, exercised rats compared to sedentary rats fed a similar diet, ^{d} $p< 0.05$ high diet fat fed rats compared to low fat diet in rats treated similarly using SNK after ANOVA.

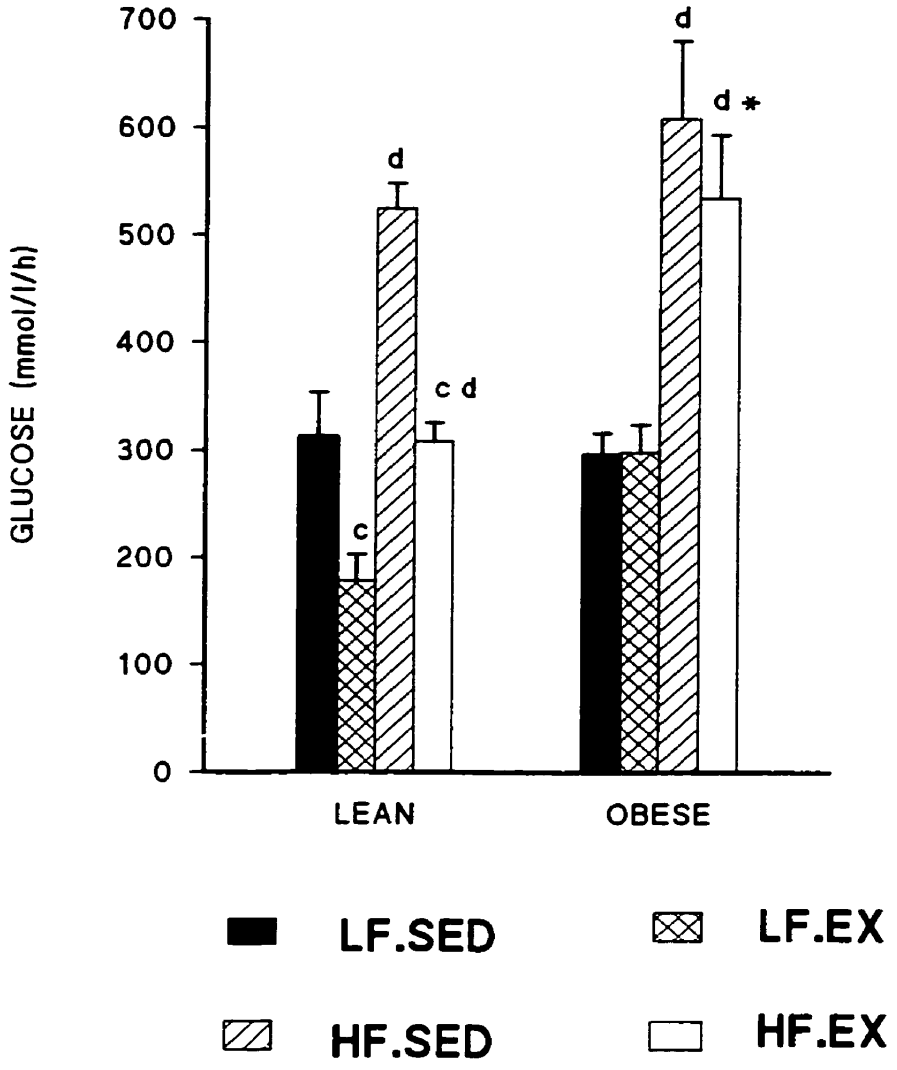

Figure 22. Effects of exercise and high fat diet on integrated glucose response during OGTT in Zucker rats. Integrated glucose response was calculated as described in Chapter 2. Data are expressed as means ± SEM of 8 rats in each group. [c] $p<0.05$ exercised rats compared to sedentary rats fed a similar diet, [d] $p< 0.05$ high fat diet compared to low fat diet in rats that received similar treatment, * $p < 0.05$ HF.EX fa/fa rat groups compared to LF.SED fa/fa rats within each phenotype using SNK after ANOVA.

4.3.1.3.4. Plasma TG and FFA concentrations

Plasma triglyceride concentrations were determined in 10 wk old rats after an overnight fast. Analysis of the data by ANOVA showed that TG were significantly affected by phenotype ($p < 0.0001$) and physical activity ($p < 0.05$). This study confirmed that Zucker fa/fa rats are hypertriglyceridemic. Sedentary fa/fa rats had approximately 17-fold higher plasma TG concentrations than did lean sedentary rats regardless of the diet composition. Exercising rats for 4 weeks reduced TG concentrations compared to those of their respective sedentary controls by 49% and 32% in LF.EX and HF.EX fa/fa rats, but this difference was statistically significant only between the LF.EX and LF.SED rats ($p < 0.05$). Feeding high fat diet to exercised rats significantly increased plasma TG concentrations ($p<0.05$). A similar effect was induced in sedentary rats, such that plasma TG concentrations in fa/fa rats were increased by 66% in HF.EX and 31% in HF.SED rats compared to LF.EX and LF.SED fa/fa rats, respectively (Table 12).

In lean groups, sedentary rats had higher mean plasma TG concentrations than exercised rats regardless of diet composition. Exercise did not affect plasma TG concentrations in low fat fed rats but significantly reduced TG concentrations of in HF.EX rats by 65% compared to HF.SED rats ($p<0.05$). Diet composition had no effect on plasma TG concentrations in either exercise or sedentary lean rats. Plasma FFA concentrations were measured in 10 wk old fed and fasted animals. Plasma FFA concentrations were significantly influenced by phenotype and fasting (all $p < 0.05$), exercise ($p < 0.02$) and interaction between diet and exercise

($p< 0.01$). There was no difference in ambient FFA concentrations between LF.SED, HF.EX and HF.SED fa/fa and lean rat groups. LF.EX fa/fa rats had higher FFA concentrations than did LF.EX lean rats ($p <0.05$).

The fasted FFA concentrations of fa/fa rats were more than 2-fold higher than those in the lean group regardless of the diet or activity. Overnight fasting had no on plasma FFA concentrations in LF.SED fa/fa rats and the high fat fed rats.. A significant difference between the fed and fasted FFA concentrations was observed only in LF.EX fa/fa rats ($p< 0.05$). Exercise induced a significant increase of 44% in plasma FFA concentrations of LF.EX rats compared to that of LF.SED fa/fa rats ($p <0.05$, unpaired t-test). However increasing dietary fat content in the exercised rats decreased plasma FFA concentrations of exercised fa/fa rats in the fed state by 44% ($p< 0.05$, unpaired t- test). In lean rats plasma FFA concentrations were significantly lower in the fasted rats than in the fed state ($p<0.05$) (Table 12).

4.3.1.3.5. Summary of the *in vivo* results

In vivo results of the exercise and diet experiments are summarized in Table 14. In fa/fa rats exercise reduced caloric intake, weight gain and TG concentrations only in low fat fed rats. However, exercise did not affect fasting glucose and insulin concentrations or the integrated responses during OGTT in LF.EX rats. Dietary fat increased caloric intake and weight gain without significantly affecting fasting glucose; however the maintenance of normoglycemia was at the expense of high fasting insulin concentrations in both HF.EX and HF.SED fa/fa rats. Dietary fat

worsened the insulin resistance already present in these rats. The main changes observed in fa/fa rats were in the integrated insulin and glucose responses where the effects of exercise and dietary fat opposed each other. Exercise tended to improve both glucose and insulin responses while high fat diet induced some degree of insulin resistance.

In lean rats both diet and exercise had few metabolic effects with the exception of the opposing effects on glucose tolerance, with exercise improving and high fat diet decreasing glucose tolerance.

4.3.2. *In vitro* pancreatic responses

4.3.2.1. Glucose response in isolated pancreatic islets

In this study we measured both basal insulin secretion and GSIS in isolated islets from lean and fa/fa rats after 4 wk of feeding a high fat diet and a swimming regimen as described earlier. In response to various glucose concentrations, insulin release from isolated islets of different experimental rat groups was compared to that from LF.SED in each phenotype. Neither basal (2.8 mM glucose) insulin secretion nor GSIS were different in isolated islets from both phenotypes. Exercise did not significantly affect GSIS in isolated islets from fa/fa rats. Even when insulin release was expressed as absolute insulin secretion instead of as a percent release of the total pancreatic insulin content, both exercise and diet had no effect on basal insulin secretion and GSIS in fa/fa rats (Figure 23). In pancreatic islets isolated from lean rats, basal insulin secretion and GSIS were similar in all lean rat groups

Table 14.

Summary of the in vivo results of exercise and diet in Zucker rats.

Animals	Total Kcal intake	Wt gain	Corticosterone	Insulin	Glucose	TG	FFA	Integrated OGTT response	
								Insulin	glucose
Lean rats									
LF.EX	–	–	–	–	–	–	–	↓	↓
HF.EX	–	–	–	–	–	↓	–	–	–
HF.SED	–	–	–	–	–	–	–	↑	↑
Obese rats									
LF.EX	↓	↓	–	–	–	↓	–	–	–
HF.EX	–	↑	–	↑	–	–	–	–	↑
HF.SED	↑	↑	–	↑	–	–	–	↑	↑

Parameters are compared to LF.SED for each phenotype: _ no change, ↓ decreased and ↑ increased.

(Figure 23 A). Pancreatic islet glucose sensitivity (EC_{50}) was calculated for all groups and the results are shown in Table 15. In obese rats, neither physical activity nor diet composition had any effect on pancreatic islet glucose sensitivity.. Even though there was no significant difference between the EC_{50} of fa/fa rats and lean rats, EC_{50} of fa/fa rat islets tended to be on the lower side. In lean rats islet sensitivity to glucose was not different among all the groups.

Pancreatic insulin content in fa/fa rats was more than double that of lean rats regardless of physical activity or diet composition ($p < 0.005$). Within the fa/fa rat groups there no differences in the pancreatic insulin content. In lean rats pancreatic insulin content of HF.SED was significantly higher than that of all other rat groups ($p < 0.05$). The insulin content of HF.SED rats was more than double that of HF.EX rats and approximately 72% higher than LF.SED rats (Table 15).

4.3.2.2. Mannoheptulose response

Glucose (16.5 mM)-stimulated insulin secretion (GSIS) from isolated islets of LF.EX, LF.SED, HF.EX and HF.SED lean and fa/fa rats was measured in the absence or presence of several MH concentrations (1-100 mM). Glucose-stimulated insulin secretion in response to MH was significantly influenced by exercise, phenotype and MH concentration (all, $p < 0.0001$), and diet and exercise interaction ($p < 0.04$). A three factor interaction was observed between diet, exercise and phenotype ($p < 0.05$). Insulin secretion in response to 16.5 mM glucose in LF.SED and LF.EX was not affected by any MH concentrations used

Table 15.

