THE RELATIONSHIP BETWEEN THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS AND THE DEVELOPMENT AND PERSISTENCE OF PANCREATIC ISLET DEFECTS IN OBESE ZUCKER (fa/fa) RATS.

A thesis

submitted to the Graduate Faculty

in partial fulfilment of the requirements

for the degree of

Master of Science

in the Department of Anatomy and Physiology

Faculty of Veterinary Medicine

University of Prince Edward Island.

Molly J. Twinomwe Kibenge

Charlottetown, P.E.I

March 1994

ISBN 0-315-88449-5

Canada

CONDITIONS OF USE OF THE THESIS

The author has agreed that the Library, University of Prince Edward Island, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Chairperson of the Department or the Dean of the Faculty in which the thesis work was done. It is understood that the recognition will be given to the author and the University of Prince Edward Island in any use of the material in this thesis. Copying or publication or any other use of the thesis for financial gain without any approval by the University of Prince Edward Island and the author's written permission is prohibited.

Request for permission to copy or make any other use of material in this thesis in whole or in part should be addressed to:

Chairman of the Department of Anatomy and Physiology

Faculty of Veterinary Medicine

University of Prince Edward Island

Charlottetown, P.E.I.

Canada, CIA 4P3

SIGNATURE PAGES

iii-iv

REMOVED

ABSTRACT.

Adult obese fa/fa Zucker rats are hyperinsulinemic, hyperlipidemic, insulin resistant and normoglycemic. Increased insulin secretion in response to glucose is observed in 3 wk old rats but fasting hyperinsulinemia is not evident until 5 wk. Developmental studies were carried out to investigate the possibility that pancreatic islet lesions are present in the B-cells of fa/fa rats prior to the observed increased response to stimulants. Using isolated pancreatic islets from adult, weanling and suckling rats, the time of onset of functional changes in α_2-adrenergic receptors and abnormal regulation of glycolysis in B-cells was investigated in fa/fa rats. Lean age-matched animals served as controls.

Functional changes in α_2-adrenergic receptors were not observed in these studies. Guanabenz (an α_2-adrenergic receptor agonist) similarly inhibited glucose (15 mM) induced insulin release from isolated islets from adult and 5 wk old rats of both phenotypes. Phenylephrine (an α_1-adrenergic receptor agonist) did not affect glucose stimulated insulin secretion at any stage. Idazoxan (an α_2-adrenergic antagonist) blocked guanabenz inhibitory actions on glucose stimulated insulin release in both phenotypes but prazosin (an α_1- adrenergic receptor antagonist) did not alter guanabenz inhibited insulin release in either lean or fa/fa isolated islets at any age. These studies failed to confirm the existence of a prazosin-sensitive α_2-adrenoceptor previously reported in islets of fa/fa rats.

Changes in the glucokinase sensitivity to inhibition by mannoheptulose were present in isolated islets from both adult and 5 wk old, but not 3 wk old preobese fa/fa rats. Mannoheptulose inhibited glucose (15 mM) stimulated insulin release in a dose dependent manner in isolated islets from adult, 5 wk and 3 wk old lean rats and in 3 wk old preobese fa/fa rats. A significant reduction in glucose stimulated insulin release from isolated islets of 5 wk old fa/fa rats was observed at a 3-fold higher dose of mannoheptulose than in lean rats. The glucokinase response to mannoheptulose in pancreatic islets of adult fa/fa rats was completely lost.

From these studies it is concluded that when isolated islets from fa/fa rats are incubated in glucose (15mM) which is within the physiological range, functional changes in the α_2-adrenoceptors are not expressed. Also, the sympathetic nervous system exerts its inhibitory regulation of the pancreatic islet B-cells through α_2-adrenoceptors but not α_1-adrenoceptors. Pancreatic islet glucokinase in isolated islets from fa/fa rats have reduced sensitivity to mannoheptulose at 5 wk of age and are completely insensitive to mannoheptulose by 8 - 12 wk of age. Thus insensitivity to mannoheptulose arose concurrently with the reported onset of fasting hyperinsulinemia.

It is well established that adrenalectomy abolishes or reduces many symptoms of obesity in fa/fa rats, thereby implicating the hypothalamo-pituitary-adrenal axis in the pathogenesis of obesity. The dependence on the presence of an intact hypothalamo-pituitary-adrenal axis to the development of the reduced glucokinase response to mannoheptulose inhibition was further investigated by carrying out

adrenalectomy in 5 wk old lean and fa/fa rats. Two wk post surgery the glucokinase response to mannoheptulose inhibition was tested. Adrenalectomy was found to reduce the rate of weight gain in fa/fa rats but did not affect the pancreatic islet insulin content. There was no effect of adrenalectomy on either weight gain or islet insulin content in the lean rats. Glucose (15 mM) induced insulin release was dose dependently reduced by mannoheptulose in isolated islets from adrenalectomized lean, sham lean and adrenalectomized fa/fa rats, but mannoheptulose had no significant effects in the sham operated fa/fa rat pancreatic islets. These data provide evidence that the development and persistence of abnormal glucokinase activity in fa/fa rats is under the influence of an intact hypothalamo-pituitary-adrenal axis. Removal of corticosterone abolishes the defect. More studies on the effect of glucocorticoids on the expression of glucokinase in fa/fa rats are needed to further determine the specific nature of the lesion in the fa/fa rat.

ACKNOWLEDGEMENTS

I would like to thank the members of my supervisory committee, Drs: S. Ihle, W. Ireland and A. Tasker and in particular my supervisor Dr Cathy Chan for their help and constant encouragement during the period of this study.

I also wish to thank Ruth MacPhail and Cathy Mitton for their technical assistance and for their continuous friendship. I also acknowledge the assistance offered by Evelyn Daley, Ramona Taylor and Dianne O'connell from the Histopathology laboratory in the processing of the pancreatic tissues. I wish to thank Drs. Karen Gibson and Alan Donald for their assistance with various aspects of the thesis.

The financial support from the Canadian Diabetes Association and the Department of Anatomy and Physiology is acknowledged.

Finally, I would like to thank my husband Fred for his patience, encouragement and support during the period of this study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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
CBP	Corticosteroid binding protein
CHD	Coronary heart disease
CCK	Cholecystokinin
CO_2	Carbon dioxide
CRF	Corticotrophin releasing factor
d	Day(s)
DME	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EIA	Entero-insular axis
G-6	Guanabenz 10^{-6} M
GAT	Nucleotide bases (G=guanine, A= adenine T=thymine)
GIP	Gastric inhibitory polypeptide
GK	Goto-Kakizaki rats
$GLUT_{1-4}$	Glucose transporters one through to four
ICV	Intracerebroventricular
Id	Idazoxan 10^{-5} M
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}
HIR-A or HIR-B	Insulin receptor isoforms
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
PE	Polyethylene catheter
PP	Pancreatic polypeptide
RNA	Ribonucleic acid
SEM	Standard error of the mean
SNK	Student-Newman-Keuls test
STZ	Streptozotocin
VMH	Ventromedial nucleus of the hypothalamus
WK	Week(s)

1. GENERAL INTRODUCTION

Obesity is defined as having a high percentage of body weight contributed by adipose tissue (Bray and York, 1971). It is a major concern in developed and developing countries because of its association with several chronic diseases (Lara-Pantin, 1987). Such diseases include cardiovascular diseases (Manolio *et al.*, 1991), non-insulin-dependent diabetes mellitus (NIDDM, type 2 diabetes) (Ferrannini *et al.*, 1991), and some forms of cancer and early mortality (Laurier *et al*, 1992; Tai *et al.*, 1992; Terry *et al.*, 1992; Sjöström, 1993). Obesity is also associated with reduced productivity due to chronic sickness. The cause of obesity is energy imbalance, whereby the energy consumed is greater than that required by the individual (Bray and York, 1979; Bray *et al.*, 1989), the excess energy then being stored as body fat. Obesity affects human beings, companion and experimental animals. Energy imbalance in humans may be due to one or more factors including excess intake of energy-rich foods (hyperphagia) (Sclafani, 1993), reduced metabolism due to neurohormonal disturbances (Bray and York, 1979), or a genetic factor which is expressed under certain environmental conditions (Meyer and Stunkard, 1993). In dogs and cats obesity is common in old and less active animals that are over fed (Lewis, 1987), those which have developed hyperadrenocortism and those which have been gonadectomized (Crane, 1991).

The obese syndrome of human beings is a multifactorial, complex condition which has made many treatments unsuccessful in preventing or curing the condition. Understanding the underlying causes and treating the whole person compared to

treatment of the symptoms may give better results. Understanding body weight regulation in both normal weight and obese individuals could provide more information on how energy imbalance develops in susceptible individuals. Many investigators believe that, like other body functions, body weight is regulated at a set point and the body fights hard to maintain that weight, either by increased caloric intake after prolonged food restriction or by reduction of basal metabolic rate (Keesey, 1993). With forced high caloric intake normal individuals increase their body metabolism, resulting in maintenance of a constant body weight (Jordan, 1969). Hamilton and Brobeck (1964) observed that hypothalamic injury in monkeys produced hyperphagia and weight gain, after which the animals stopped overeating but maintained that newly acquired weight. When these monkeys were put on restricted caloric intake they lost weight, but then regained the lost weight and stabilized at the post-lesioned weight when they returned to normal feeding habits. This has also been observed in human beings who lose weight on restricted caloric diets but regain it when they resume normal eating habits (Stunkard and Penick, 1979). The body weight set point can be shifted either by changes in eating habits (Sclafani, 1993) or physical activity or by damage to the hypothalamic regulatory centre (Hoebel and Hernandez, 1993; Keesey, 1993).

1.1. Types of obesity.

An obese syndrome is etiologically classified as dietary obesity if diet change is the only underlying cause (Stone *et al.*, 1980; Levin *et al.*, 1983b), pathological if

it arises due to trauma or hypothalamic injury (Jeanrenaud, 1985; Bray and York, 1979) or genetic if it occurs in the absence of changes in diet or hypothalamic injury (Zucker and Zucker, 1961). However there is no clear separation between the causes since in most cases there is at least a transient increase in energy consumption in the genetically transmitted or hypothalamic obese syndromes (Bray *et al.*, 1989). The energy imbalance can result in enlargement (hypertrophic obesity) or multiplication (hyperplastic obesity) of the fat cells.

1.1.1. Dietary obesity.

Dietary obesity is caused by excess consumption of high energy foods combined with reduced activity for a prolonged time and is normally hypertrophic in nature, although high fat diets can cause new fat cell synthesis (Bray *et al.*, 1989). Dietary obesity generally occurs in people or animals that are genetically susceptible to obesity (Schemmel *et al.*, 1970; Stone *et al.*, 1980; Levin *et al.*, 1983b). Young and male animals are less susceptible to dietary obesity than are old and female animals (Sclafani and Gorman, 1977). Dietary obesity can be induced experimentally by manipulating diet composition, the amount of food fed or the way of delivering food to the animal, such as tube feeding or forced feeding. The availability of high energy foods coupled with reduced activity leads to weight gain in humans and experimental animals. Sclafani and Springer (1976) showed that rats allowed access to cafeteria food in addition to their regular diet ate more than the animals fed only the regular diet and gained weight. However those which had access to a running wheel gained

less weight than the sedentary ones. Of all the nutrient composition manipulations, diets with high saturated fat content have been found to induce obesity in experimental animals in the shortest time (Schemmel *et al.*, 1970). Increasing the dietary fat composition enhances the palatability of the diet, thus leading to overeating and weight gain (Sclafani, 1993). High fat diets may also induce overeating due to the poor satiating power of fat compared to carbohydrates and proteins (Friedman, 1990) therefore leading to consumption of larger quantities of food during a meal (Blundell and Burley, 1990). Because of the increased body size the obese individual has to consume more food to maintain energy balance. Dietary obesity could probably be controlled by reducing the amount of energy consumed, for example by reducing the fat content in the diet and increasing physical activity if there are no other underlying defects.