Effect of exercise and diet composition on pancreatic B-cell sensitivity to glucose (EC_{50}) and insulin content of Zucker rats.

Animals	N	EC_{50} (mM)	Insulin content (nmol/islet)
Lean rats			
LF.EX	8	8.70 ± 5.19	10.52 ± 2.75 [a]
LF.SED	5	6.28 ± 3.60	9.54 ± 2.35 [a]
HF.EX	8	6.10 ± 1.06	7.21 ± 0.71 [ac]
HF.SED	7	11.57 ± 5.37	16.81 ± 1.77 [ad]
Obese rats			
EX.LF	5	5.98 ± 2.77	23.82 ± 2.53
LF.SED	8	4.54 ± 0.22	25.52 ± 1.95
HF.EX	7	6.03 ± 2.49	28.24 ± 3.74
HF.SED	7	5.16 ± 2.54	34.31 ± 4.58

Values are means ± SEM, N= number of animals.

[a] $p < 0.05$ lean vs fa/fa rats receiving similar treatment, [c] $p < 0.05$ exercised compared to sedentary rats of the same phenotype fed same diet , [d] $p < 0.05$ high fat diet compared to low fat diet fed rats receiving similar treatment and are of the same phenotype using SNK after ANOVA.

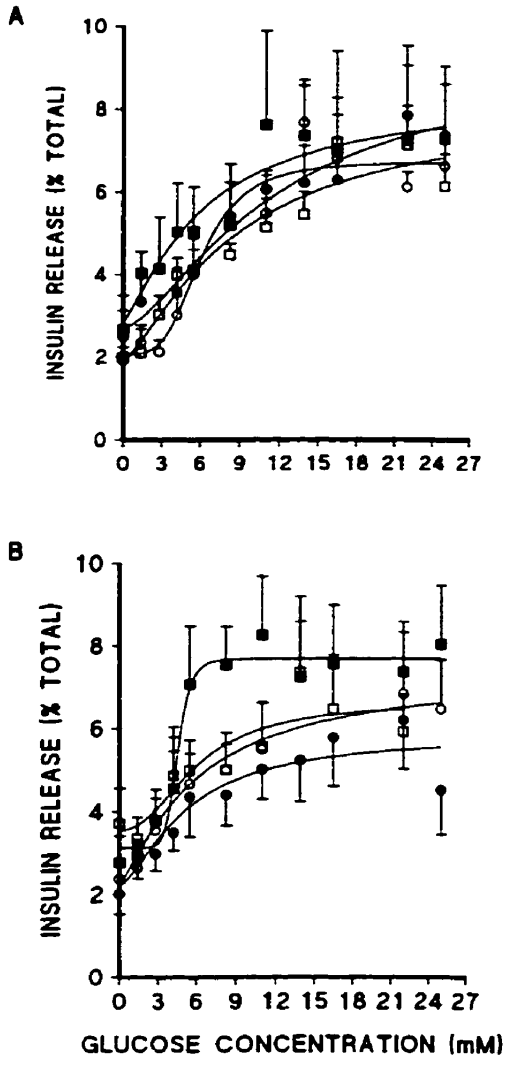

Figure 23 . Effects of exercise and high fat diet on the glucose response in isolated islets from Zucker (A) lean and (B) fa/fa rats. Insulin secretion was measured in the presence of graded glucose concentrations as described in the General Methods section. Total islet insulin content and EC_{50} for glucose for each group are reported in Table 16. Values are means ± SEM for number of animals shown in Table 16: LF.SED ■, LF.EX □, HF.SED ● and HF.EX ○ rats.

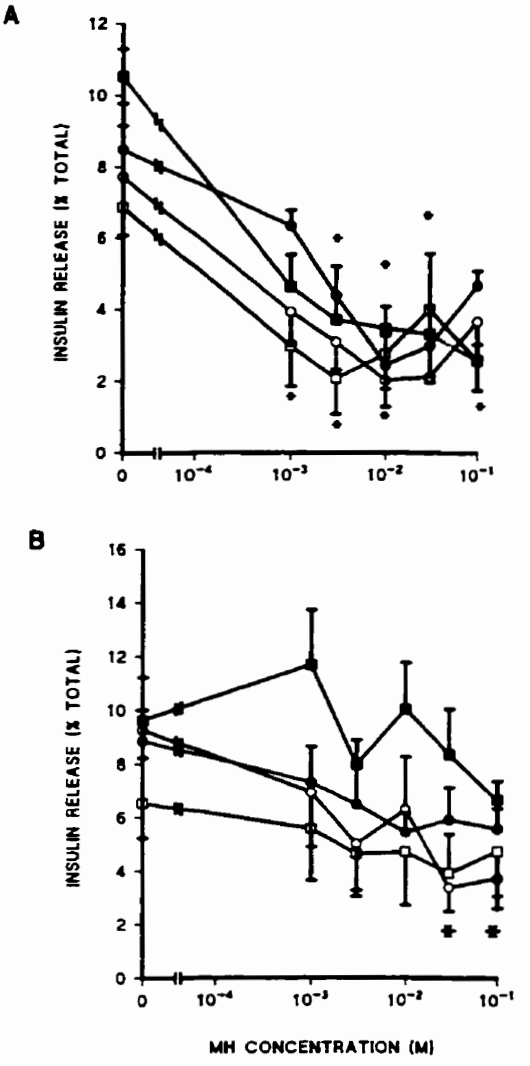

Figure 24. Effects of exercise and high fat diet on the sensitivity to MH inhibition of insulin secretion in isolated islets from Zucker (A) lean and (B) fa/fa rats. Insulin secretion in response to 16.5 mM glucose was measured in the absence (control, 0) or presence of graded MH concentrations as described in the Methods section. Values are means ± SEM of n = 7, LF.SED ■; n = 4, LF.EX □; n = 7, HF.SED ●, and n = 4, HF.EX ○ for both lean and fa/fa rats. *p< 0.05 effect of MH compared to GSIS in absence of MH in each group using SNK after ANOVA.

(1-100 mM). Although a combination of high fat diet and exercise partially restored MH inhibitory response in HF.EX rat islets, this was achieved at very high concentration of 30 mM (Figure 24 B). The action of high MH concentration may not entirely be due to inhibition of the glucokinase but may affect other cell components even though it does not affect GLUT2 (Ferrer *et al.*, 1993). In lean rats, as expected MH concentrations of 3 mM or higher induced a significant reduction in GSIS from islets of all groups regardless of diet or activity ($p< 0.05$) (Figure 24 A).

4.3.2.3. Glucose phosphorylating activity

In vitro exposure of islets to high FFA or *in vivo* intralipid infusion into normal rats increases basal insulin secretion and islet B-cell sensitivity to glucose, effects attributed to increased hexokinase and glucokinase activity in the islets (Chen *et al.*, 1994a; Cockburn *et al.*, 1997), while exercise-induced reduction of GSIS is attributed in part to reduction of glucokinase mRNA expression (Koranyi *et al.*, 1991). Feeding high fat diets tend to increase circulating FFA whereas exercise has the opposite effect (Triscari *et al.*, 1985; Hill *et al.*, 1991).

To determine if feeding a diet with 4-fold higher fat content and/or aerobic exercise would affect hexokinase and/or glucokinase kinetics (K_m and V_{max}), glucose phosphorylating activity was measured in islets isolated from Zucker lean and fa/fa rats after a combination of a 4 wk of feeding and swimming regimen. Hexokinase activity was calculated using Eadie-Hofstee analysis on results from islets in the presence of glucose concentrations of 0.05 - 0.5 mM, while results from islets in

the presence of glucose concentrations of 6-16 mM were used for calculating glucokinase kinetics (Chan *et al.*, 1995).

4.3.2.3.1. Hexokinase

In fa/fa rat islets hexokinase K_m and V_{max} were not different in the sedentary rats fed either diet. However, exercise significantly lowered hexokinase K_m from the islets of HF.EX by 88% compared to that of HF.SED rats, this was also 82 % lower than that of islets from LF.EX rats ($p<0.05$) (Table 16).

Exercise also decreased hexokinase V_{max} of LF.EX and HF.EX rats by 73% and 82% respectively, compared to their sedentary controls ($p<0.05$), leading to a significant decrease in glucose phosphorylation in the exercised rats ($p <0.05$) Figure 25B). High fat diet induced a 50% reduction on hexokinase V_{max} in islets from HF.EX compared to that of islets from LF.EX rats (0< 0.50), without having any effect in the control rats (Table 16 and Figure 25 B).

In lean rats, hexokinase K_m was not different among the rat groups. Exercise significantly reduced hexokinase V_{max} in rat chow fed rats (Table 16 and Figure 25 A). Increasing dietary fat reversed the effects of exercise by increasing hexokinase V_{max} of islets from HF.EX rats by 2-fold compared to that of LF.EX rat islets ($p< 0.05$). The lack of difference in hexokinase K_m among the lean groups was also reflected in similar basal insulin secretion in these rat groups.

Exercise and high dietary fat content appear to augment each others' actions in the regulation of hexokinase kinetics in fa/fa rats but oppose one another on

hexokinase V_{max} in the lean rats (Table 16) .

4.3.2.3. 2. Glucokinase

In fa/fa rats, glucokinase V_{max} values were not different among all rat groups.. However, exercise significantly increased glucokinase K_m in high fat fed rats by 60% ($p<0.05$) but had no effect in low fat diet fed rats (Table 16). Glucokinase K_m of islets isolated from LF.EX was 50 % lower than that of HF.EX rats ($p<0.05$).

Glucose sensitivity of glucokinase tended to increase in lean HF.SED rats as seen from the reduction in the K_m by 78% as compared to the results from LF.SED rats ($p<0.05$). A similar trend was observed in HF.EX vs LF.EX rats, although the adaptation was smaller in magnitude. Exercise decreased glucokinase K_m values by 57% in LF.EX but increased it by 180% in HF.EX, respectively, compared to their sedentary controls ($p<0.05$). Increasing dietary fat did not affect the effects of exercise on glucokinase glucose sensitivity in exercised rats. Glucokinase K_m of the HF.SED rats was 64% lower than that of HF.EX rats ($p <0.05$).

There was no statistically significant difference between V_{max} among the four lean rat groups (Table 16). In summary, as shown in Figure 26 A, large changes in the overall GK activity as a result of diet or exercise were not observed in the lean islets.

Table 16.

Effects of exercise and high fat diet on hexokinase and glucokinase kinetics V_{max} (pmol/μg protein /h) and K_m (mM) in islet B-cells of Zucker rats.