1.1.2. Pathological or Hypothalamic Obesity.

Hypothalamic obesity, also known as regulatory obesity, is a result of injury to specific areas in the hypothalamus. Body weight increases due to excess accumulation of fat in already existing fat cells. The first case of hypothalamic obesity in humans was reported by Mohr (1840) and was later confirmed by Babinski in 1900 and Frohlich in 1901(reviewed by Bray *et al.*, 1989). They documented that tumours in the region of the hypothalamus were associated with obesity in humans (Babinski, 1900; Frohlich, 1901). However a better understanding of this syndrome has been gained through the use of experimental animals. Hypothalamic injury can

be caused by mechanical means such as knife cuts (Sclafani and Kirckgessner, 1986), electrolytic lesions (Bray and York, 1979) or by chemicals such as gold thioglucose or monosodium glutamate (Olney, 1969; Debons *et al.*, 1977; Takasaki, 1978; Blair *et al.*, 1993) or bipiperidyl mustard (Rutman *et al.*, 1966). Injury to the ventromedial nucleus of the hypothalamus (VMH) produces obesity with or without an increase in food consumption in different animals including humans (Karakash *et al.*, 1977; Bray and York, 1979; Bray, 1984), while injury to the paraventricular nucleus produces obesity with hyperphagia (Weingarten *et al.*, 1985).

Because the hypothalamus regulates energy balance, feeding mechanisms, and other physiological functions through autonomic nervous system outflow and hormonal feedback, any disruption in the neuronal connections can have a great impact on energy balance of the animal. In the VMH lesioned rat or mouse the first observation is increased insulin secretion followed by increased food intake and body weight gain (Morrison, 1977; Jeanrenaud, 1985; Blair *et al.*, 1993). Increased insulin secretion enhances lipogenesis in the liver and adipose tissue (Jeanrenaud, 1985) while reduced physical activity contributes to the obese syndrome.

Development of hyperinsulinemia is thought to be due to increased parasympathetic activity and reduced sympathetic activity in the pancreatic B-cells, because hyperinsulinemia after VMH lesions can be eliminated by subdiaphragmatic vagotomy (Berthoud and Jeanrenaud, 1979). Reduced sympathetic activity after VMH lesioning is demonstrated by reduced norepinephrine turnover at the sympathetic nerve terminals and reduced brown adipose tissue thermogenesis in

response to diet or cold exposure in weanling rats (Vander Tuig *et al.*, 1982) but not in adult rats (Young and Landsberg, 1980). VMH lesioned rats also exhibit other hormonal changes, including disruption in the reproductive cycle, blunted diurnal rhythm for corticosterone secretion and reduced growth hormone secretion (Bray and York, 1979). Adrenalectomy reverses hypothalamic obesity, and corticosterone replacement prevents the effects of adrenalectomy; thus, development of this obese syndrome depends on the presence of glucocorticoids (King and Smith, 1985). The effects of adrenalectomy are probably due to elimination of negative feedback of the corticosterone on corticotropin releasing factor (CRF) (Arase *et al.*, 1989a). Infusion of CRF into the third ventricle reduces food intake and enhances thermogenesis in normal rats (Arase *et al.*, 1989a) and increases circulating norepinephrine (Brown *et al.*, 1982). Similar findings were observed in VMH lesioned rats (Arase *et al.*, 1989b). Arase and others (1989b) also reported an increased rate in sympathetic nerve firing to the brown adipose tissue following intraventricular infusion of CRF in obese rats.

1.1.3. Genetically transmitted obesity.

In humans, obesity runs in some families, and in certain ethnic and social groups, but the genetic origin is not yet clearly defined. Obesity often occurs in children of families in which one or both parents are obese (Greenwood and Turkenkopf, 1983; Price *et al.*, 1989; Eck *et al.*, 1992). Childhood obesity has a higher chance of being genetic in origin than does adult onset obesity (Brook *et al.*, 1975). Childhood onset obesity is characterized by an increase in number of fat cells

(hyperplastic obesity) and is more resistant to treatment than adult onset obesity, which is considered to be due to enlargement of existing fat cells (hypertrophic obesity) (Krotkiewski *et al.*, 1977; Sjöström, 1993). It has been observed that obese children have greater numbers of adipose cells at any age compared to lean ones and that the increase in fat cells in obese children occurs during a period that is quiescent in normal-weight children (Knittle, 1975; Knittle *et al.*, 1977). Knittle observed two groups within the obese children; in one group the fat cell numbers were in the range of those found in adult while the second group had fat cells numbers that exceeded those of the adults. He suggested that the group with fat cells that exceeded normal adult values were more likely to become obese adults, while those with fat cell numbers in the normal adult range would outgrow the so-called " baby fat" (Knittle, 1975).

Studies involving adopted children show that the children's body weights are correlated to those of their biological parents rather than those of the adoptive parents (Stunkard *et al.*, 1986; Sorensen *et al.*, 1992) and that environmental factors do not have much influence on their weight (Sorensen *et al.*, 1989). Bouchard and others (1988) observed that there was possible familial obesity but not genetic obesity, although there was a genetic contribution to various obesity indices (5% for subcutaneous fat and body mass index and 25% for fat mass, fat free mass and percentage body fat) (Bouchard *et al.*, 1988; Bouchard, 1991). Sorensen and colleagues (1992) found the heritability correlation for body mass index (BMI, in kg/m^2) to be between 20 and 40%. Studies involving identical twins show that their

weight is normally in the same range whether they are raised together or apart (Stunkard *et al.*, 1986; Stunkard *et al.*, 1990) with few exceptions. However, body weight changes in fraternal twins were different. Body weight change in monozygous twins who were fed an extra 1,000 calories per day for 12 weeks was found to be similar in members of each pair, although there was a difference in weight gain in the group as a whole (ranging from 4.3 to 13.3 kg). A high correlation of body fat distribution between a twin pair was also reported (Bouchard *et al.*, 1988; 1990). Development of obesity in human beings is therefore under the influence of both genetic and environmental factors, such that increased intake of highly palatable foods and reduced physical activity will lead to obesity in susceptible individuals (Bouchard, 1991; Meyer and Stunkard, 1993).

Studies in animal models of genetically transmitted obesity have helped to shed more light on the pathogenesis of human obesity (Bray *et al.*, 1989). The most widely studied model is the Zucker obese (fa/fa) rat, in which the obesity is transmitted as a single Mendelian recessive gene (fa/fa) from mating of two heterozygous (Fa/fa) lean rats (Zucker and Zucker, 1961). This model will be described in further detail below (see section 1.4).

1.2. Complications of obesity in human beings.

Obesity is associated with many health risks including abnormal metabolism of carbohydrates and lipids and development of NIDDM (Widen *et al.*, 1992; Salans, 1987). Obesity's association with coronary heart disease (CHD) is well documented

from longitudinal studies by many investigators (Van Itallie and Hashim; 1975; Brunzell, 1983; Manson *et al.*, 1990; Lissner *et al.*, 1991; Beard *et al.*, 1992; Sjöström, 1992b; Terry *et al.*, 1992; Sjöström, 1993), although others have failed to find a definite connection (Larsson *et al.*, 1981; Hoffmans and Kromhout, 1989; Stevens and Lissner, 1990). The risk of CHD increases with an increase in abdominal and upper body fat accumulation (White *et al.*, 1986; Pi-Sunyer, 1991; Folsom *et al.*, 1991). Also, obese subjects are at higher risk of developing NIDDM than are normal weight subjects. Approximately 60% - 90% of people with NIDDM are obese (Felber, 1992), and obese individuals without NIDDM have impaired glucose tolerance and other endocrine dysfunction (Salans, 1987). Severely obese patients with NIDDM have a higher risk of developing CHD or hypertension than does the normal population because of higher plasma triglyceride, cholesterol and low density lipoprotein (LDL) levels, and changes in composition and proportion of lipoproteins (Barakat *et al.*, 1992). The LDL in NIDDM subjects are reported to be smaller and denser than in lean and non-diabetic obese subjects and their high density lipoproteins (HDL) contained a greater HDL_3 : HDL_2 ratio than the lean subjects. Obesity is also implicated as a factor in diseases like breast cancer (Schapira *et al.*, 1991), endometrial and ovarian cancer in women (Dunn and Bradbury, 1967; Folsom *et al*,. 1989), and prostate cancer in men (Nomura *et al.*, 1985). Obesity also aggravates arthritis, especially of the hip and knee joints, varicose veins (Seidell *et al.*, 1985), and diseases of the liver, gallbladder and lungs (Dwyer and Feldman, 1975; Pi-Sunyer, 1991). The incidence of unexplained sudden death is higher in severely obese

individuals than in the general population (Sjöström, 1992b).

Regardless of the cause, all obesity syndromes are accompanied by hyperinsulinemia and insulin resistance. A correlation between hyperinsulinemia and increased body weight has been reported in many human studies (Krotkiewski *et al.*, 1983; Peiris *et al.*, 1986; Peiris *et al.*, 1992) and hyperinsulinemia is a common denominator in all genetically transmitted obesity in animal models (Bray and York, 1971; Bray and York, 1979; Bray *et al.*, 1989). Hyperinsulinemia is also associated with many diseases such as CHD, hypertension, NIDDM (Ferrannini *et al.*, 1991), and hyperlipidemia (Widen *et al.*, 1992). With persistent hyperinsulinemia, insulin resistance develops in insulin sensitive target tissues, mainly affecting glucose disposal by skeletal muscles, liver and adipose tissue while lipogenesis is increased in both liver and adipose tissue (Jeanrenaud, 1985; DeFronzo, 1992). Coppack and colleagues (1992) reported the failure of insulin to regulate the activities of both hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) in obese subjects in the fed and fasting state, resulting in high levels of circulating nonesterified free fatty acids (NEFA) despite the hyperinsulinemic state of these subjects.

The causes of hyperinsulinemia in obesity are not clearly understood. However, body fat distribution seems be a significant factor in the increase of insulin secretion even in moderately overweight individuals (Kissebah *et al.*, 1987; Bonora *et al.*, 1993; Kohrt *et al.*, 1993). In addition some endocrine disorders of the adrenal and pituitary glands (Cushings disease and acromegaly) in humans and animals are accompanied by excess insulin secretion (Klink and Estrich, 1964; Perley and Kipnis,

1966). In experimental obesity, injury to the VMH nucleus is immediately followed by increased insulin secretion and this increase is abolished by vagotomy (Jeanrenaud, 1985). This implies that abnormal regulation by the central nervous system may be the primary cause of increased insulin secretion in obesity syndromes. Obesity in VMH lesioned animals develops secondary to hyperinsulinemia since insulin augments lipid synthesis and storage in both liver and adipose tissues.

1.3. Regulation of insulin secretion.

Insulin is a peptide hormone which is synthesized, stored and secreted by the B-cells of the endocrine pancreas (Orci, 1985). It regulates carbohydrate, lipid and protein metabolism in animals. In normal physiological situations, insulin secretion is influenced by different substances, such as fuel metabolites (glucose, amino acids, fatty acids) and neurotransmitters and hormones (Efendic *et al.*, 1991).

Glucose is the major physiological stimulant of insulin secretion following transport into the B-cell and metabolism through the glycolytic pathway and Kreb's cycle. Its metabolite, ATP, induces insulin release by closure of the ATP-sensitive potassium channels, which depolarizes the B-cell plasma membrane. The depolarization then opens the voltage-gated calcium channels. Extracellular calcium enters the cell to increase intracellular calcium, which activates Ca^{2+} -calmodulin dependent proteins regulating the insulin secretory machinery (Wollheim and Sharp, 1981; Efendic *et al.*, 1991). High levels of plasma insulin are detected after a carbohydrate rich meal and low levels are detected after fasting in normal subjects.

Glucose induced insulin release can be blocked by inhibitors of glucose metabolism such as mannoheptulose (Pipeleers *et al.*, 1973).

Insulin secretion is also regulated by the central nervous system, which determines the autonomic nervous system tone reaching the pancreas. While the parasympathetic nervous system enhances glucose stimulated insulin secretion, the sympathetic nervous system inhibits secretion through alpha$_2$- adrenergic receptors (Rorsman *et al.*, 1991). The endocrine pancreas also receives input from the entero-insular axis (EIA, composed of neural, endocrine, paracrine and neurocrine entities) and this plays a significant role in regulating meal-stimulated insulin release. The effect of the EIA is observed when oral ingestion of glucose results in greater insulin secretion than does intravenous administration of the same amount of glucose (Hampton *et al.*, 1986; Bailey and Flatt, 1988).

After insulin is secreted by pancreatic islet B-cells in response to stimulants, insulin binds to its receptors in target tissues where it induces both immediate and long term effects. The immediate actions do not result in synthesis of any new proteins but include increased transport of metabolites (glucose, lipids and amino acids) by mobilizing transporter proteins from cell cytoplasm into the cell plasma membrane in skeletal muscles and adipose tissues (Darnell *et al.*, 1991). However prolonged insulin exposure results in DNA and RNA induction, and lipid and protein synthesis (Jefferson and Kimball, 1989; Saltiel, 1989). In some cells insulin promotes cell proliferation and growth. After prolonged exposure to high levels of the hormone, receptor down regulation will occur thus resulting in insulin resistance in

many target tissues (Jeanrenaud, 1985). Prolonged exposure to hyperinsulinemia results in many abnormalities involving lipid, protein and glucose metabolism that have been widely documented (see section 1.2).

1.4. Animal models of genetic obesity.

There are several animal models of genetically transmitted obesity which have been reviewed extensively (Bray and York, 1971; 1979; Bray *et al.*, 1989). Obesity is either transmitted as a single autosomal dominant trait such as in the yellow obese mouse (A_y) and the adipose mouse (Ad) or as a double autosomal recessive trait as in the obese mouse (ob/ob), diabetic mouse (db/db), and the fatty rat (fa/fa) (Bray and York, 1971). Most genetically obese animals are not distinguished from their lean littermates until 3 to 5 wk of age, with the exception of the diabetic mouse which becomes hyperinsulinemic as early as 2 wk (Coleman and Hummel, 1967; Bray and York, 1971). Genetic obesity in rodents is characterized by hyperphagia. However, food restriction does not prevent the development of obesity. These animals exhibit reduced energy expenditure, have increased adipocyte size and number, and are hyperinsulinemic (Bray and York, 1971; 1979). The most studied animal models of obesity are the obese mouse (ob/ob), which is both hyperinsulinemic and hyperglycemic, and the Zucker obese (fa/fa) rat, which is hyperinsulinemic but normoglycemic (Zucker and Zucker, 1961; Bray, 1977). Early development of severe hyperglycemia in the obese ob/ob mouse makes it difficult to study the effects of long standing hyperinsulinemia so most investigators use the fa/fa rat as a model for both

childhood onset human obesity and for development of early metabolic changes in NIDDM (Bray, 1977; Terrettaz and Jeanrenaud, 1983; Krief and Bazin, 1991).

1.4.1. The obese syndrome of the Zucker fa/fa rat.

Obesity in the Zucker obese rat is genetically transmitted as a double autosomal Mendelian recessive trait (fa/fa) (Zucker and Zucker, 1961; Bray, 1977; Argilés, 1989; Krief and Bazin, 1991). The fa/fa rat arose from a cross between the Merck stock M and Sherman rats (Zucker and Zucker, 1961) and is easily recognised at 4 to 5 wk by its weight and shape. After that time the fa/fa rat gains weight faster than its lean littermates and accumulates fat throughout its life span. The young adult Zucker fa/fa rat is hyperinsulinemic, hyperphagic and hyperlipidemic (Debant *et al.*, 1987; Argilés, 1989), and insulin resistant in some metabolic pathways (Bray, 1977; Jeanrenaud, 1985). These rats have abnormal function of the reproductive system. All females rats and approximately 80% of the males are sterile (Krief and Bazin, 1991).

Although fa/fa rats are hyperphagic and hyperinsulinemic they remain normoglycemic or only slightly hyperglycemic. Adult fa/fa rats show decreased to normal food intake but are more energy efficient and less active than their lean littermates, which helps the fa/fa rats maintain the obese state (Keesey, 1993). A study by McCaleb and Sredy (1992) reported severe hyperglycemia in some male fa/fa rats and these rats also developed some complications observed with NIDDM. This variation of the fa/fa rat has been designated ZDF/Dtr-fa (Friedman *et al.*,

1991). The obese syndrome of Zucker fa/fa rats is characterized by both increased fat cell size and number, resembling human childhood onset obesity (Cleary *et al.*, 1980). The hyperplasia in the adipocyte of both fa/fa rats and obese children occurs at a time when there is no fat cell increase in normal weight children and rats (Lemonnier and Alexieu, 1974; Knittle, 1975).

Increased lipid synthesis in both liver and white adipose tissues is reported in the first postnatal week in the preobese pups (York and Bray, 1973; Godbole *et al.*, 1978; Krief and Bazin, 1991), which suggests that the fa/fa rat syndrome is driven by an overactive lipid synthesis and storage abnormality. The young pups also have abnormal thermoregulation which is maintained in the adult stage, and is expressed as lower body temperature and reduced oxygen consumption (Krief and Bazin, 1991).

1.4.2. Causes of hyperinsulinemia in the fa/fa rat.

The primary cause of increased insulin secretion in fa/fa rats after weaning is not clearly understood despite extensive investigation of this defect. Because in normal physiological situations insulin secretion is regulated or influenced by several different pathways, abnormalities in any of them could lead to excess secretion. A few hypotheses have been proposed by Jeanrenaud (1979) and Beloff-Chain (1987) which include the following:

1. Hyperphagia (increased food intake)
2. Insulin resistance
3. Overactive entero-insular axis

4. Abnormal regulation by the central and/or autonomic nervous system (imbalance of autonomic nervous system and overactive hypothalamo-pituitary-adrenal (HPA) axis).

5. Primary pancreatic islet lesions.

1.4.2.1. Hyperphagia.

The Zucker obese rat becomes hyperphagic and hyperinsulinemic immediately after weaning and remains so most of its life (Zucker and Zucker, 1961; Bray, 1977; Bray and York, 1979). Increased food intake has been observed as early as the second week if preobese pups are allowed access to solid food (Bell and Stern, 1977). However hyperinsulinemia is not observed until the third week. The fact that obese fa/fa rats are constantly eating both day and night is likely to result in high levels of circulating metabolites (glucose, amino acids and free fatty acids) in the blood reaching the endocrine pancreas (Bray and York, 1971; Argilés, 1989), leading to constant stimulation of pancreatic B-cells and hyperinsulinemia. However when preobese pups are raised on restricted low calorie diets from day 10 or are pair fed to the lean rats, the preobese pups still become obese and hyperinsulinemic (Zucker, 1967; Bray *et al.*, 1973; Radcliffe and Webster, 1976; Cleary *et al.*, 1980). This implies that hyperphagia is not the primary defect leading to hyperinsulinemia in Zucker fa/fa rats.

1.4.2.2. Insulin resistance.

Development of hyperinsulinemia in fa/fa rats is accompanied by insulin resistance in skeletal muscle, adipose tissue and liver (Czech *et al.*, 1978; Bray and York, 1979; Kemmer *et al.*, 1979; Krief and Bazin, 1991). It is not clear whether insulin resistance precedes hyperinsulinemia or vice versa. Insulin resistance is observed in both lean and preobese pups during the suckling stage, and then disappears in the lean rats but persists in fa/fa rats after weaning (Pénicaud *et al.*, 1988). Insulin resistance in the suckling stage could be due to different factors, such as a high glucagon/insulin ratio. High glucagon levels inhibit insulin actions on the liver, thus maintaining elevated gluconeogensis in the liver despite high levels of insulin during insulin infusion (Del Prato *et al.*, 1987). A lower proportion of insulin-dependent tissues in the suckling stage and to some extent an alteration in insulin action (Issad *et al.*, 1987) may also contribute to insulin resistance. In skeletal muscles, the resistance may be due to the type of muscle fiber predominant during this developmental stage because it is known that insulin influences muscle fiber composition (James *et al.*, 1985; Kahn, 1992a; Holmäng *et al.*, 1993). Insulin resistance in white adipose tissue is suggested to be due to diet (high fat) and the developmental stage of the animals (Pénicaud *et al.*, 1988). When the pups are weaned to a high fat diet, glucose utilization in the presence of insulin is still lower than when the pups are weaned to a high carbohydrate diet (Pénicaud *et al.*, 1991).

Concentrations of $GLUT_4$ mRNA and lipogenic enzymes in the white adipose tissue are reported to increase in response to high levels of circulating insulin after

weaning to a high carbohydrate diet (Pénicaud *et al.*, 1988; Girard *et al.*, 1992a). A recent study by Kahn and Pedersen (1993) found that Sprague-Dawley rats made obese by feeding a high fat diet had reduced expression of $GLUT_4$ mRNA and $GLUT_1$ mRNA and their respective proteins in the skeletal muscles, while rats made obese by feeding a high calorie/carbohydrate diet did not differ in $GLUT_4$ expression from the controls. In the same study, they observed no difference in the $GLUT_4$ protein levels and mRNA in muscles of 5 and 20 wk lean and obese Zucker rats, and in 20 wk old fa/fa rats made diabetic by treating them with streptozotocin. The $GLUT_4$ protein concentration in white adipose tissue of Zucker obese rats is reported to be increased before 20 wk compared to lean animals, after which there is a decline (Friedman *et al.*, 1990; Hainault *et al.*, 1991; Kahn *et al.*, 1991).

Insulin stimulation of glucose utilization was reported to be higher in the white adipose tissues of preobese rats, but when the whole-body utilization was considered it was lower than in the lean littermates. Hepatic glucose production was not inhibited by insulin to the same extent as in the lean pups, showing that even at an early stage of development fa/fa rats have reduced insulin sensitivity in the liver and muscles but normal or increased sensitivity in the white adipose tissues (Pénicaud *et al.*, 1988). Insulin resistance of fa/fa rats is genetically determined early in life but is masked by the high fat diet during the suckling period which after weaning to high carbohydrate diet becomes fully expressed (Pénicaud *et al.*, 1991). Preweaning insulin resistance therefore cannot be the primary cause of hyperinsulinemia in the adult fa/fa rat.

Insulin resistance can occur due to reduced or defective insulin receptors in the target tissues (Garvey *et al.*, 1989). Two isoforms of insulin receptors (HIR-A and HIR-B) have been identified in skeletal muscles of prediabetic and NIDDM patients. In normal subjects HIR-A is predominant while in relatives of diabetics, subjects with varying degrees of insulin resistance, and NIDDM subjects increased HIR-B isoform expression is seen (Mosthaf *et al.*, 1993). The predominance of HIR-B insulin receptor isoform in muscles of prediabetic human subjects (Handberg *et al.*, 1993) and in skeletal muscles of ob/ob mice (Le Marchand-Brustel *et al.*, 1985) account for the reduced insulin binding activity that has been reported. However this defect have not yet been reported in fa/fa rats.