Animals	N	Hexokinase		Glucokinase	
Lean rats		V_{max}	K_m	V_{max}	K_m
LF.EX	8	53.1 ± 6.6 [a c]	0.119 ± 0.037	911 ± 40	8.30 ± 0.8 [c]
LF.SED	8	80.0 ± 6.4 [a]	0.195 ± 0.031	904 ± 180	19.4 ± 5.9 [a]
HF.EX	8	108 ± 9.8 [a d]	0.239 ± 0.04	1108 ± 180	11.8 ± 3.5 [a c]
HF.SED	8	124.5 ± 18.4 [a]	0.245 ± 0.066	671 ± 63	4.20 ± 1.3 [a d]
Obese rats					
LF.EX	8	100 ± 14 [c]	0.265 ± 0.064 [c]	1384 ± 164	11.5 ± 2.4
LF.SED	8	376 ± 51	0.378 ± 0.080	951 ± 158	7.95 ± 3.0
HF.EX	8	47 ± 1.4 [c d]	0.047 ± 0.001 [c d]	994 ± 76	23.3 ± 2.8 [c]
HF.SED	8	257 ± 57	0.407 ± 0.138	898 ± 74	9.10 ± 1.5

V_{MAX} (pmol/μg protein/h) and K_m (mM). Values were calculated using Eadie-Hofstee analysis (V vs V/S) using glucose concentrations of 0.05-0.5 mM for hexokinase and 6-16 mM for glucokinase after correcting for hexokinase activity at glucose concentration of 0.5 mM. Data are means ± SEM, N= animals in each group. [a] $p<0.05$ lean rats compared to fa/fa rats receiving similar treatment or diet , [c] $p<0.05$ exercised rats compared to sedentary rats fed a similar diet , [d] $p<0.05$ high fat diet fed rats compared to low fat diet rats receiving similar treatment using SNK after ANOVA.

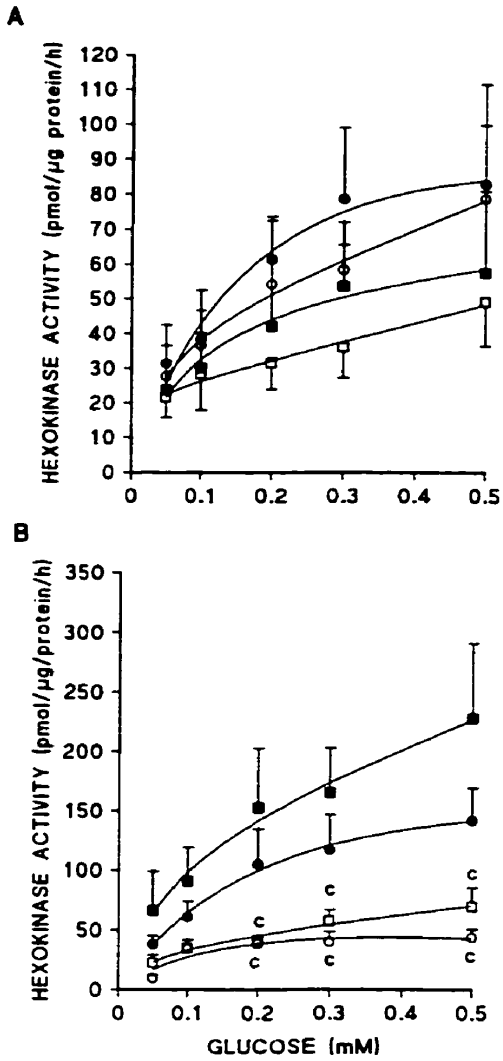

Figure 25. Effects of exercise and dietary fat on pancreatic B-cell hexokinase activity (pmol/µg protein/h) in Zucker (A) lean and (B) fa/fa rats. Hexokinase activity was measured in disrupted islets in the presence of 0.05-0.5 mM glucose and kinetic (V_{max} and K_m) values were obtained using Eadie-Hofstee analysis (V_{max}= (V/(1+Km/S))). Data are expressed as means ± SEM of 8 rats per group (LF.SED ■, LF.EX □, HF.SED ●, HF.EX ○ rats).^{c} $p<0.05$ exercised compared with sedentary rats fed similar diet and is for both rat chow and high fat diet.

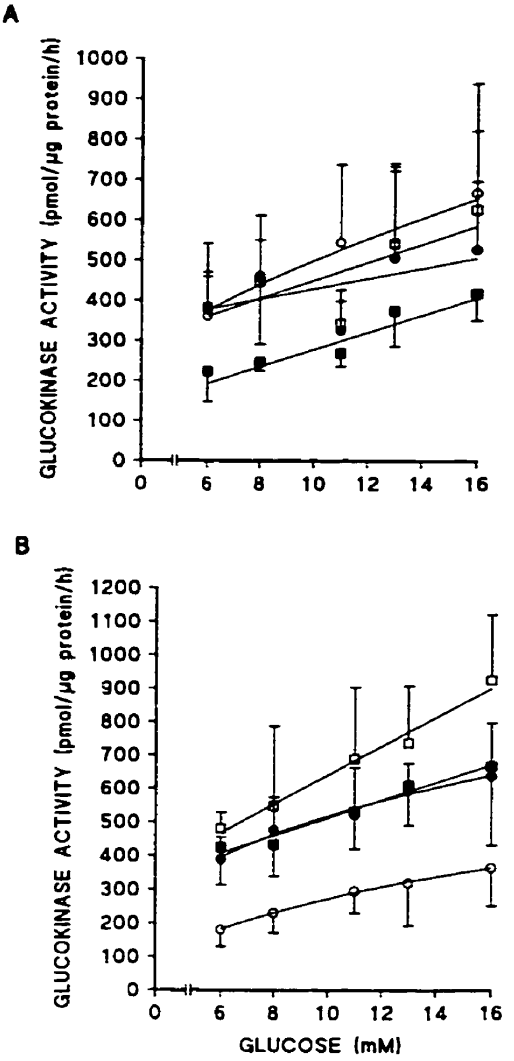

Figure 26. Effects of exercise and dietary fat on pancreatic B-cell glucokinase activity (pmol/µg protein/h) in Zucker (A) lean and (B) fa/fa rats. Glucokinase phosphorylating activity was measured in disrupted islets using 6-16 mM glucose concentrations and the kinetic (V_{max} and K_m) values were calculated employing Eadie-Hofstee analysis $V_{max} = (V/(1+K_m/S))$ after subtracting hexokinase activity at 0.5 mM glucose. Data are expressed as means ±SEM of 8 rats per group (LF.SED ■; LF.EX □; HF.SED ● and HF.EX ○ rats).

4.3.2.4. FFA oxidation in pancreatic islet B-cells

The mechanism by which FFA enhances basal insulin secretion and inhibits GSIS after prolonged exposure is by increasing lipid metabolism in the B-cells through ß-oxidation in the inner mitochondrial membrane (Vara and, Tamarit-Rodriguez , 1986; Zhou *et al.*, 1996). The FFA oxidation in freshly isolated islets from Zucker rats was measured by quantifying CO_2 production from [1-^{14}C] palmitic acid in the presence of low (3.0 mM) and high (25 mM) glucose concentrations and the results are shown in Table 17.

In pancreatic islets isolated from fa/fa rats there were no effects of diet, exercise or glucose concentration on FFA oxidation. High dietary fat content increased FFA oxidation by 50% in HF.SED compared to that in LF.SED lean rat islets in the presence of low glucose concentration. This effect was significantly reduced by exercise and by a high glucose concentration ($p < 0.05$) (Table 17).

4.3.3. Summary

Results of the *in vitro* experiments are summarized in Table 18. The most striking difference between the lean and fa/fa rat islet adaptations to either diet or exercise were opposite effects on GK function. In fa/fa rat islets, a combination of high dietary fat and exercise reduced GK sensitivity to glucose and increased the islet response to MH inhibitory actions but separately, exercise or a high dietary fat content did not affect GK kinetics or MH action in fa/fa rat islets.

Table 17.

The effects of exercise and high fat diet on pancreatic islet CO_2 production from [1-^{14}C] palmitic acid.

Animals	N	CO_2 production (fmol/islet/h)	
		3 mM glucose	25 mM glucose
Lean rats			
LF.EX	8	5.05 ± 1.37	3.73 ± 1.48
LF.SED	8	8.38 ± 3.26	6.39 ± 2.45
HF.EX	8	2.67 ± 0.72 [c]	2.97 ± 1.04
HF.SED	8	16.3 ± 11.3	3.31 ± 0.68 [e]
Obese rats			
LF.EX	8	3.91 ± 0.74	2.72 ± 0.54
LF.SED	8	4.93 ± 1.26	5.20 ± 1.30
HF.EX	8	4.63 ± 1.22	3.10 ± 1.02
HF.SED	8	4.50 ± 1.21	3.31 ± 0.88

Data are expressed as means ± SEM, N= animals per group.

[c] $p < 0.05$ exercise compared to sedentary rats fed similar diet,

[e] $p < 0.05$ 25 mM glucose compared to 3 mM within the same rat group using SNK after ANOVA.

Table 18.

Summary of the *in vitro* results of exercise and high fat diet.

Animals	Insulin secretion (%Total)		EC_{50}	Insulin content	Hexokinase		Glucokinase		FFA oxidation	MH
	(2.8 mM	25 mM			V_{max}	K_m	V_{max}	K_m		
Lean rats										
LF.EX	3.02 ± 0.46	6.94 ± 1.04	–	–	↓	–	–	–	–	–
LF.SED	4.14 ± 1.25	7.30 ± 1.31								
HF.EX	2.10 ± 0.25	6.63 ± 0.56	–	–	–	–	–	↓	↓	–
HF.SED	3.78 ± 0.63	7.38 ±1.65	–	↑	–	–	–	↓	↑	–
Obese										
LF.EX	5.01 ± 1.03	8.60 ± 1.90	–	–	↓	↓	–	–	–	–
LF.SED	3.80 ± 0.73	8.04 ± 1.42								
HF.EX	3.55 ± 0.77	6.47 ± 1.19	–	–	↓	↓	–	↑	–	↑
HF.SED	2.99 ± 0.42	4.52 ± 1.07	–	–	–	–	–	–	–	–

The parameters are compared to LF.SED for each phenotype: _ no change, ↓ decreased and ↑ increased.

However, exercise increased HK glucose sensitivity while reducing its rate of phosphorylation in fa/fa rats. Both exercise and dietary fat increase GK sensitivity to glucose in islets from fat fed lean rats as compared to islets from LF.SED lean rats without affecting the maximal velocity. High dietary fat tended to modulate FFA oxidation in lean islets while in fa/fa rat islets, FFA oxidation showed no response to dietary change.