The most likely site of the metabolic defect is the insulin sensitive glucose transporters ($GLUT_4$) in both skeletal muscles and adipose tissue. These transporter proteins are mobilized from the cytoplasm to the cell plasma membrane after insulin binding; this allows transport of glucose into the cell. Reduced glucose transporter $GLUT_4$ has been observed in skeletal muscles of diabetic human patients (Kahn , 1992b; Vogt *et al.*, 1992), diabetic fatty Zucker rats (ZDF/Drt-fa) (Friedman *et al.*, 1991) and SHR/N-cp rats (Marette *et al.*, 1993). However, insulin resistance in the muscles of adult fa/fa rats probably results from impaired translocation of the glucose transporter $GLUT_4$ from the cytosol to the cell plasma membrane (Horton *et al.*, 1990). There is no reported difference in the number of transporters and in $GLUT_4$ mRNA expression in skeletal muscle of fa/fa rats and lean controls (Horton *et al.*, 1990; Knott and Hesketh, 1992; Seino *et al.*, 1992; Zarjevski *et al.*, 1992; Kahn and

Pedersen, 1993;). The reported defective translocation of the glucose transporters in presence of insulin may be a result of defective tyrosine receptor kinase activity of the insulin receptor (Horton *et al.*, 1990; King *et al.*, 1992). Defective signal transduction due to defects in the autophosphorylation of insulin receptor kinase has been reported in livers of old Wistar rat exhibiting insulin resistance (Nadiv *et al.*, 1992). Exercise increases both translocation and turnover rate of glucose transporters in skeletal muscles of fa/fa rats (King *et al.*, 1993). This suggests that insulin and exercise effects on glucose metabolism in the muscle use different signal transducing pathways which can also explain the synergistic actions of insulin and muscle contraction on glucose utilization in fa/fa rats (Dolan *et al.*, 1993).

Insulin resistance and obesity have been induced by prolonged administration of high doses of insulin to normal rats, which means that a high level of circulating hormone may be the main cause of insulin resistance in simple obesity or obesity associated with NIDDM (Inoue and Bray, 1977; Cusin *et al.*, 1990). In VMH lesioned rats excess insulin secretion precedes the development of insulin resistance (Jeanrenaud, 1985) indicating that insulin resistance does not cause hyperinsulinemia in these animals.

1.4.2.3. Overactive entero-insular axis.

Another possible cause of hyperinsulinemia in fa/fa rats is an overactive entero-insular axis (EIA). The EIA is a network of neural and endocrine communications between the alimentary tract and pancreatic islets that promotes insulin secretion in

response to feeding (Marks and Morgan, 1984; Bailey and Flatt, 1988). Glucose-stimulated hyperinsulinemia is apparent in 3 wk old fa/fa preobese rats (Chan *et al.*, 1985) wl ch coincides with weaning and the onset of hyperphagia (Bray and York, 1979). A similar developmental phase is seen in the ob/ob mouse, in which the EIA has been reported to contribute to its hyperinsulinemic state (Flatt *et al.*, 1984; Bailey and Flatt 1988). Oral nutrient ingestion, especially of carbohydrates, in ob/ob mice and obese humans is reported to result in a greater insulin release (Flatt and Bailey, 1982; 1984; Hampton *et al.*, 1986) than does parenterally-administered glucose (Flatt and Bailey, 1981) indicating that there might be factors other than hyperglycemia stimulating insulin secretion by B-cells. Most of the peptide hormones of the EIA such as neurotensin, gastrin, secretin, cholecystokinin, glucagon-like peptide and gastric inhibitory polypeptide (GIP) are reported to be increased in the intestines and plasma of ob/ob mice (Flatt *et al.*, 1984; Bailey and Flatt, 1986). These hormones increase insulin secretion in the presence of physiological concentrations of glucose *in vitro* (Bailey and Flatt, 1988).

Intestinal levels of GIP in ob/ob mice are reported to be double that in lean mice. During feeding the plasma levels may increase to more than 10 fold greater than those of the lean animals (Flatt *et al.*, 1983). Gastric inhibitory polypeptide enhances insulin secretion when the glucose concentration is raised above basal in lean mice, but ob/ob mice and fa/fa rats also respond to GIP at glucose concentrations below basal levels (Chan *et al.*, 1984; Flatt *et al.*, 1984). Chan *et al.* (1985) reported that the perfused pancreas of fa/fa rats showed hyperresponsiveness

to GIP (at low glucose concentrations) at 35 d but not earlier. This finding indicates that fasting hyperinsulinemia observed in young adult fa/fa rats may be partly due to increased response of the pancreas to GIP at low or normal glucose concentrations. Gastric inhibitory polypeptide secretion *per se* is not altered (Chan *et al.*, 1984) nor are intestinal cell populations different between lean and fa/fa rats (Chan *et al.*, 1987).

Neuropeptide Y (NPY), peptide YY and pancreatic polypeptide (PP), are other EIA neuropeptides or hormones. They have been reported to increase gastrointestinal pressure and motility in rats (Wager-Page *et al.*, 1993). This could modify the rate of nutrient absorption from the gut and result in hyperinsulinemia. Neuropeptide Y mRNA is reportedly higher in paraventricular nucleus, arcuate nucleus and other parts of the brain involved in the regulation of appetite, energy balance and endocrine gland activity in fa/fa rats (Sanacora *et al.*, 1990) and cp/cp rats (Beck *et al.*, 1992; Williams *et al.*, 1992). Beck and his colleagues (1992) were able to produce an obese syndrome similar to that of fa/fa rats in Long Evans rats by chronic, continuous intracerebroventricular infusion of NPY. The actions of NPY seem to be central rather than peripheral in its contribution to development of hyperinsulinemia and obesity in both the fa/fa rat and the cp/cp rat (Williams *et al.*, 1992), because NPY inhibits insulin secretion in both isolated islets and perfused pancreas while it stimulates insulin secretion when centrally injected (Moltz and McDonald, 1986; Dunbar *et al.*, 1992).

Cholecystokinin (CCK) has both peripheral and central actions to cause satiety

in animals. Obese Zucker fa/fa rats show a reduced response to CCK around 4-5 wk in that high exogenous CCK is required to suppress feeding in these rats (McLaughlin and Baile, 1980). Further, the pancreatic exocrine secretory response to CCK stimulation is reduced, an effect that is attributed to reduced receptor binding capacity (McLaughlin *et al.*, 1984; Praissman and Izzo, 1986). Cholecystokinin binding is variable in different parts of the brain in both fa/fa rats and ob/ob mice. Cholecystokinin binding is normal in the hypothalamus but increased in the cortex of both animals. However CCK binding is also increased in the midbrain and hippocampus of fa/fa rats (Hays *et al.*, 1981; Finkelstein *et al.*, 1984). Different responses to CCK in fa/fa rats (reduced in peripheral tissues but increased in brain) may contribute to hyperinsulinemia in these animals. Because CCK is also a potent hypothalamo-pituitary-adrenal (HPA) axis stimulant (Kamilaris *et al.*, 1992; Katsuura *et al.*, 1992) it may contribute to the maintenance of corticosteronemia in fa/fa rats thus maintaining hyperinsulinemia and obesity in these animals (see below, section 1.4.2.4)

1.4.2.4. Abnormal regulation of the central and/or autonomic nervous system.

That hyperinsulinemia in Zucker fa/fa rats may be a result of defects in the central nervous system was proposed because of some resemblance to VMH obesity (Bray and York, 1979; Jeanrenaud, 1985). Both syndromes can occur with or without hyperphagia (Bray and York, 1979; Bray *et al.*, 1989). They depend on the presence of glucocorticoids because adrenalectomy (ADX) prevents or reduces the symptoms

of both VMH and fa/fa obese syndromes, and glucocorticoid replacement reverses ADX effects (Bray and York , 1979; *King et al.*, 1983; King and Smith, 1985; Castonguay *et al.*, 1986; Fletcher, 1986a; Fletcher and McKenzie, 1988). The central nervous system (CNS) controls peripheral metabolic activities through the autonomic nervous system (ANS) with the control centre located in the hypothalamus. Adrenergic, noradrenergic and cholinergic neuropathways are present in different hypothalamic areas which control both food intake and endocrine activity. The actions of the parasympathetic and sympathetic nervous system depend on the type of organ and receptors stimulated by the parasympathetic or sympathetic nervous system neurotransmitters. In most cases the parasympathetic nervous system is stimulatory while sympathetic nervous system actions are more variable. In the endocrine pancreas the parasympathetic nervous system stimulates insulin release when acetylcholine binds to muscarinic receptors on the B-cell membrane while the sympathetic nervous system is inhibitory through α_2-adrenergic receptors and stimulatory through ß-adrenergic receptors (Efendic *et al.*,1991). In brown adipose tissue sympathetic nervous system increases metabolism when noradrenaline binds to $ß_3$- adrenoceptors (Ilyés *et al.*, 1991; Yoshida, 1991).

Injury to the VMH nuclei has been reported to increase parasympathetic stimulation through the vagus nerve and reduce sympathetic activity in the periphery (Jeanrenaud, 1985), and it is believed that this imbalance of ANS tonicity reaching the periphery, including the endocrine pancreas, leads to development of hyperinsulinemia and obesity in VMH lesioned rats and fa/fa rats. In fact truncal

vagotomy abolishes hyperinsulinemia, reduces gastric acid secretion and ameliorates the obese syndrome in VMH lesioned rats (Powley and Opsahl, 1974; Inoue and Bray, 1977). The fa/fa rat obese syndrome was reduced but not abolished by bilateral subdiaphragmatic vagotomy (Opsahl and Powley, 1974).

Increased activity of the parasympathetic nervous system has been demonstrated in 5 d old preobese fa/fa pups. Atef *et al.* (1991) reported higher acetylcholine stimulatory effects on glucose-induced insulin secretion in perfused pancreas from preobese but not lean rats. Rohner-Jeanrenaud *et al.* (1983) reported higher insulin and glucagon secretion from 17 d old preobese pups in response to arginine and glucose; the increased secretion was eliminated by pretreatment with atropine.

Evidence of reduced sympathetic nervous system activity was first reported in 16 d and 17 d old fa/fa rats. These rats have reduced brown adipose tissue (BAT) thermogenesis when exposed to cold temperature (Greco *et al.*, 1987). This is reflected by reduced binding of guanosine 5'-diphosphate to the mitochondrial membranes (Bazin *et al.*, 1984) or diminished noradrenaline turnover in the BAT and at sympathetic nerve endings (Levin *et al.*, 1981; Krief *et al.*, 1989). These preobese fa/fa rats also have lower rectal temperature compared to the lean littermates (Godbole *et al.*, 1978). Lower rectal temperature was later detected in 6 d and 7 d old fa/fa rats (Schmidt *et al.*, 1984; Planche *et al.*, 1983). Also, 2 d old preobese rats have low oxygen consumption and diminished cold induced-thermogenesis (Moore *et al.*, 1985), while a diet-induced thermogenesis deficit develops later demonstrating

that a defective sympathetic nervous system is among the earliest abnormalities to develop in the Zucker fa/fa rat obese syndrome.

Altered BAT thermogenesis and other metabolic abnormalities in obese Zucker fa/fa rats are abolished or reduced by surgical or chemical ADX, and glucocorticoid replacement reverses ADX effects (Bray and York, 1979; Marchington *et al.*, 1983; Castonguay *et al.*, 1986; Fletcher, 1986b; Fletcher and McKenzie, 1988; Bray *et al.*, 1989; Holt and York, 1989). Also injection of CRF into the cerebral ventricles reduces food intake, weight gain and plasma insulin levels, and increases energy expenditure and BAT thermogenesis in fa/fa rats (Arase *et al.*, 1988; Arase *et al.*, 1989a; Holt and York, 1989; Rohner-Jeanrenaud *et al.*, 1989; Glowa *et al.*, 1992). Intravenous (IV) injection of CRF in human obese and non-obese subjects has been shown to increase energy expenditure and plasma noradrenaline (Chong *et al.*, 1992). In fa/fa rats, low doses (500 pmol) of IV CRF reduced insulin secretion and blood glucose in response to oral food intake but not in response to IV glucose administration (Rohner-Jeanrenaud and Jeanrenaud, 1992).

Young adult obese Zucker fa/fa rats have an overactive HPA axis with a defective negative feedback (Guillaume-Gentil *et al.*, 1990). The HPA axis is composed of the hypothalamus which forms the neuroendocrine part of CNS, the pituitary gland and the adrenal glands. Hypothalamic neurons contain cells which synthesize and secrete CRF. Corticotrophin releasing factor stimulates the synthesis and secretion of adrenocorticotropic hormone (ACTH) by the pituitary which stimulates the synthesis and release of glucocorticoids from the zona fasciculata of the

adrenal cortex. Hypothalamo-pituitary-adrenal axis activity is controlled by diurnal rhythm, stress, pain and emotions and plasma glucose levels. High plasma glucocorticoid levels exert a negative feedback on both ACTH and CRF secretion while low levels will lead to positive feedback (Wilson and Foster, 1985).

In the rat the main glucocorticoid is corticosterone. Corticosterone plasma levels in fa/fa rats have been reported to be either normal (Yukimura *et al.*, 1978; Gibson *et al.*, 1981) or increased (Fletcher *et al.*, 1986; Guillaume-Gentil *et al.*, 1990), while adrenocorticotrophic hormone plasma levels are normal or reduced (Yukimura *et al.*, 1978). Cunningham and his colleagues (1986) observed an increased rate of corticosterone turnover and the presence of morning hypercorticosteronuria in fa/fa rats. Because corticosterone is transported in circulation bound to corticosteroid binding protein (CBP) and albumin, the ratio between the bound and free corticosterone may be important. However, Shargill and his colleagues (1987) found that both free and bound hormone concentrations in 4 and 10 wk old fa/fa rats are not different from those of lean rats, and this means that glucocorticoid availability is similar in both phenotypes. Evidence of an overactive HPA axis is more pronounced when fa/fa rats are subjected to stressful situations such as cold exposure or immobilization (Guillaume-Gentil *et al.*, 1990).

Glucocorticoid actions are mediated through cystolic receptors present in target tissues. Liver glucocorticoid receptor numbers and binding capacity are not different between fa/fa rats and lean rats (Shargill *et al.*, 1987; Langley and York, 1992). However, in the hippocampus and hypothalamus, both receptor number and

glucocorticoid binding to both type I and type II receptors are increased in fa/fa rats compared to lean littermates. After ADX, type II glucocorticoid receptor numbers in lean rats showed an initial rapid rise followed by a steady rise which stabilized within 1-2 wk. In fa/fa rats, however, only the initial rise was reported, suggesting the presence of a defect in corticosterone binding activity in the brain tissues (Langley and York, 1990b; 1992). The obesity-inducing actions of glucocorticoids are thought to be mediated through inappropriate inhibition of hypothalamic CRF. This conclusion is based on findings that either (1) intracerebral infusion of CRF in intact fa/fa and VMH lesioned rats or (2) administration of the glucocorticoid receptor antagonist RU846 results in weight loss, reduced food intake, reduced insulin secretion and fat deposition, and increased sympathetic activity in the BAT thus increasing energy expenditure through thermogenesis (Arase *et al.*, 1989a; 1989b; Holt and York, 1989; Langley and York, 1990a). Adrenalectomy results in an increase of CRF activity in the hypothalamic area, which then acts on distal sites in the food intake regulation process and/or sympathetic nervous system to modulate energy balance in the animals (Bray *et al.*, 1989).

The mechanisms by which corticosterone might directly cause excess insulin secretion in fa/fa rats are not clear since the synthetic glucocorticoid dexamethasone directly decreases insulin secretion *in vitro* but enhances insulin secretion *in vivo* (Ludvik *et al.*, 1993). Philippe *et al.* (1992) showed that dexamethasone increased proinsulin mRNA both *in vivo* and *in vitro* only when the pancreatic islets were intact. When islet cells were separated, dexamethasone decreased insulin mRNA in the cells.

The observed inhibitory effects of dexamethasone on insulin secretion and insulin mRNA expression *in vitro* are reported to be due to a lack of intracellular cAMP in the isolated cells since addition of a cAMP analogue eliminated the inhibitory effect of glucocorticoids (Philippe *et al.*, 1992). Isolated islets of fa/fa rats have decreased sensitivity to chronic dexamethasone exposure (Chan and Lejeune, 1992) which may contribute to hyperinsulinemia in these rats.

Glucocorticoids enhance hepatic glucose production via gluconeogenesis and this glucose could produce a constant B-cell stimulation *in vivo* thereby increasing insulin secretion. Zucker fa/fa rats show reduced insulin clearance by the liver and hepatic glucose production in these rats is not suppressed by insulin (Jeanrenaud, 1985). Glucose regulates the synthesis and activity of glucokinase in the pancreatic tissues *in vitro* (Liang *et al.*, 1991; Fernandez-Mejia and Davidson, 1992) and *in vivo* (Koranyi *et al.*, 1992). Increased plasma glucose as a result of increased hepatic production may increase glucokinase activity in the B-cells and thus contribute indirectly to increased insulin secretion.

1.4.2.5. **Primary pancreatic islet lesions.**

Although many factors can increase insulin secretion in the normal B-cells, the increased response to the stimulant may be to due the presence of a defect within the B-cells. This defect or defects may involve the synthesis, storage or release mechanisms of insulin. Pancreatic islets of fa/fa rats are larger than those of the lean rats, a defect observed as early as 7 d (Chan *et al.*, 1985); however increased size

alone does not account for persistent hyperinsulinemia in these rats. Observation that islet size and numbers are increased in all other animals and human beings at the onset of obesity, but that the B-cells of these obese species then progressively lose their insulin secretory capacity means that there must be a protective mechanism in the fa/fa rat B-cells allowing them to maintain high insulin secretion throughout their life span (Fürnsinn *et al.*, 1991).

Heterogeneity in the B-cell population has also been postulated as a cause of increased islet B-cell activity in obese (Shino *et al.*, 1973) and pregnant rats (Aerts and Van Assche, 1975), animals treated with cortisone (Bencosme and Martinez-Palormo, 1968) and animals with growth hormone-producing tumours (Bencosme *et al.*, 1971; Pipeleers, 1992). In these different situations, animals have an increased number of pale-over-dark mature granulated B-cells and B-cells with pale granules are observed to respond to basal or low glucose levels.

In ob/ob mice pancreatic islet defects in the function of potassium and calcium channels (Rosario *et al.*, 1985; Fournier *et al.*, 1990) and abnormal regulation of cAMP in the B-cell (Black *et al.*, 1988) have been reported to contribute to hyperinsulinemia. However, similar cation channel defects or abnormal cAMP regulation have not been found in islets from fa/fa rats (Chan and MacPhail, personal communication; Cawthorn and Chan , 1991). Islets from fa/fa rats were found to lack a glucose threshold in response to GIP hormone (Chan *et al.*, 1984) and this glucose threshold was lost at the time of onset of fasting hyperinsulinemia (Chan *et al.*, 1985).

Cawthorn and Chan (1991) observed that catecholamine inhibitory actions on

B-cells were normal, but that adrenaline inhibitory actions on glucose induced insulin release in the presence of pertussis toxin, (which acts by permanently inactivating inhibitory G_i protein), were less pronounced in fa/fa than in lean rats. It was speculated that loss of this catecholamine inhibitory action through the pertussis toxin-independent pathway could contribute to the development of hyperinsulinemia in these rats. Catecholamine inhibitory actions in the B-cells are mediated predominantly through the α_2-adrenergic receptors (Rorsman *et al.*, 1991). The function of α_2- adrenoreceptor has been reported to be altered in fa/fa rats. In studies by Chan and MacPhail (1992) insulin secretion and cAMP production were inhibited by clonidine, an α_2-adrenoceptor agonist, in islets from both lean and fa/fa rats but this inhibition was antagonized by prazosin, an α_1-adrenoceptor antagonist only in islets from fa/fa rats (Chan and MacPhail, 1992). In this respect the α_2-adrenoceptor on the fa/fa islets resembled an prazosin-sensitive α_2 receptor subtype designated α_{2B} that has been described in both the gastrointestinal tract and kidneys (Docherty, 1989). Changes in adrenoceptor expression on the pancreatic islet B-cells in fa/fa rats could lead to hyperinsulinemia by changing responsiveness to adrenaline action in these cells.

Glucose metabolism by the B-cell results in insulin secretion. Any defect in this process could result in changes in B-cell secretory activity. Glucose enters the B-cell by facilitative diffusion via the low affinity glucose transporter $GLUT_2$. These transporters have been reported to be reduced in male diabetic Zucker fatty fa/fa rats (Johnson *et al.*, 1990; Orci *et al.*, 1990), non-obese NIDDM Goto-Kakizaki rats (GK)

(Ohneda *et al.*, 1993) and in some diabetic humans (Unger, 1991). In all the animal models and human patients studied so far $GLUT_2$ reduction occurs when hyperglycaemia begins. This protein is reported to be normally expressed in non-diabetic fa/fa rats (Johnson *et al.*, 1990). Glucose is metabolized through the glycolytic pathway and Kreb's cycle before inducing insulin secretion. Glucokinase catalyses the first reaction of glycolysis. Blockade of this enzyme's activity *in vitro* by mannoheptulose inhibits glucose induced insulin secretion in islets from normal animals (Zawalich, 1979) but not in obese fa/fa rats (Chan *et al.*, 1993) or cp/cp corpulent rats (Timmers *et al.*, 1992). Glucokinase has been referred to as the B-cell glucose sensor (Matschinsky, 1990), thus any changes in its activity could result in a shift of B-cell metabolism and insulin secreting capacity (Meglasson and Matschinsky, 1984).

Defective glucokinase genes have been identified in subjects with NIDDM and maturity-onset diabetes of the young (MODY) (Hattersley *et al.*, 1992). In black Americans with NIDDM, a 3' Z+4 allele is commonly found while in Mauritian Creoles a 3' Z+2 allele is more common (Chiu *et al.*, 1992a; 1992b). Both Z+4 and Z+2 alleles are more common in Japanese NIDDM subjects (Noda *et al.*, 1993) compared to the non-diabetic subjects. In one family with MODY, substitution of a single base (from GAG to TAG) resulted in transcription of a truncated glucokinase mRNA, and therefore translation of a non-functional enzyme (Vionnet *et al.*, 1992). Glucokinase gene linkage to NIDDM has been identified in two separate families in England (Hattersley *et al.*, 1992) and in French families (Froguel *et al.*, 1992).

Glucokinase defects which result in a reduction in enzyme activity have been identified in mild early-onset NIDDM (onset in the third decade) characterized by reduced B-cell function (Permutt *et al.*, 1992). Expression of a defective glucokinase gene in *E. coli* resulted in synthesis of an enzyme with reduced catalytic activities as observed in NIDDM patients (Gidh-Jain *et al.*, 1993).

However, the situation is different in fa/fa rats since B-cells maintain their activity in old age and these animals generally do not become diabetic (Fürnsinn *et al.*, 1991). Chan (1993) reported that glucokinase affinity for glucose in pancreatic islets from the fa/fa rats was increased, thus allowing the islets to secrete insulin at lower glucose concentrations. This could contribute to the presence of fasting hyperinsulinemia in these rats. Pancreatic glucokinase activity is regulated by glucose (Iynedjian *et al.*, 1989b; Liang *et al.*, 1990), in that high glucose concentrations increase glucokinase activity and low glucose levels result in reduced enzyme activity (Liang *et al.*, 1992). Starvation of adult fa/fa rats however has been shown not to reduce insulin secretion to the same extent as in lean controls, suggesting a loss of glucose regulation of glucokinase activity in these animals (Chan *et al.*, 1993). It seems likely that defective B-cell glucose sensing, the presence of other pancreatic islet biochemical lesions, and genetic background predispose the fa/fa rat to develop and maintain a hyperinsulinemic state.