4.4. Discussion

The hypothesis of this study was that exercise would produce effects in the pancreatic islet B-cells of fa/fa rats similar to those induced by ADX and that 4 wk feeding of a high fat diet would negate those effects as well as other *in vivo* metabolic, hormonal and behavioral abnormalities of fa/fa rats. The data showed our hypothesis was partially true in that exercise induced small positive effects similar to those induced by ADX in fa/fa rats in some *in vivo* parameters but not in most of the *in vitro* ones. The data showed exercise improved *in vivo* glucose and insulin responses and lipid profiles in fa/fa rats. A high fat diet/low fiber diet reduced these effects of exercise and these opposing effects were greater in exercised rats than what was observed in ADX rats. However, adaptations of B-cell function *in vitro* revealed only modest changes. Notably, a combination of a diet with a high fat and low fiber content and exercise decreased glucokinase sensitivity to glucose and partially restored MH sensitivity in fa/fa rats while it worsened insulin resistance in fa/fa rats.

4.4.1 Effects of exercise on pancreatic islet B-cell function

Our hypothesis was that the *in vitro* islet response to glucose would be normalized by exercise as observed in ADX studies but worsened by dietary fat. Also, moderate daily physical activity would result in weight loss and improve *in vivo* glucose and insulin responses in fa/fa rats while increasing dietary fat would antagonize the exercise effect. The purpose of this study was to investigate the effects of and potential interactions between exercise and dietary fat on GSIS and mannoheptulose activity in pancreatic islets of fa/fa rats.

Exercise was reported to reduce GSIS by inhibiting glucokinase activity in B-cells (Koranyi *et al.*, 1991) and dietary fat inhibited glucose regulated insulin biosynthesis and secretion in mice and rats (Capito *et al.*, 1992; Kim *et al.*, 1995). Most of the ADX beneficial effects on regulation of energy balance and endocrine function are attributed to increasing SNS activity in the periphery (Arase *et al.*, 1989, Bray *et al.*, 1989).

The data showed that exercise improved *in vivo* glucose and insulin responses and lipid profiles in fa/fa rats and high fat diet reduced these effects. However, adaptations of B-cells did not appear to be a major factor in inducing metabolic improvement since direct measurement of B-cell function *in vitro* revealed only modest changes. Exercise has been shown to increase insulin sensitivity to many tissues including muscle and fat cells regardless of intensity of the exercise (Cortez *et al.*, 1991) and this would increase glucose clearance thus improving glucose tolerance.

4.4.1.1. *In vitro* responses

The isolated pancreatic islet study was carried out to assess the effects of exercise and/or dietary fat on the glucose-stimulated insulin response in the absence of internal environment. Exercise reduces insulin secretion (Simonson *et al.*, 1984), and this action has been attributed to increased sympathetic stimulation of B-cells (Houwing *et al.*, 1995). Exercise training by running was been reported to decrease expression of glucokinase and proinsulin mRNA leading to reduced insulin secretion in rats (Koranyi *et al.*, 1991). The exercise-induced decrease on both glucokinase and proinsulin mRNA expression and GSIS would be due to reduced glucose metabolism by the pancreatic B-cells since glucokinase activity and proinsulin expression are positively regulated by glucose (Liang *et al.*, 1992). Pancreatic islets isolated from Zucker fa/fa rats exhibit high sensitivity to basal glucose and loss of MH inhibitory action on GSIS and these defects are normalized by ADX (Kibenge and Chan, 1996). Because after ADX, central CRH levels are increased (Plotsky *et al.*, 1992), then regulate ANS function resulting in an increased sympathetic nervous tone in the peripheral tissues including the endocrine pancreas (Stubbs and York 1991). Also noting that exercise stimulate the secretion of CRH (Tabata *et al.*, 1991, Duclos *et al.*, 1998) and SNS activity in many tissues (Howlett *et al.*, 1999), we speculated that exercise and ADX may have similar effects on islets of fa/fa rats.

4.4.1.1.1 Insulin release and glucose phosphorylating activity

Exercise induced very no significant changes in the pancreatic response to

glucose in both phenotypes. This could be because fasting glucose concentrations of the this study were higher enough to maintain normal pancreatic islet B-cell function including glucokinase activity. Although hexokinase activity is abundant in exocrine and other islet cells (Schuit *et al.*, 1999), an exercise-related decrease in glucose phosphorylating rate by hexokinase was also reflected in the lower basal insulin release. Dietary fat, on the other hand, significantly lowered glucose sensitivity in the lean rats. As observed in the ADX study, exercise and a high fat diet had no effect on lean rat islets' sensitivity to MH (glucokinase inhibitor).

In the fa/fa rats exercise partially restored GSIS by MH (20%-40%) in LF.EX fa/fa rats while 100 mM MH significantly inhibited GSIS in the HF.EX fa/fa rats. Since ADX restores the inhibitory effect of MH in fa/fa rat islets (Chapter 3; Kibenge and Chan, 1996) and exercise had only a partial effect, the results of this study indicate that an intact HPA axis has a regulatory role in the maintenance of this defect in fa/fa rats, but other factors such as low peripheral sympathetic nervous system tone may be important. Reduced response to exercise has been observed in obese humans where exercise induced lower norepinephrine and epinephrine concentrations compared to normal subjects (Vettor *et al.*, 1997). In this study we did not monitor SNS activity and the intensity of the exercise, however both could be a factor in our lack of significant effect.

Dietary fat significantly increased islet insulin content in lean rats and modestly increased it in fa/fa rats. These results are in disagreement with those of Kim *et al.* (1995) and Capito *et al.* (1992) who showed that prolonged high fat feeding to rodents

reduced both insulin synthesis and GSIS. However the difference in results might be attributed to the differences in the dietary fat content used in the three studies. Also the reduced insulin synthesis could also be a response to the relatively low carbohydrate content of the diets used in the above mentioned studies which was below 40%. The results seen in sedentary high fat fed rats are similar to what is reported to take place during the dynamic stage of obesity development (McGarry, 1992) when both hyperplasia and hypertrophy of the pancreatic islets occur in order to handle the increased demand for insulin in response to extra calories. *In vitro* exposure of normal pancreatic islets to high FFA concentrations gives a similar effect to that observed in pancreatic islets isolated from obese animals, thus indicating that high FFA concentrations reaching the pancreas might contribute to increased insulin synthesis leading to the high pancreatic insulin content (Milburn *et al.*, 1995; Hirose *et al.*, 1996). In lean control rats fed a high fat diet, the small non significant increase in the fasting FFA concentration compared to that of the low fat fed rats might have contributed to the observed increase in the pancreatic insulin content. The *in vitro* and *in vivo* effects of FFA on insulin secretion are variable and dependent on the length of exposure. Short term (1-3 h) exposure of islets to FFA results in enhancement of both basal and GSIS. However, long term exposure of islets to FFA (more than 6 h) results in enhanced basal insulin secretion but inhibits GSIS (Elks, 1993).

The acute FFA enhancement of both basal secretion and GSIS is attributed to the increased activity of the glucose phosphorylating enzymes hexokinase and

glucokinase (Cockburn *et al.*, 1997) and enhanced calcium uptake by activating calcium channels (Warnotte *et al.*, 1994) in islets from normal rats (Chen *et al.*, 1994d). The inhibition of GSIS after prolonged exposure to FFA is due to increased FFA oxidation by the B-cell since the inhibition caused by long term FFA exposure is abolished by inhibitors of lipid ß- oxidation (Chen *et al.*, 1994; Prentki and Corkey, 1996).

The lack of effect of dietary fat on the hexokinase K_m in the isolated islets from lean and fa/fa rats in the current studies could be due to the fact that the diet did not raise plasma FFA concentrations to the concentrations (2 mM) used in *in vitro* studies (Lee *et al.*, 1994) or the levels achieved *in vivo* during lipid infusion (Elks, 1993). Failure of dietary fat to influence hexokinase K_m in fa/fa rats was reported by Hirose *et al.* (1996) who showed that FFA exposure of islets from fa/fa rats and prediabetic Zucker diabetic rats islets failed to increase hexokinase activity (low K_m enzyme) as it did in islets from Wistar rats; they theorized that islets from obese rats had already compensated in order to meet the high insulin demand of the obese state.

Exercise reduced total hexokinase activity in fa/fa rats regardless of the diet; however, this was due to decreased maximal response (V_{max}) since glucose sensitivity (K_m) was enhanced. These results conflicted with the glucose response data in that the LF.EX fa/fa rat islets secreted more insulin (as a % of the total content) in the presence of 2.8 mM glucose than did islets from the control rats. The conflicting *in vitro* and *in vivo* results were also observed in the HF.EX rats, where the overall

hexokinase activity was not different from that of LF.EX rats, but HF.EX rats exhibited fasting insulin concentrations which were 4-fold greater that of LF.EX rats. These results indicate that the activity of hexokinase does not correlate well with insulin secretion (Schuit *et al.*, 1999). However, both increased hexokinase and glucokinase sensitivity to low glucose and persistent insulin resistance contributed to the observed hyperinsulinemia in fa/fa high fat fed exercised rats. In sedentary fa/fa rats high dietary fat content decreased the phosphorylation rate without affecting the glucose sensitivity, and exercise tended to decrease glucose sensitivity in these rats. Insulin output from the isolated islets was only lower in the HF.SED rats and these results agree with the *in vivo* results because there was relatively little increase in plasma insulin concentrations throughout OGTT.

Overall, glucokinase phosphorylating activity was not affected by diet or exercise in the lean rats even though high fat diet significantly increased glucose sensitivity and reduced the maximal phosphorylating rate in sedentary lean rats.