1.5. Objectives of the study.

The pancreatic islet lesions in α_2-adrenoceptor and glucokinase function described above have been observed in isolated islets from young adult fa/fa rats when obesity and the hyperinsulinemia are already established. It is possible, however, that the lesions may be present at a younger age and contribute to the development of obesity. Many metabolic abnormalities have been reported to be suppressed by the high fat diet (dam's milk) during the suckling stage (Turkenkopf *et al.*, 1982). The reduced sympathetic and increased parasympathetic activity observed in fa/fa rats favours high energy efficiency, fat deposition and hyperinsulinemia and is shown to be dependent on the presence of glucocorticoids (Bray and York, 1979; Bray *et al.*, 1989). These findings led to the hypothesis that the development of the pancreatic B-cell lesions is secondary to altered HPA axis function and that an intact HPA axis is crucial to the development and persistence of these lesions in Zucker fa/fa rats.

The objectives of the current research were:

1. To determine the time of onset of lesions in glucokinase activity and α_2 - adrenoceptor function in fa/fa rats by conducting a developmental study using 3 (suckling), 5 (weanling) and 8-12 (young adult) wk old rats.

2. To determine the dependence of pancreatic islet lesions on the presence of an intact HPA axis by adrenalectomizing the rats in the age group where the lesion(s) was first detected and then testing for the presence or absence of the lesion(s) 2 wk post surgery.

2. GENERAL METHODS AND MATERIALS.

2.1. Experimental animals.

In all experiments lean (Fa/?) and obese (fa/fa) female rats were used. The animals were divided into different age groups: the adult lean and obese (8-12 wk), the weanling group (5 wk), and the suckling group (3 wk). The 5 wk old and adult rats were obtained from Charles River Laboratories, Canada. Breeding pairs were obtained from the same source and bred to obtain the animals in the suckling group. Animals were housed in an artificially lit room with a 12 h light/dark cycle and a temperature of 22° C. They were fed commercial rodent laboratory chow 5001 (with composition of 23.4% crude protein, 4.5% crude fat, 5.8% crude fibre, 7.3% ash, 49% carbohydrate and 10% water) (Purina) and tap water *ad libitum*. Adrenalectomized and sham-operated rats were given saline/sugar (0.9% sodium chloride and 40 g/l glucose) *ad libitum*, in addition to the regular diet. The breeding rats were similarly housed and the pups were weaned and sexed at 21 d.

2.2. Pancreatic islet preparation.

2.2.1. Isolation and culture of pancreatic islets.

Isolated islets were prepared by a modification of the method of Van Der Vliet *et al* (1988) as described by Cawthorn and Chan (1991). Siliconized glassware was used in all steps to prevent sticking of islets to the glassware and all other instruments were sterilised to maintain aseptic conditions.

Rats were fasted overnight and anaesthetized with sodium pentobarbital (65

mg/kg body weight I.P.). All procedures in islet isolation were performed in a laminar flow hood (Purifier Clean Bench, Labconco Corporation, Kansas). The abdomen was disinfected with 70% ethanol and the abdominal cavity was opened through a ventral midline incision. After identification of the pancreas and the bile duct, both the distal (at the pyloric stomach) and proximal ends (at the hilus of the liver) of the bile duct were ligated. Cardio-respiratory arrest was induced by opening the diaphragm. A polyethylene catheter (PE 50 for adult rats and PE 10 for young rats) was used to cannulate the bile duct distal to the ligature at the hilus of the liver. Five to 10 ml of ice-cold collagenase type XI (0.32 mg/ml) dissolved in Hanks's Balanced Salt solution (HBSS) (supplemented with 10 mM of N-{2-Hydroxyethyl} piperazine-N'-{2-ethanesulfonic acid} (HEPES), 0.2% bovine serum albumin (BSA) and 2 mM glutamate) was injected into the pancreas (Van der Vliet *et al.*, 1988). The pancreas was carefully dissected out of the animal, put into a petri dish, and chopped in small pieces using scissors. An extra 15-20 ml of collagenase solution was added before incubation in an orbital 37°C water bath (150 rpm). Due to age and phenotype differences in sensitivity to collagenase action, the incubation times were adjusted to obtain maximal islet yield. Preliminary trials with different age or treatment groups showed that the times shown (Table 1) gave the best islet yield. Collagenase digestion was stopped by adding 35-40 ml of cold HBSS and the digest was centrifuged (Beckman J-6M/E centrifuge, TY JS 4.2 rotor at speed of 463 g for 5 min at 22^0 C). The same rotor (TY JS 4.2) was used in all centrifugation procedures. After centrifugation the supernatant was discarded by decanting and the

pellet was resuspended in 25 ml of fresh collagenase and incubated a second time (Table 1). The pancreatic digest was centrifuged as before and washed with HBSS as described above and then filtered through 800 μm Nitex screen to remove any undigested tissues. The filtered tissue was centrifuged (463 g for 5 min at 22^0 C), the supernatant was discarded by decanting and the pancreatic tissue was then separated using a dextran step-density gradient (industrial grade MW 70,000). To prepare the gradient, the pellet was suspended in 10 ml of 27% dextran with 6 ml of the 27% dextran gently layered below. Two layers, 10 ml of 23% dextran and 10 ml of 14% dextran were added on top. Centrifugation (463 g, 15 min at 22^0 C) resulted in enrichment of islets in the interface between 23% and 14% dextran. This layer was collected by aspiration, washed with HBSS, and centrifuged for 5 min at a speed of 463 g at 20^0 C. The pancreatic tissue pellet was resuspended in HBSS with 0.2% BSA and transferred to a petri dish. Pancreatic islets were hand picked under a dissecting microscope, 5 islets/well for all lean groups and 3 wk old rats and 3 islets/well for 5 - 12 wk old obese rats, respectively, in order to normalize insulin content per well. They 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) and 10% calf serum. Islets were cultured overnight in a humidified incubator at 37°C in 5% CO_2.

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

Age and treatment	Phenotype	1st incubation	2nd incubation
Adult intact/sham	lean	20	10
ADX	lean	18	10
Adult intact/sham	fa/fa	10	7
ADX	fa/fa	12	7
5 week	lean	12	6
5 week	fa/fa	7	4
3 week	lean	8	-
3 week	preobese	8	-

ADX = adrenalectomized rats

Sham = sham operated rats

2.2.2. Insulin release.

To measure insulin release the culture medium was replaced with 1.0 ml of fresh DME containing 15 mM glucose and 0.1% gelatin. Guanabenz (an α_2-adrenoceptor agonist), idazoxan (an α_2-adrenoceptor antagonist), phenylephrine (an α_1-adrenoceptor agonist) and mannoheptulose (glucokinase inhibitor) were all dissolved in DME medium so islets incubated with 15 mM glucose alone were used as controls. Prazosin (an α_1-adrenoceptor antagonist) was dissolved in dimethyl sulfoxide (DMSO), effect of DMSO on insulin release was also investigated. The samples were centrifuged (824 g for 5 min at 20^0 C) and the supernatant was discarded by aspiration and replaced with one millilitre of fresh DME medium containing 15 mM glucose and 0.1% gelatin. Twenty-five μl of guanabenz (10^{-9}-10^{-6} M), idazoxan (10^{-5} M), phenylephrine (10^{-7} -10^{-5} M) and mannoheptulose (10^{-4} M - $3x10^{-2}$ M) or 2.5 μl of prazosin (10^{-7} M - 10^{-5} M) or DMSO were added to duplicate islet samples. The samples were statically incubated for 90 min after which the supernatant was collected by aspiration (referred to as " releases" in RIA assay) after centrifugation (824 g for 5 min at 20^0 C) and reserved. The pancreatic islet pellet was boiled for 5 min in 3% acetic acid (referred to as " totals" in RIA assays) and then stored at - 20°C until assayed for insulin.

2.3. Insulin radioimmunoassay.

Insulin was measured using an RIA employing iodinated (I^{125}) porcine insulin as the tracer, which was diluted to give approximately 10,000 cpm per 100 μl, and rat

insulin as the standard with sensitivity ranging from 37.5 to 1200 p mol. The insulin antiserum (Gp 01, raised in guinea pigs in 1978) was a gift from Dr R. A. Pederson (University of British Columbia, Vancouver, BC, Canada). It was reconstituted from lyophilized form using 0.04 M phosphate buffer pH 7.5 (containing 5% charcoal extracted equine plasma) to obtain a final dilution of 1:1,000,000. Samples, along with the standard curve, were diluted using 0.04 M phosphate buffer pH 7.5, (dilution factors were 1: 100 or 1:200 for most lean rat and obese rat totals respectively while 1:10 for most of adult and 5 wk old releases and releases for the 3 wk old rat islets were done in undiluted and 100 μl of reconstituted antiserum was added to all the tubes except the total counts tubes and the non specific binding tubes of the standard curve. After incubation at 4° C overnight, 100 μl of ^{125}I-labelled porcine insulin was added followed by an additional 24 h incubation at 4°C. 250 μl of activated dextran-coated charcoal was added to all tubes except the total counts. After centrifugation (1854 g for 15 min at 4° C) the supernatant was discarded by decanting and the pellet, which contained the free ^{125} I-insulin, was counted using a Packard Riastar gamma counter (with an efficiency of 69%-73% for ^{125}I) after draining for 20 -25 min. Samples were measured in duplicate while the standard curve were measured in triplicate. Raw data (cpm) were converted to pmol/L by standard software Riastar (Packard, details and equations are reported in Appendix 1). In order to calculate the insulin in either the release or total portions, the number read off the computer was multiplied by the dilution factor. Due to variability in pancreatic islet size, mostly those from obese rats, insulin release was expressed as a percent of total islet insulin

content.

Total insulin content =

Insulin present in the release + insulin in the totals in a single sample.

$$\text{Insulin release} = \frac{\text{Insulin in the supernatant}}{\text{Total islet insulin content}} \times 100$$

2.4 Statistical analysis.

In the results (n) refers to the number of animals from which islets were isolated and each data point is an average of duplicate samples from one animal. Data are given as means ± SEM for the number of animals indicated. The data were analyzed using two way analysis of variance (general linear model) after arcsine transformation (Zar, 1974) of the actual percent data shown in the figures when testing for interactions between test drugs (referred to as treatments in the results), or experimental variables such as age, phenotype, adrenalectomy or sham-operated. Insulin release was measured in response to different doses of the same drug tested; thus from one rat donor several data points were obtained and when analyzing data using two way analysis of variance (general linear model because the data sets were not equal), the data points are taken as different animals, thus the large degrees of freedom indicated in the results. One way analysis of variance was when comparing effects of different doses of test drugs within phenotypes using Minitab version 7.1 (Ryan, Joiner and Ryan, 1985), which was followed by Student-Newman-Keuls test (SNK).

Unpaired t-test was used when comparing response produced by the same treatment between phenotypes. All results were considered significant at $p < 0.05$ (Glantz, 1987).

2.5. Materials and sources.

Sources of individual materials are listed in Appendix 2.

3. IDENTIFICATION OF PANCREATIC ISLET DEFECTS IN fa/fa RATS: A DEVELOPMENTAL STUDY.

3.1. Introduction.

The obese syndrome and hyperinsulinemia of fa/fa rats becomes evident after weaning (Bray and York, 1971; Chan *et al.*, 1985). However, many abnormalities including altered fat metabolism (Bell and Stern, 1977; York *et al.*, 1981; Turkenkopf *et al.*, 1982; Lavau *et al.*, 1985), abnormal body temperature regulation (Godbole *et al.*, 1978; Planche *et al.*, 1983; Schmidt *et al.*, 1984), and parasympathetic nervous system activity in the pancreas (Rohner-Jeanrenaud *et al.*, 1983; Atef *et al.*, 1991) have been observed in the first or second week of life in pre-obese animals. Some pancreatic islet lesions have been identified in weanling and adult fa/fa rats. These include loss of a glucose threshold for GIP hormone stimulatory actions (Chan *et al.*, 1984; 1985), lack of a pertussis toxin-independent pathway of catecholamine inhibition action (Cawthorn and Chan, 1991), abnormal α_2-adrenoceptor function (Chan and MacPhail, 1992) and abnormal glycolysis regulation (Chan, 1993; Chan *et al.*, 1993). In this study the time of onset of abnormal α_2- adrenoceptor function and abnormal glycolysis regulation in fa/fa rat islets was investigated using isolated islets from adult (8-12 wk), weanling (5 wk) and suckling (3 wk) rats.

3.2 Methods.

3.2.1. Identification of obese fa/fa pups.

3.2.1.1. Colonic temperature measurement.

Zucker fa/fa rats have decreased thermogenesis which is evident by the second wk of their lives. This defect has been used to identify phenotypes (Godbole *et al*, 1978; Chan *et al.*, 1985). Colonic temperature was measured using a microprocessor-based (microcomputer) thermometer (Cole-Parmer, Labcor Inc. Quebec) between 11.00 a.m and 1.00 p.m on three consecutive days following weaning (d 21). Pups with temperature 1°C lower than the litter average temperature were considered preobese (normal rat temperature ranges from 35°C to 38° C). However, since the temperature differences were small, predictions were then confirmed by measuring the islet size (Chan *et al.*, 1985).

3.2.1.2. Islet diameter measurement.

Adult Zucker obese rats have enlarged pancreatic islets and enlargement has been observed in preobese pups as early as 7 d (Chan *et al.*, 1985). The pancreas removal was carried out as described in the general methods section 2.2.1. In order to measure the pancreatic islets, a small piece of the pancreas was fixed in Bouin's solution (75 ml picric acid, 25 ml formalin and 5 ml glacial acetic acid) for 2 h, then washed two times in 70% ethanol. The samples were then sent to the Histopathology Laboratory of the AVC where they were processed, embedded in paraffin wax and cut into 4 μm sections; 5 slides were made for each pancreatic tissue. The slides

were then stained with haematoxylin and eosin according to standard procedures (Chan, 1985, PhD thesis). Islets were measured using a commercial image analysis hard ware and software (Bioquant IV., R&M, Biometric Inc Nashville, TN). Because not all islets were spherical, the average diameter of the islet was used. The longest and the shortest distances were measured and then averaged for every islet. Then the average diameters of all the islets from the same rat were again added together to obtain the final average diameter of islet of that rat. The average of the diameters was used to separate the lean from the preobese rats. In these studies a 150 μm islet diameter, which is more conservative than the 100 μm diameter previously used (Chan *et al.*, 1985), was choosen as the cut off point. Animals with any islets with a diameter greater than 150 μm were considered preobese and those with all islets equal to or less than 150 μm were considered lean (see Appendix 4).

3.2.1.3. Islet isolation and insulin release.

Zucker female lean and obese rats were divided into 3 groups; adult (13 lean and 18 fa/fa), weanling (10 lean and 14 fa/fa) and suckling (15 lean and 7 preobese) rats. All animals were used to measure pancreatic islet insulin content but not all were used to assess responses to test drugs. Pancreatic islet preparation, insulin release experiments and statistical analyses were carried out as described in Chapter 2 (general methods, sections 2.2, 2.3 and 2.4).

3.3. Results.

3.3.1. Phenotype identification.

3.3.1.1. Colonic temperature.

Average colonic temperature from 21-23 d of age was calculated for 27 female pups. There was no temperature difference between the pups (36.7°C ± 0.2, n = 7 and 36.7°C ± 0.1, n = 15) in the preobese and lean female pups respectively after using the islet diameter to sort the rats by phenotype (Table 2).

3.3.1.2. Islet diameter measurement.

Animals with one or more islets with a diameter greater than 150 μm were considered as preobese. Using this cut off 9 rat pups were identified as preobese and 15 pups as lean. Three pups could not be placed in any category. These three pups and two preobese pups (due to lost insulin release data) were not included in any further experiments. The average islet diameter was significantly higher in the preobese than in the lean pups (83.4 ± 2.2 compared to 53.3 ± 3.1 μm respectively). The average weight and colonic temperature of 3 wk old lean and preobese pups were not significantly different (Table 2).

3.3.2. Insulin release from isolated islets.

3.3.2.1. Total islet insulin content.

Glucose (15 mM) induced insulin release data analysis were carried out as outlined in the methods (Section 2.2, 2.3 and 2.4). Total islet insulin content was

more than 2 fold greater in the fa/fa rats ($p < 0.05$) compared to lean rats in the weanling and the adult groups (Table 3). There was no significant difference in total islet insulin content of the 3 wk old preobese and lean pups. The total islet insulin content showed a greater increase from 3 wk to 5 wk in the fa/fa rats than in the lean rats (176% compared to 33.7%) which was statistically significant only in fa/fa rats ($p < 0.05$). There was a statistically significant increase in total islet insulin content in lean rats ($p < 0.05$) but not fa/fa rats from 5 wk to adult stage (63.8% compared to 32.8%). Pancreatic islet insulin content increased 2.13 and 3.36 fold from 3 wk to adult stage in lean and fa/fa rats, respectively. The greater increase in pancreatic islet insulin content in fa/fa rats may be due to both increased islet size and numbers that occurs in these rats (Bray and York, 1971; Chan *et al.*, 1985). Age ($F(2,71) = 26.97$; $p < 0.0001$) and phenotype ($F(1, 71) = 33.83$; $p < 0.0001$) had a statistically significant effect on the pancreatic islet insulin content of the rats. Also, the age and phenotype interaction was statistically significant ($p = 0.010$).

Table 2. Colonic temperature (°C), weight (g) and islet diameter (μm) of 3 wk old pups.

	Lean	Preobese
Colonic temperature (°C)	36.7 ± 0.13	36.7 ± 0.2
Weight (g)	42.4 ± 0.93	39.4 ± 0.1
Islet diameter (μm)	53.3 ± 3.18	3.4 ± 2.2*
Number of pups	15	7

Data are expressed as means ± SEM

* $p < 0.05$ compared to lean pups (unpaired t-test)

Table 3. Islet insulin content (mean ± SEM) in pmol/islet in lean (Fa/?) and fa/fa rats.

Age of the rats	Lean	Obese
Adult (8-12 wk)	6.6 ± 0.7 (13)	13.1 ± 1.2 (18)*
Weanling (5 wk)	4.0 ± 0.4 (10)§	10.2 ± 1.0 (14)*
Suckling (3 wk)	3.14 ± 0.4 (15)α	3.9 ± 0.9 (7) §α

Data are expressed as means ± SEM, (n) = number of animals used

* $p < 0.05$, comparison between phenotypes of the same age group (unpaired t-test)

§ $p < 0.05$ compared to the next older age group within the same phenotype.

α $p < 0.001$ compared to adult of the same phenotype.

3.3.3. Development of α_2-adrenoceptor function changes in isolated islets of fa/fa rats.

It was previously shown that islets from adult fa/fa rats exhibited a change in α_2- adrenoceptor sensitivity to the α_1- adrenoceptor antagonist prazosin (Chan and MacPhail, 1992), so the purpose of these experiments was to see if this change in sensitivity occurs in fa/fa islets before the animals were mature and their obese syndrome is fully developed. The effect of α_2- and α_1-adrenoceptor agonists and antagonists on 15 mM glucose-induced insulin release from isolated islets of weanling (5 wk) and adult (8-12 wk) lean and fa/fa rats was determined as described above (Section 2.2.2).

3.3.3.1. Effects of α-adrenergic agonists.

Glucose (15 mM) induced insulin secretion was measured in the absence or presence of four graded doses of guanabenz (α_2- adrenergic agonist; 10^{-9} -10^{-6} M) and three graded doses of phenylephrine (α_1- adrenergic agonist; 10^{-7}-10^{-5} M). Analysis of the data for interactions showed that guanabenz significantly inhibited 15 mM glucose-induced insulin release ($F(4,160) = 20.04$; $p < 0.0001$) while phenylephrine at all concentrations used did not have any significant effect on glucose-induced insulin secretion ($F(3,130) = 2.53$; $p = 0.06$) from isolated islets of adult and 5 wk old lean and fa/fa rats (Figures 1- 4). There was no significant interaction among the animal groups. Analysis of data within phenotypes showed all concentrations of guanabenz (10^{-9} to 10^{-6} M) used produced a significant reduction in 15 mM glucose-

induced insulin release ($p < 0.05$). There was no significant difference in the inhibitory action of the four different concentrations of guanabenz (10^{-9} M to 10^{-6} M) in 15 mM glucose-induced insulin release. Phenylephrine did not have a significant effect on insulin release from any group of rats ($p > 0.1$). These data indicate that α_2 but not α_1- adrenoceptors are involved in regulation of insulin release in isolated B-cells and that catecholamine inhibiting activity in B-cells of both lean and fa/fa rats is similar.

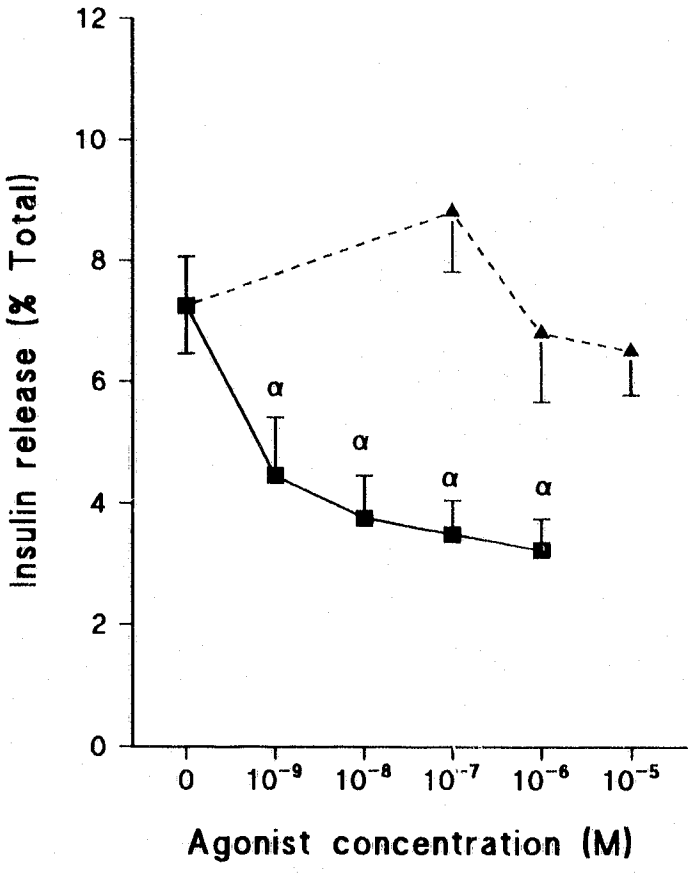

Figure 1. The effect of α-adrenergic agonists on 15 mM glucose-induced insulin secretion in isolated islets from adult lean rats. Glucose induced insulin secretion was measured in the absence (0) or presence of different doses of the α_2-adrenoceptor agonist guanabenz (■, n = 8 rats for 15 mM glucose and guanabenz 10^{-8} M, 7 rats for both 10^{-7} M and 10^{-6} M and 6 rats for 10^{-9} M) and the α_1-adrenoceptor agonist phenylephrine (▲, n = 8) as described in Section 2.2.2. Data are expressed as the Mean ± SEM. $^{\alpha}$ $p < 0.05$ compared to the control (0) using SNK.