4.4.1.1.2. FFA oxidation

Increased FFA oxidation in pancreatic cells and other tissues is blamed for the fasting hyperinsulinemia observed in most obese and NIDDM subjects (McGarry, 1992). This has been demonstrated in *in vivo* and *in vitro* experiments (Elks, 1993; Lee *et al.*, 1994; Boden, 1997). Our results in freshly isolated islets of fa/fa rats showed no increase in FFA ß- oxidation either at low or high glucose concentrations. However, higher FFA ß-oxidation was observed in islets from lean HF.SED rats

exposed to the lower glucose concentration indicating a potential increase in the activity of CPT-1. In support of this, a recent report by Assimacopoulos-Jeannet *et al.* (1997) indicated that low concentrations of FFA (0.35 mM of either palmitate, oleate or linoleate) induced CPT-1 gene expression in the pancreatic B- cell line INS1 within 1 h of exposure and reached maximum expression after 3 h. The increased expression and activity of CPT-1 in this cell line was accompanied by increased FFA oxidation, increased basal insulin secretion and enhanced GSIS but GSIS was reduced after 24 h FFA exposure (Assimacopoulos-Jeannet *et al.*, 1997). The fasting plasma FFA concentrations of the lean HF.SED rats in this study were greater than that used in the above INS-1 study. However, in the above mentioned study and all other *in vitro* studies, cells were in direct contact with the same FFA concentration for the entire experiment, while *in vivo*, it is unlikely that pancreatic B-cells are exposed to the same concentration of FFA all the time and could explain our failure to observe most of the reported effects of increasing plasma FFA concentrations. In our study no rise in FFA concentration was observed in HF.SED rats compared to LF.SED. However, components of the diet other than the fat content could have induced the changes we observed . The diet was low in fiber, mineral and vitamin content and diets with low fiber content have been reported to a play significant role in the development of glucose intolerance (Bakker *et al.*, 1998), which would lead to some form of adaptation in the islet B-cell metabolism, and this adaptation could be the cause of the increased insulin response observed during OGTT without affecting fasting insulin concentrations . The increased palmitic acid oxidation in the presence

of 3 mM glucose was reduced by both exercise and a higher glucose concentration indicating that high glucose concentrations inhibit FFA oxidation. However in order to confirm that enhanced FFA oxidation at low glucose concentrations was responsible for the increased *in vivo* responses and the increase in pancreatic insulin content of lean HF.SED rats, further studies which incorporate inhibitors of FFA oxidation in experimental protocol are warranted.

4.4.2. *In vivo* responses to exercise and high fat diet

4.4.2.1. Caloric intake and body weight management

A moderate regimen of exercise was chosen because it was reported to increased insulin sensitivity in peripheral tissues in rats (Burstein *et al.*, 1992) and reduce blood pressure in hypertensive obese patients (Tanaka *et a l.*, 1997). Therefore it would not overly stress the obese rats and also would simulate what would be feasible in humans. Exercise reduced food intake in fa/fa rats fed rat chow although it did not eliminate hyperphagia. This could be due to the short duration of the study and length of the exercising period since Jen *et al.* (1992) reported a near-normalization of food intake of fa/fa rats to that of lean rats after 35 wk of swimming 2 h/day, 5 days a week. The LF.EX fa/fa rat caloric intake was lower than the sedentary controls during the first three weeks of the study and along with a presumed increase in energy expenditure, resulted in the rats gaining 26% less weight than LF.SED. However exercise was not effective in blocking excess caloric intake during this period in the fa/fa rats fed a diet with 16% fat content. Even though

by the end of the study HF.EX rats consumed a similar number of calories to that consumed by LF.EX and LF.SED rats, the HF.EX rats' final weight was similar to the HF.SED rats which maintained a high caloric intake. The difference in weight gain might be attributed to the timing of hyperphagia and the developmental stage of the rats, because LF.EX fa/fa rats reduced their caloric intake during the 5 to 8 wk of age period which coincided with the dynamic growth period. In comparison, the other 3 fa/fa rat groups increased caloric intake during this time (Figure 17). These results indicate that swimming exercise on its own when there is no dietary restriction does not lead to weight loss in obese animals. Similar results were reported in humans (Gwinup , 1987). Caloric intake of lean rats showed very little variation compared to that of fa/fa rats. These data confirm results of human studies where a combination of low fat diet and exercise resulted in caloric deficit and weight maintenance as long as there was no compensation for energy used during exercise (Keim *et al.*, 1990) with high fat diet eliminating this effect (King *et al.*, 1996).

Exercise prevents or reduces weight gain by increasing energy expenditure during and after exercise energy or by increasing resting metabolic rate (Poehlman and Danforth 1991; Toth and Poehlman, 1996). Exercise stimulates the HPA axis and two pivotal neuropeptides (i.e, CRH and NPY) secreted by hypothalamic nuclei are involved in body energy homeostasis (Richard, 1995). CRH reduces food intake (Rothwell, 1989) while NPY stimulates feeding (Stanley *et al.*, 1989). The evidence that the anorectic effect of exercise is mediated by CRH is shown that by the fact that exercise-induced anorexia can be prevented by administration of the CRH antagonist

(α-helical CRH_{9-41}) (Rivest and Richard 1990). Both exercise and administration of CRH also influence food selection by reducing dietary fat intake in rats (Larue-Achagiotis *et al.*,1994; Miller *et al.*, 1994). Exercise increases CRH which then inhibits PNS activity and potentiates peripheral SNS by increasing plasma epinephrine (Poehlman and Danforth 1991). CRH effects on energy balance may also result from increased energy expenditure because CRH increases BAT thermogenesis via the SNS (Arase *et al.*, 1989; Holt and York, 1989).

The failure of high fat fed rats to reduce their caloric intake and weight gain could be due to low intensity of the exercise. However, high fat diets also decrease sympathetic nervous system activity (Lu *et al.*, 1998). Both effects could have evaluated by monitoring their cardiac output (heart rate before and after) or measure plasma norepinephrine immediately after exercise (Strobel *et al.*, 1998). Also both high fat diet (Tannebaum *et al.*, 1997) and exercise (Inder *et al* ., 1995; Wittert *et al.*, 1996; Inder *et al.*, 1998) were shown to stimulate corticosterone secretion which would directly or indirect stimulate caloric intake and fat storage. However, corticosterone concentrations in the HF.EX rats were not different from those in the LF.EX fa/fa rats. Since obese Zucker rats already have high concentrations of NPY (Bchini-Hooft van Hhuijsduijnen *et al.*, 1993) and an overactive HPA axis (Plotsky *et al.*, 1992), the additional combined stress of exercise and high dietary fat could easily override the beneficial effects of exercise on caloric intake and weight reduction. Evidence that exercise is capable of increasing NPY concentrations is shown by the presence of high concentrations of NPY in chronically exercised rats' arcuate,

dorsomedial, medial preoptic and lateral hypothalamic nuclei. Similar effects are produced by prolonged starvation (Lewis *et al.*, 1993). The activation of the NPY system by exercise might occur as a result of reduced energy stores and insulin concentrations, effects that are known to occur both in humans and rats (Richard and LeBlanc, 1980). NPY is also suggested to play a role in the regaining of energy balance through stimulation of energy intake and reduction of sympathetic nervous system-mediated thermogenesis (Richard, 1995). Although Zucker fa/fa rats already have high concentrations of NPY (Bchini-Hooft van Huijsduijnen *et al.*, 1993) the interaction of exercise and high fat on the NPY concentrations in these rats needs to be investigated.

In comparison to the ADX data (Chapter 3), swimming induced less changes in weight gain of rat chow fed fa/fa rats and was without any effect on the rats fed a high fat diet. This emphasizes three points: 1) the fa/fa rat obese syndrome requires the presence of corticosterone for maintenance, 2) a more strenuous regimen of exercise may be required for weight loss if dietary fat is increased and 3) exercise in combination with low fat diet could be used successfully to manage energy imbalance of genetic origin.

Since fa/fa rats are a model for childhood onset obesity (Foldes *et al.*, 1992), introducing exercise and a low fat diet to the routine of a young child at risk for obesity could be a significant tool to reduce excess weight gain during the developmental period. Better compliance to diet and exercise regimes in obese children versus adults has been reported and weight loss was maintained 10 years after the initial

intervention (Epstein *et al.*, 1994; 1995). Exercise did not reduce weight gain in the high fat fed rats, likely because of increased food intake that compensated for energy utilized during exercise, less effort exerted during exercise, or a compensatory reduction in non-exercise energy expenditure.

High fat diet accelerated weight gain in both control and exercised fa/fa rats independent of physical activity while lean HF.EX rats gained more weight than HF.SED rats. This illustrates different ways of adaptation to excess caloric intake or exercise by lean and obese rats. The difference could result from the fact that lean rats increased lipid catabolism whereas in fa/fa rats most of the dietary fat was directed to storage as triglycerides (Bessesen *et al.*, 1995). Similar results have also been shown in human experiments where in lean subjects exercise tends to increase caloric intake with no extra weight being gained (Wood *et al.*, 1985). This study also showed that lean rats and fa/fa rats respond in a similar manner to dietary fat when they are first exposed to the diet by increasing caloric intake (Gale *et al.*, 1981).

4.4.2.2. Insulin and glucose responses

Obese fa/fa rats were hyperinsulinemic and although exercise induced a consistent, but insignificant, 25% reduction in plasma insulin concentrations, obese fa/fa rats' insulinemia was greater than that of lean rats. The lack of effect on plasma insulin concentrations could be related to the low intensity and the short duration of the exercise or type of exercise. Treadmill training of old and young fa/fa rats was shown to reduce both basal and post-glucose challenge insulin and to prevent the

development of insulin resistance associated with age in these rats (Becker-Zimmermann *et al.*, 1982). The exercise-mediated reduction in plasma insulin was not observed in the fa/fa rats that were fed a high fat diet. Exercise improved *in vivo* insulin and glucose responses in lean rats as well. In humans, moderate exercise is shown to decrease plasma insulin concentrations after a prolonged period of 10 wks (Escobar *et al.*, 1999) and the benefits are normally lost within a few days of stopping the exercise program (King *et al.*, 1988).

Increased dietary fat induced mild insulin resistance in lean rats and exacerbated existing insulin resistance in fa/fa rats. As in the ADX study, the insulin response was significantly higher in the high fat fed rats indicating the detrimental effect of dietary fat. As opposed to the ADX study, the exercised rats in this study still had high concentrations of corticosterone. In fact both exercise and high fat diets have been shown to stimulate the HPA axis (Tabata *et al.*, 1991; Tannenbaum *et al.*,1997; Inder *et al.*, 1998). Even though our data did not show any increase in corticosterone in fa/fa rats, the combination of exercise and high fat diet with the already abnormally-regulated HPA axis of these rats might worsen insulin resistance. However, this hypothesis will need to be further investigated using a different types of exercise regimen and a diet with significantly higher fat /high fiber content than used in this study.

Although the fa/fa rats had higher fasting plasma insulin concentrations than did the lean rats, their values did not change from 10 - 60 min post glucose gavage; this could reflect decreased insulin clearance. Decreased insulin clearance in the

presence of hyperinsulinemia has been shown in both ob/ob mice and fa/fa rats (Jeanrenaud, 1985). A similar effect was observed in normal humans when plasma concentrations of C-peptide were measured after 14 h intralipid infusion during euglycemic and hyperglycemic clamp. They were able to show that the high plasma insulin concentrations in presence of high glucose concentration were mainly due to the reduction of endogenous insulin clearance rather than increased insulin secretion as was observed under basal conditions (Hennes *et al.*, 1997). C-peptide is co-secreted with insulin at a similar concentration, but unlike insulin, C-peptide has a long half life in circulation and thus enables monitoring of pancreatic B-cell insulin secreting capacity *in vivo* (Polonsky *et al.*, 1986; Van Cauter *et al.*, 1992)

In comparison to the response observed in fa/fa rats, increasing dietary fat content increased insulin secretion in lean rats as shown by a significant increase of plasma insulin concentrations in HF.SED lean rats 10 min after glucose administration during OGTT. Although the dietary fat content used in our study was low compared with many other studies, by the end of 4 wk lean rats had signs of insulin resistance on this diet as shown by increased insulin output in the presence of a high glucose concentration during OGTT.

Exercise lowered both fed and fasting plasma glucose concentrations and improved glucose handling during OGTT in fa/fa rats. Increased dietary fat reduced the exercise effect. Exercise's improvement of glucose handling has been attributed to increased glucose clearance by skeletal muscle (Yamanouchi *et al.*, 1992) and adipose tissue (Craig *et al.*, 1981; Arner, 1995) and to increased effectiveness of

insulin action. Exercise increases glucose clearance in the muscle by increasing blood flow and thus improving insulin delivery (Armstrong and Laughlin, 1984). Exercise also increases transcription of glucose transporter (GLUT4) gene (Neufer and Dohm, 1993), translocation of GLUT4 to the plasma membranes of skeletal (King *et al.*, 1993) and heart muscles (Douen *et al.*, 1989; Slot *et al.*,1991), and GLUT-4 turnover rate (Goodyear *et al.*,1992; Goodyear and Kahn, 1998). Combined, these effects would accelerate removal of glucose from the circulation. These same beneficial effects of exercise have been documented in fa/fa rats (Cortez *et al.*, 1991; King *et al.*, 1993). Via activation of the SNS, exercise increases both lipolysis and fat oxidation (Friedlander *et al.*, 1998), while lipogenesis is blocked as a result of decreased insulin secretion. Exercise also stimulates secretion of glucagon, which plays a major role in glucose production during exercise by promoting glycogenolysis and gluconeogenesis in the liver (Wasserman *et al.*, 1995; Lavoie *et al.*, 1997). Also increased glucocorticoid secretion as a result of activation of the HPA axis may augment the ability of catecholamines to increase lipolysis and glycogenolysis (Wilmore and Costill, 1994). Our result showed no hypercorticosteronemia 24 h after exercise; however, it can not be ruled out that corticosterone concentrations were not elevated during and immediately after exercise in our study.

Exercise also increases lean body mass, which would further increase fat oxidation (Poehlman *et al* ., 1991; Owens *et al.*, 1999) and prevents the development of insulin resistance in muscle (Storlien *et al.*, 1991). Exercise increases the rate of lipolysis and utilization of FFA regardless of the intensity of activity as was shown in

women exercising at both low (45%) and high (65% or 75%) rates of oxygen consumption (Friedlander *et al.*, 1998). Although the length of exercise program is correlated to the observed beneficial effects, fa/fa rats that were exercised for 8 wk by swimming 2 h/d 6 d/ wk (Walberg *et al.*, 1983) or by treadmill running for 10 wk (Seelback *et al.*, 1985) failed to normalize adipose cell size to that of lean rats'. This could imply that the fat cells of fa/fa rats are not as sensitive to the lipolytic actions of exercise as those of lean rats. Exercise increased adipose and muscle lipoprotein lipase activity and lipolysis sensitivity to epinephrine (Wardzala *et al.*, 1982; Walberg *et al.*, 1983) and reduced muscle insulin resistance (Ivy *et al.*, 1986). Thus there is evidence that exercise activity performed with consistency and for a prolonged period, leads to improvement of metabolic profiles of both humans and rodents (Barlow *et al.*, 1995; Pescatello and Murphy, 1998).

4.4.2.3. FFA and triglycerides

Dietary fat increases circulating TG and FFA concentrations, inhibits glucose metabolism (Storlien *et al.*, 1991) and has an inhibitory effect on GLUT4 in skeletal muscle (Kahn and Pedersen, 1993), thus inducing insulin resistance. During high fat feeding, insulin resistance is first manifested in the liver, then in skeletal muscle and lastly the adipose tissue (Storlein *et al* ., 1991; Storlein *et al*, 1996).

Although plasma TG concentrations in fa/fa rats were not normalized to the lean rat concentrations, exercise did induce a decrease in both chow and high fat fed rats. The significant decrease in TG concentrations in LF.EX rats compared to the

control might be the cause of the small improvement observed in the glucose tolerance. Within the fa/fa phenotype, fasting FFA concentrations were not significantly affected by either diet. However, fasting significantly reduced FFA concentrations in LF.EX rats. Failure of fasting to induce a significant change in FFA concentrations in LF.SED and the high fat fed fa/fa rats indicate the abnormal metabolic condition of fa/fa rats. The lack of change with fasting might also mean that in obesity the production and clearance of FFA are in equilibrium or both mechanisms are defective. In lean rats, fasting significantly reduced plasma FFA concentrations which might indicate increased FFA usage in the presence of low production. Thus these *in vivo* results show beneficial effects of exercise on the regulation of energy balance in fa/fa rats; however increasing dietary fat content erases most of the benefits in both lean and fa/fa rats.

4.4.3. Summary and Conclusion

Overall this study showed that moderate dietary fat content interacted with genetic susceptibility of fa/fa rats to worsen the insulin resistance. Although the fa/fa obese syndrome is due to lack of normal leptin receptors causing defective communication between the periphery and CNS energy-regulating centers (Chua *et al.*, 1996b) physical activity in combination with a low fat diet might partially overcome this genetic disorder as shown by our results. Although a single episode of exercise was shown not to affect plasma leptin concentration in fa/fa rats (Pagano *et al.*, 1999), prolonged exercise might have different effects if it results in reduction

of body weight. This effect has been observed in genetically obese SHHF/Mcc-fa(cp) rats (Friedman *et al.*, 1997), diet-sensitive Osborne-Mendel and diet-resistant S5B/P1 rats (Zachwieja *et al.*, 1997) and in humans (Okazaki *et al.*, 1999). In the above mentioned studies, exercise training reduced ob gene expression, leptin concentrations and weight gain. In our study mild exercise reduced food intake and weight gain in fa/fa rats.

The failure of exercise to normalize plasma insulin concentrations may be related to persistently high TG concentrations even though these concentrations were somewhat reduced. Increasing the intensity as well as the length of the exercise might give more pronounced effects both *in vivo* and *in vitro* (Cortez *et al.*, 1991). The small benefits observed in the *in vivo* parameters were not accompanied by parallel changes in B-cells. Therefore, these beneficial effects could not be attributed to adaptations in B-cell biochemistry, since changes in islet function were very small.

In conclusion, our study showed exercise has a place in the regulation of energy balance, glucose and insulin responses in genetically transmitted obesity and that lean rats also benefit from exercise as it prevented weight gain and improved the glucose and insulin response. However, the presence of an abnormally regulated HPA axis has a dominant effect in the regulation of pancreatic islet function in fa/fa rats.

Chapter 5. GENERAL DISCUSSION AND CONCLUSION

These studies were carried out to test 3 hypotheses. (1) Feeding of a high fat diet would negate the beneficial effects of ADX on pancreatic islet B-cell function previously reported in fa/fa rats and would induce adaptive changes in islets of lean rats. (2) Since most of ADX actions are thought to be mediated by increased SNS activity in the periphery, and exercise is an activator of the SNS, then regular exercise would produce pancreatic islet B-cell benefits similar to those of ADX in fa/fa rats. (3) ADX and exercise would produce their effects on pancreatic islet B-cell function in fa/fa rats via key regulatory enzymes including glucokinase or by regulating FFA oxidation levels.

5.1. Adrenalectomy and dietary fat

5.1.1. Effects of ADX on pancreatic B-cell function

Previously we showed that in fa/fa rats fed rat chow, ADX of 5 wk old rats induced beneficial effects on pancreatic islet B-cell function 2 wk post surgery. These effects included (1) reduction of pancreatic islet B-cell sensitivity to glucose; (2) normalization of the lost mannoheptulose inhibitory action on pancreatic B-cell GSIS and (3) increased pancreatic islet glucokinase K_m (Kibenge and Chan, 1996).

In fa/fa rats fed stock rat chow, ADX normalized pancreatic B-cell function to that of the lean rats 2 wk post surgery by reducing pancreatic glucose sensitivity (EC_{50}) and increasing glucokinase K_m (Kibenge and Chan, 1996). Our

present results did not fully agree with those previously reported, in that the normalization of pancreatic GSIS by ADX was correlated with reduction of glucokinase phosphorylating rate (glucokinase V_{max}) rather than glucokinase sensitivity (glucokinase K_m). Pancreatic islet glucose sensitivity (EC_{50}) of islets isolated from ADX, sham fa/fa and lean rats were not different from each other; however, both basal insulin secretion and GSIS were higher in islets from fa/fa sham rats than those from ADX fa/fa and lean rats, indicating that islets from sham fa/fa rats were more responsive to low glucose concentrations.

In lean rats, glucokinase sensitivity to glucose tended to be reduced in both ADX.LF and SH.LF rats with glucokinase K_m values greater than what is considered the normal range (5-10 mM) (Lenzen *et al.*, 1987). While reduced glucokinase sensitivity in ADX rats may be explained by the *in vivo* low glucose concentration, it does not explain that in sham rats. The higher values obtained in this study were not expected but are not unusual since higher glucokinase K_m values (35.4 mM) have been reported in another lean rat strain (LA/N) (Chan *et al.*, 1995) using the same method . However, they make it difficult to reach any conclusion because of lack of correlation between the *in vivo* and *in vitro* pancreatic responses in lean sham rats since fasting glucose concentrations in there rats were in the normal range (6 mM). Although the sham lean rats' diet included 4% sucrose/ 0.9% NaCL which was not available to the LF.SED rats in the exercise program, their glucokinase K_m values are comparable indicating that at this low concentration sucrose did not alter glucokinase sensitivity in control rats. The high values may reflect the methodology of GK assay as we did not

cover wide range of glucose concentrations (Chapter 2, Section 2.6.1) used in this study. Because high sucrose diets tend to induce hypertriglyceridemia and hyperinsulinemia (Soria *et al.*, 1996), the 4% sucrose in the ADX study could have resulted in the differences between the *in vivo* and *in vitro* islet responses.

Overall glucokinase activity of isolated pancreatic islets was not significantly modulated by ADX. However GSIS was depressed in ADX rats, results similar to those observed after ADX in Sprague-Dawley rats where ADX decreased glucose metabolism, calcium uptake, insulin and cAMP content in pancreatic islets (Borelli et al., 1982). Administration of dexamethasone to ADX rats normalized all except pancreatic islet insulin content (Borelli *et al.*, 1982). That ADX reduction of GSIS is mediated through modulation of ANS was shown in ob/ob mice where ADX-reduced GSIS was abolished by icv administration of dexamethasone to ADX mice and by addition of 10 μM acetylcholine to islets incubated with 10 mM glucose (Okuda and Rosmos, 1994).

After ADX in fa/fa rats, the MH inhibitory effects on glucokinase were restored to the same magnitude as that observed 2 wk post surgery in low fat fed rats. Thus a MH concentration of 3 mM or higher significantly inhibited GSIS in islets from ADX fa/fa rats while this same MH concentration had no effect in sham fa/fa rats. As previously reported ADX had no significant effects on pancreatic insulin content in fa/fa and lean rats. The results of this study emphasized the significance of the HPA axis regulatory role in the persistence of abnormal pancreatic islet B-cell function in one model of genetic obesity. However, the data also showed that there is continuous change within the

pancreatic B-cell, since in 7 wk old ADX rats normalization of pancreatic GSIS was through decreased pancreatic islet glucose sensitivity (EC_{50}) and glucokinase sensitivity (K_m) while in this study (10 wk old rats) reduced insulin secretion was associated with changes in the glucokinase phosphorylating rate (V_{max}). Even though the reduction of glucokinase sensitivity (K_m) did not reach a significant level, a small change in the enzyme activity could have significant effects on glucose metabolism in the islets and insulin secretion *in vivo* (Matschinsky, 1996).

ADX induces time-dependent adaptations of glucokinase, first a change of the sensitivity (K_m) of the existing enzyme, then an alteration in the enzyme phosporylation activity (V_{max}), which might be a normal response to reduced whole body glucose metabolism in the face of low glucocorticoids. However, glucokinase mRNA expression does not appear to reduce with age compared to insulin and GLUT2 biosynthesis and GSIS which declines with age in rodents (Tsuchiyama *et al.*, 1991; Perfetti *et al.*, 1995) and humans (Wang *et al.*, 1988b; Dechenes *et al.*, 1998). Basal insulin secretion is increased with development of obesity and NIDDM and under these conditions hexokinase activity was shown to be increased as seen in Zucker diabetic fatty, fa/fa and lean rats, while glucokinase activity did not change between the ages of 5 wk and 12 wk within each group of these rats (Cockburn *et al.*, 1997). Similar results were observed in this study in fa/fa sham rats fed a low fat diet but not those fed a high fat diet. Although the activity of hexokinase is more evident in exocrine and other islet-cells (Schuit *et al.*, 1999), high activity in fa/fa rats pancreatic tissues may reflect

increased glucose use at low concentration and may play a role in the development of hyperinsulinemia.

The reduction of GSIS that accompanies age (Castro *et al.*, 1993) and ADX (Borelli *et al.*, 1982) appears to be mediated by the failure of the islets to metabolize glucose. An investigation of interactions between age and ADX on pancreatic B-cell function whereby the amount of gene expression and activity of all components that are involved in the process of GSIS is required. This would involve monitoring the effect of ADX on the gene expression of enzymes and activity of the glycolytic pathway, Krebs cycle and all the components of the nutrient-insulin secretory coupling process in pancreatic islet cells of ADX and control lean and fa/fa rats. Such a study would help to elucidate which of the components is predominantly affected by ADX.

Defects in pancreatic islet B-cell glucose responsiveness have been attributed to hypertriglyceridemia in obesity and NIDDM. FFA oxidation and lipogenic capacity are increased in most tissues including islets of obese animals (Lee *et al.*, 1997). Higher FFA ß-oxidation in pancreatic islets is associated with increased basal insulin secretion and inhibition of GSIS due to inhibition of glucose metabolism (Zhou and Grill, 1994). However the necessity of FFA for proper functioning of B-cells has been also documented (Dobbins *et al.*, 1998). Therefore reduction of basal insulin secretion and GSIS in ADX fa/fa rats could be attributed to the reduction of FFA oxidation in islets. Our data showed that higher FFA oxidation levels in islets from SH.LF fa/fa rats were reduced by ADX. This increased FFA oxidation was not associated with significant changes in total

hexokinase nor glucokinase activity, even though glucokinase sensitivity to glucose was increased in these islets. Although it has been shown that in rodent models of diabetes islet FFA oxidation is increased prior to the development of hyperglycemia and that islet TG content mirrors the plasma levels, our results from SH.LF and SH.HF fa/fa rats, which had equal concentrations of plasma TG, did not show similar levels of FFA oxidation. Also, there was failure to adjust to increasing glucose concentrations in islets of these rats. In islets isolated from lean rats there was no effect of ADX on both FFA oxidation and TG content. Therefore increased TG content and FFA in low fat fed fa/fa rats might contribute the persistent hyperinsulinemia of these rats and ADX might reduce insulin secretion by reducing plasma TG and FFA concentrations and islet usage of FFA.

In vivo responses to ADX were more pronounced in this study than after 2 wk post surgery in the reduction of weight gain. After 2 wk, the ADX rats weighed 11% less than sham rats while in this study the ADX rats weighed 45% less than the sham rats. Although in the previous study food intake, TG and FFA concentrations were not measured, it could be assumed that they were reduced by the same magnitude as the body weight. At 5 wk post surgery, weight gain, TG and FFA concentrations of ADX fa/fa rats were normalized to those of the intact lean rats. Insulin and glucose responses during OGTT were improved, indicating an improvement in insulin resistance in fa/fa rats.

5.1.2. Effects of dietary fat on pancreatic B-cell function in ADX rats

Our hypothesis was that post-surgical feeding of diet with 4-fold higher fat content would negate the effects of ADX in fa/fa rats and induce metabolic abnormalities in lean rats. The data did not fully support this hypothesis in that ADX benefits on pancreatic functions were reduced but not completely eliminated by feeding a high fat diet. Increased dietary fat increased pancreatic islet glucose sensitivity in both lean as reflected in the acute insulin response in lean rats during OGTT and in high basal insulin secretion in fa/fa rats however this may have been due to low fiber content of the diet (Ludwig *et al.*, 1999).

Increasing dietary fat content appears to augment ADX effects on hexokinase kinetics while preventing ADX-induced reduction of glucokinase V_{max}. Because only glucokinase V_{max} was modified by high fat diet in ADX, possibly this effect was responsible for reducing MH inhibitory action on GSIS from ADX.HF rat islets. In SH.HF fa/fa islets glucokinase V_{max} was not affected by high fat diet but glucokinase K_m was significantly increased compared to that of SH.LF. However, the overall glucose phosphorylation rate was not different from that of SH.LF rats which could be attributed to having similar fasting glucose concentrations (Table 4). An increase of dietary fat content increased hexokinase sensitivity to glucose (lowered K_m), but phosphorylating rate (V_{max}) was reduced; thus the observed high basal insulin secretion in SH.HF fa/fa rats could not be explained by increased hexokinase activity. Because pancreatic islet glucose sensitivity (EC_{50}) in SH.HF fa/fa rats was increased, this lowered glucose threshold in these cells likely played a major role *in vivo*

hyperinsulinemia observed in SH.HF rats since there is little hexokinase activity in B-cells (Schuit *et al.*, 1999). These results are supported by the finding in Zucker diabetic fatty rats where increased basal insulin secretion is inhibited by MH and CPT-1 inhibitor etomoxir indicating that this increased B-cell secretory activity was due to increased fuel flux through glycolysis rather than increased hexokinase activity (Zhou *et al.*, 1999).

Contrary to reports that high fat diet reduces pancreatic insulin content (Capito *et al.*, 1992, Kim *et al.*, 1995), we did not observe any difference between low fat and high fat fed rats. The different results might be due to the differences in the diet compositions, in both the above studies the diets had low carbohydrate content (35%) and a very high fat content (30%) whereas the diet used in this study had 44% carbohydrates and 16% fat content (Chapter 2). However, pancreatic insulin content in sham lean and fa/fa rats fed a high fat fed rats tended to be higher than that of low fat fed rats. To determine whether the differences would have reached a significant difference had the animals stayed on the high fat diet for an extended period or if the dietary fat content were increased would require further investigations.

Feeding a high fat diet reduced pancreatic islet TG content without affecting FFA ß-oxidation in ADX fa/fa rats. High carbohydrate diets increase lipogenesis both in the liver and adipose tissue, while high fat diets tend to inhibit lipogenic enzymes (Cheema and Clandinin, 1996; Kim and Freake, 1996). ADX might prevent TG deposition in the islets by reducing overall plasma lipid profiles thus limiting the concentration reaching the islets.

In vivo results also showed a dominant role of ADX over high fat content, in that caloric intake, weight gain, fasting insulin, glucose, FFA and TG content were reduced in fa/fa rats. In ADX.HF fa/fa rats, the integrated insulin response was increased despite a reduced glucose response. Therefore there is active interaction between HPA and high fat diet in the regulation of energy balance and the pancreatic response to glucose during OGTT. Since ADX also prevents the development of dietary obesity, then HPA axis regulatory action is the predominant influence on pancreatic islet glucose metabolism and energy balance.

In lean rats high dietary fat increased glucokinase K_m but reduced the phosphorylating rate without affecting the overall glucokinase activity in islets from SH.HF rats. Neither ADX nor a high fat diet had any effect on the pancreatic islet FFA oxidation capacity. *In vivo*, increasing dietary fat content increased TG concentrations, and insulin and glucose responses during OGTT in sham rats. These effects were reduced by ADX. ADX effects on food intake and weight gain were not modulated by a high fat diet.

Although some effects of ADX were altered by a high fat diet, it is concluded that reduction of corticosterone concentrations has beneficial effects on the metabolic, hormonal and abnormalities of fa/fa rats. Compared to studies in adult rats (Bray *et al.*, 1992) where high fat diet increased energy intake and body weight gain in ADX rats, in preweaned rats (Fletcher and MacKenzie, 1988b) and 5 wk old fa/fa rats, effects on weight gain pattern of ADX were not modulated by post-surgical feeding of a high diet. However, complete

normalization of insulin resistance of fa/fa rats was not achieved in these studies.

5.2. Exercise and dietary fat

This study was carried out to compare the effects of exercise with those of ADX on the functional changes in pancreatic B-cells and to investigate the interaction between the effects of exercise and dietary fat on energy balance and pancreatic function. Both exercise and chronic feeding of a high fat diet were reported to reduce insulin synthesis and impair signal transduction in pancreatic B-cells by inhibiting GLUT2, glucokinase and proinsulin mRNA expression, resulting in a reduction of GSIS in rats and mice (Koranyi *et al.*, 1991; Capito *et al.*, 1992, Kim *et al.*, 1995). The effect of high fat diet might be attributed to inducing an increase in FFA concentration reaching the pancreatic islets since prolonged exposure of islets to FFA resulted in similar effects (Gremlich *et al.*, 1997) and effects of exercise might be attributed to reduced glucose metabolism (Zawalich *et al.*, 1982).

5.2.1. Effects of exercise on pancreatic islet B-cell function

Our present data showed that exercise did not modify pancreatic function in fa/fa rats and this could be because fasting glucose concentrations were above the glucose threshold for insulin secretion. ADX reduced glucokinase activity mainly by reducing the phosphorylating rate in low fat diet fed fa/fa rats. The lack of effect of exercise on glucokinase activity in islets from fa/fa rats was

manifested by similar GSIS of exercised and sedentary fa/fa rats fed a low fat diet and failure of exercise to restore MH inhibitory activity in fa/fa rats. However, similar to ADX, exercise did not affect pancreatic insulin content nor glucose sensitivity.

Exercise did induce a modest reduction in fasting plasma FFA and TG concentrations in low fat fed fa/fa rats although these were still higher than those of lean rats. Failure of exercise to induce similar effects to those of ADX is probably due to the presence of an intact HPA axis, since the obese syndrome of fa/fa rats is dependent on the presence of glucocorticoids (Castonguay *et al.*, 1986; Fletcher and MacKenzie, 1988b). The presence of an intact abnormal HPA axis might have inhibited the effects of exercise; for example LF.EX lean rats showed improved insulin and glucose response during OGTT while no effect was seen in fa/fa rats. The lack of effect of exercise could be because fa/fa rats did exercise hard enough to stimulate SNS activity which would lead to adaptation in pancreatic islet B-cells function. Alternatively the effect could just be a function of obesity rather than the intensity of exercise, because obese human exercising at the same intensity as lean subjects had low exercised - induced plasma epinephrine increment (Yale *et al.*, 1989). The fact that we did not measure plasma catecholamines in both studies (an index of SNS activity) makes it impossible to estimate to what extent SNS was activated by ADX or exercise. The difference in the pancreatic B-cell responses in exercise versus ADX could be attributed to the timing of experimentation, which was carried out 24 h after the last exercise period was carried out. This might be enough time for

the metabolic and hormonal parameters to return to resting levels. Compared to ADX where there was no disruption by glucocorticoid replacement, the cumulative effects of exercise would be less than those of ADX, which is what we observed.

Failure to prevent hyperinsulinemia with exercise in our study might be due to the nature of the exercise regimen: low intensity and/or short duration. However, similar results have been reported by others (Walberg *et al.*, 1984) using a longer duration for the exercise sessions (2 h/d) and for a prolonged time (8 wk). The possibility that adding strength training to aerobic exercise might reduce plasma insulin and glucose concentrations has been illustrated in human studies (Eriksson *et al.*, 1998). A circuit-type resistance training for 3 months improved insulin sensitivity and glucose metabolism compared to lack of improvement in these parameters in individuals who trained aerobically for 6 months. However, aerobic training increased aerobic power and HDL-cholesterol (Eriksson *et al.*, 1998). In our study exercise reduced plasma levels of TG in low fat fed rats to a lesser extent than in high fat fed fa/fa rats.

Exercise did not induce significant modulation of the *in vitro* pancreatic B-cell function. *In vivo*, some beneficial effects were observed in lean rats. Insulin and glucose responses in OGTT were improved, demonstrating that the intensity level of the exercise was at least sufficient to modify peripheral insulin sensitivity.

5.2.2. Effects of high fat diet in exercised Zucker rats

Exercise and increased dietary fat and were shown to reduce GSIS by modifying pancreatic insulin synthesis, GLUT2 and glucokinase gene expression (Koranyi *et al.*, 1991; Kim *et al.*, 1995). Our data showed that exercise reduced overall hexokinase activity without effects on glucokinase in rat chow fed fa/fa rats. Feeding a high fat diet did not modulate the effects of exercise on hexokinase activity, pancreatic islet glucose sensitivity, nor insulin content. However, high fat diet increased glucokinase K_m and partially restored MH inhibitory action on GSIS in islets from HF.EX rats. However, on its own, high fat had no significant influence on hexokinase and glucokinase activity in fa/fa rats. *In vivo*, a high fat diet increased fasting insulin, and glucose responses during OGTT, and increased weight gain in exercised fa/fa rats.

In sedentary fa/fa rats, feeding a high fat diet resulted in reduced GSIS, without significant changes in pancreatic enzyme kinetics or pancreatic islet FFA oxidation capacity. High fat diet impaired the pancreatic response to glucose stimulation. However, the failure to secrete insulin was not due to lack of insulin since the pancreatic islet content HF.SED fa/fa rats was increased by 34% compared to that of LF.SED fa/fa rats. Also pancreatic islet glucose sensitivities were not different in high fat and low fat fed f/fa rats. *In vivo* hyperinsulinemia of high fat fed rats could not be attributed to hexokinase activity but to a combination of abnormal lipid profiles and severe insulin resistance in these rats. High fat diet reduced both *in vitro* and *in vivo* GSIS in fa/fa rats even though

glucokinase kinetics were not different from the those of the chow fed fa/fa rats. A worsened glucose intolerance, and increased insulin and glucose responses were induced during OGTT in high fat fed sedentary rats.

In vivo, both insulin and glucose responses were increased during OGTT in lean rats. The increased glucokinase glucose sensitivity and FFA ß-oxidation at low glucose concentrations in islets of these rats might be responsible for increased insulin displayed during OGTT. These results agree with those reported in the literature whereby increased pancreatic islet FFA oxidation is suggested to be the cause of the fasting hyperinsulinemia observed in most obese and NIDDM humans and animals (Milburn *et al.*, 1995; Zhou *et al.*, 1996b; Hirose *et al.*, 1996). Chronic high fat feeding increased pancreatic islet insulin content of HF.SED lean rats contrary to previous reports (Capito *et al.*, 1992; Kim *et al.*, 1995). However, these results are similar to those reported *in vitro* by (Milburn *et al.*, 1995), where exposure of islets of normal rats to high FFA concentrations resulted in an increase of pancreatic islet insulin content. The reason our diet did not result in reduced insulin content might be due to differences in the dietary fat content used and failure to raise plasma FFA to those obtained in most reported studies. The fat content of those diets used in studies were above 30%, compared to our diet which contained only 16% fat. However, this diet also had a low fiber content which could have contributed to the adaptive changes induced in lean rats.

In both fa/fa and lean rats the adaptive changes observed were not a

result of changes in the pancreatic B-cell chemistry, at least in what we measured. However, this diet might have modified other B-cell components involved in insulin processing and secretion that were not looked such as those recently reported in islets isolated from mice fed a high fat diet. These mouse islets exhibited both reduced glucose utilization and glucokinase activity (V_{max}) without any change in glucokinase K_m. However, this was also accompanied by an increased lysophospholipase activity which would lead to reduced lysophosphatidylcholine concentrations thus changing islet plasma membrane phospholipid composition and therefore playing a role in high fat diet induced-reduction of islet glucose metabolism and GSIS (Capito *et al.*, 1999). In these mouse islets, calcium ion channels, and enzymes such as phosphofructokinase, hexokinase, glucose 6-phosphatase and mitochondrial glycerophosphate dehydrogenase were not affected by high fat feeding (Capito *et al.*, 1999). However, these enzymes and others such as citrate synthase (Liu *et al.*, 1998), acetyl CoA carboxylase (Brun *et al.*, 1997) and pyruvate dehydrogenase, which have been shown to be modulated in the presence of both high FFA and glucose levels, requires further investigation in fa/fa rats.

The results of these studies did not full explain the relationship between the *in vivo* and *in vitro* effects of ADX or exercise on pancreatic B-cell function although some similar trends were observed. Variation of glucokinase kinetics might reflect the low glucose concentrations used in enzyme assays, thus an assay using a wide range of glucose concentrations might give better results.

Also failure to account for the enzyme localization could lead to misinterpretation of results such those of hexokinase activity. Additionally the diets in the ADX study and the exercise study were not identical in that, the rats in the ADX study were also given 4% sucrose/0.9% NaCl. In the ADX study SH.LF rats had high plasma TG concentrations compared to LF.SED rats but whether this difference can be attributed to the 4% sucrose is not clear since very high sucrose -rich diet (63%) have been shown to increase plasma TG, glucose and insulin concentrations in rats (Soria *et al.*, 1996). However, this very low sucrose concentration may have selectively affected TG concentrations in both lean and fa/fa rats because plasma fasting insulin concentrations in sham rats were lower than those of LF.SD rats. Also differences in metabolic parameters might be attributed to stress induced during surgery of sham rats, which the exercised rats did not experience.

Lastly, detrimental changes induced by the high fat diet may not related to the fat content of the diet but may reflect the unbalanced nature of the diet since it had low fiber, mineral and vitamin contents. However, despite these short comings in the methods, the study shows that both ADX and exercise induced some similar changes that resulted in reducing insulin resistance in fa/fa rats.

5.3. Conclusion

Adrenalectomy's ability to normalize energy intake, energy expenditure and insulin secretion in obese animals is supposedly due to increased SNS

activity (VanderTuig *et al.*, 1984; Onai *et al.*, 1995; Routh *et al.*, 1994). Exercise is also a SNS stimulator and causes increased plasma catecholamine levels (Houwing *et al.*, 1995), so it was hypothesized that chronic exercise should give similar results to those seen with ADX in obese rats.

Contrary to our hypothesis, exercise did not normalize pancreatic islet function. However, exercised fa/fa rats fed rat chow reduced their caloric intake and weight gain and TG concentrations. Both exercise and ADX induced small changes in fasting insulin concentrations of rats fed rat chow. However exercise failed to eliminate hyperphagia in fa/fa rats fed a high fat diet as compared to ADX. The persistent hyperphagia is dependent on high corticosterone concentrations since blocking the action of glucocorticoid with RU 486 prevents the development of obesity in fa/fa rats (Langley and York, 1990) and high fat induced obese syndrome (Kusunoki *et al.*, 1995). From these studies, it is concluded that:

1) ADX is more effective in regulating insulin secretion in fa/fa rats than exercise because it induces bigger changes in B-cell function, (2) High fat diet blocks some effects of both ADX and exercise, and (3) Changes in B-cell biochemical parameters and insulin secretion patterns were small and are unlikely to account for more than a minor component of the *in vivo* changes seen.

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