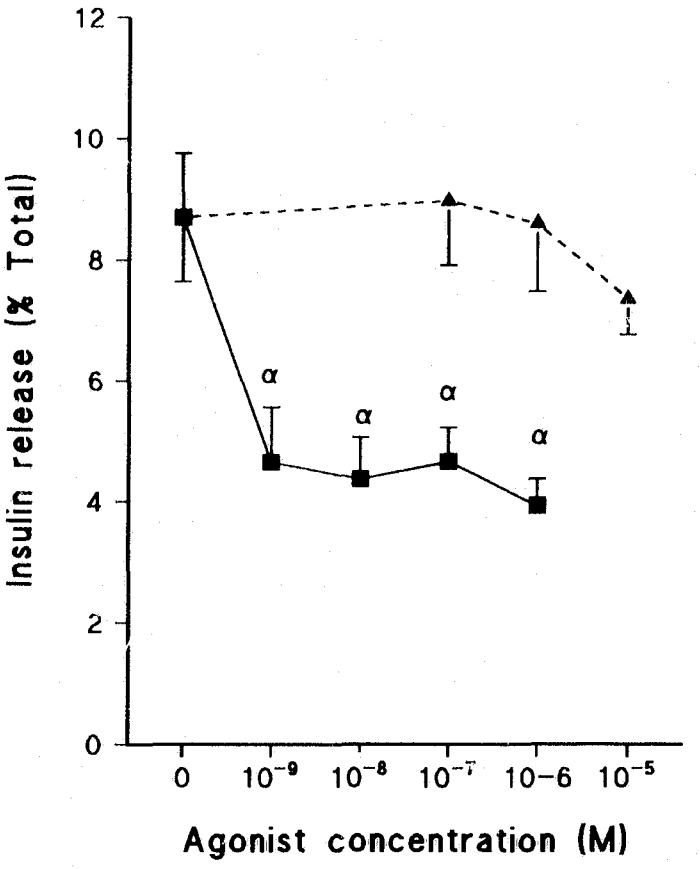

Figure 2. The effect of α-adrenergic agonists on 15 mM glucose-induced insulin secretion in isolated islets from adult fa/fa rats. Glucose-induced insulin secretion was measured in the presence of different doses of the α_2-adrenoceptor agonist guanabenz (■, n = 9 except for guanabenz 10^{-6} M where n = 8) and the α_1-adrenoceptor agonist phenylephrine (▲, n = 10) as described in section 2.2.2 Insulin release is expressed as a percentage of the islet insulin content. Data are expressed as the Mean ± SEM.

[α] $p < 0.05$ from the control (0)

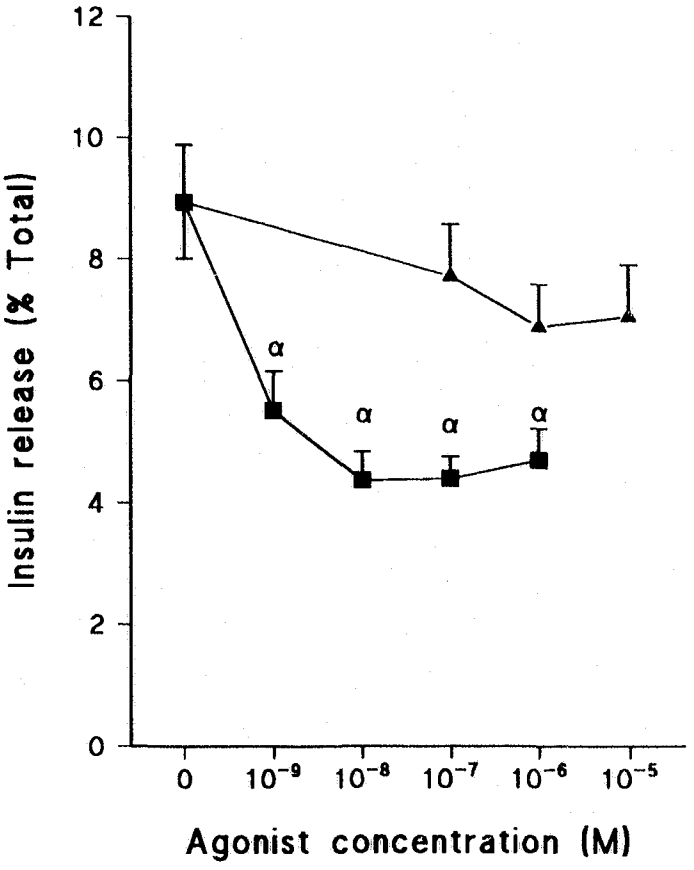

Figure 3. The effect of α-adrenergic agonists on 15 mM glucose-induced insulin secretion in isolated islets from 5 wk old lean rats. Glucose-induced insulin secretion was measured in the presence of different doses of the α_2-adrenoceptor agonist guanabenz (■, n = 9, except response to 10^{-6} M where n = 7) and the α_1-adrenoceptor agonist phenylephrine (▲, n = 8 for both 10^{-6} M and 10^{-5} M but n= 9 for 10^{-7} M) as described in section 2.2.2. Data are expressed as the Mean ± SEM. $^{\alpha}$ $p < 0.05$ compared to the control (0)

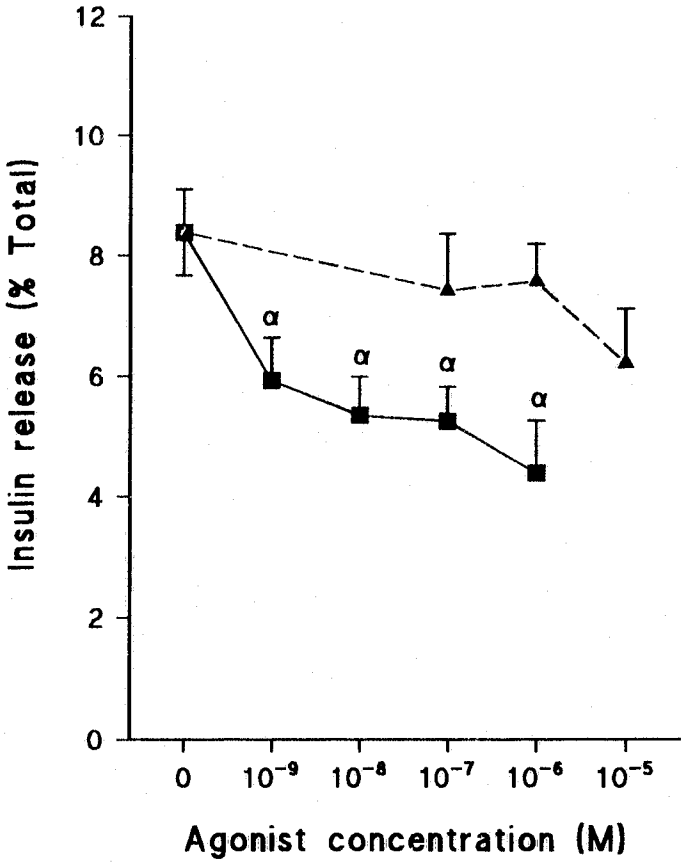

Figure 4. Effect of α-adrenergic agonists on 15 mM glucose-induced insulin secretion in isolated islets from 5 wk old fa/fa rats. Glucose-induced insulin secretion was measured in the presence of different doses of the α_2-adrenoceptor agonist guanabenz (■, n = 12 for 15 mM glucose, guanabenz 10^{-8} M and 10^{-6} M,n = 11 for 10^{-9} M and 10 for 10^{-6} M) and the α_1-adrenoceptor agonist phenylephrine (▲, n = 11, except for 10^{-5} M where n = 10) as described in section 2.2.2. Data are expressed as the Mean ± SEM. $^{\alpha}$ $p < 0.05$ compared to the control (0)

3.3.3.2. Actions of α-adrenergic antagonists.

Glucose (15 mM) induced insulin release was measured in the presence of 10^{-6} M guanabenz plus 10^{-5} M idazoxan, an α_2-adrenoceptor antagonist, or graded doses (10^{-7} -10^{-5} M) of prazosin, an α_1- adrenergic antagonist. Idazoxan blocked the inhibitory actions of guanabenz in isolated islets of adult lean and fa/fa rats and 5 wk old rats (Figures 5 and 6). Analysis of the data showed that idazoxan had a statistically significant antagonistic effect on guanabenz inhibited release in both phenotypes regardless of the age ($F(2,96) = 30.79$; $p < 0.0001$). Individual group data analysis confirmed the significant effect of idazoxan on guanabenz inhibited glucose induced insulin release ($p < 0.05$). There was no significant difference in the percent insulin release from pancreatic islets incubated with 15 mM glucose alone or that with glucose plus guanabenz and idazoxan in all four groups of rats (glucose alone (7.26 ± 0.78 (n = 8), 8.69 ± 1.05 (n = 9), 8.95 ± 0.94 (n = 9), 8.39 ± 0.72 (n =12), glucose, guanabenz, idazoxan (6.04 ± 0.65 (n= 7), 7.39 ± 0.77 (n = 8), 7.48 ± 1.34 (n = 9) and 8.41 ± 0.87 (n = 12) in adult lean, adult fa/fa, 5 wk old lean and 5 wk old fa/fa rats, respectively). This indicated that idazoxan completely antagonized the guanabenz inhibition.

Since prazosin was dissolved in DMSO, 15 mM glucose-induced insulin release was measured in the presence of DMSO alone and it was found that DMSO did not have a significant effect on the percentage of glucose-induced insulin release in the isolated islets in any groups. (% insulin release in presence of 15 mM glucose alone was 7.26 ± 0.80 (n = 8), 8.69 ± 1.05 (n = 9), 8.95 ± 0.94 (n = 9), 8.39 ± 0.72 (n

= 12), glucose and DMSO 7.26 ± 0.50 (n = 9), 8.68 ± 1.39 (n = 9), 8.51 ± 1.3 (n = 8), 7.50 ± 0.36 (n = 8), for adult lean, adult fa/fa, 5 wk old lean and 5 wk old fa/fa rats, respectively). Glucose-induced insulin release in the presence of DMSO is referred to as the control when comparing the effects of guanabenz and prazosin on insulin release. Guanabenz (10^{-6} M) in the absence and presence of DMSO significantly inhibited 15 mM glucose induced insulin release in adult lean and fa/fa rats, and 5 wk old lean and fa/fa rats (Figures 7 and 8). Analysis of the data for interactions between different concentrations, age 5 wk and adult rats and phenotype performed for 6 different treatments (15 mM glucose, DMSO, guanabenz 10^{-6} M and prazosin 10^{-7} M, 10^{-6} M and 10^{-7} M) or 5 treatments (DMSO, guanabenz 10^{-6} M and prazosin 10^{-7} M, 10^{-6} M and 10^{-7} M) indicated a statistically significant effect of treatments in all the phenotype/age groups. For 6 treatment groups (glucose, DMSO, guanabenz 10^{-6} M and prazosin 10^{-5} to 10^{-7} M) ($F(5,192) = 17.90$; $p < 0.0001$) and for only 5 treatment groups (DMSO, guanabenz 10^{-6} M, and prazosin 10^{-5} to 10^{-7} M) ($F(4,156) = 11.52$; $p < 0.0001$) indicating no effect of DMSO on insulin secretion under the conditions tested. There was no significant interaction between age and phenotype and treatments ($p > 0.1$). Comparison of means of different treatments in all groups showed that percent insulin release in the presence of glucose/DMSO was significantly different from that released in the presence of 10^{-6} M guanabenz and all prazosin doses (10^{-7} to 10^{-5} M). However, there was no significant difference between the mean % insulin release in the presence of guanabenz 10^{-6} M and all the prazosin concentrations used ($p > 0.05$) in all the groups. Thus addition of prazosin

10^{-7} M to 10^{-5} M to islets in the presence of guanabenz 10^{-6} M had no effect on the glucose-induced insulin secretion in response to guanabenz 10^{-6} M (Figures 7 and 8).

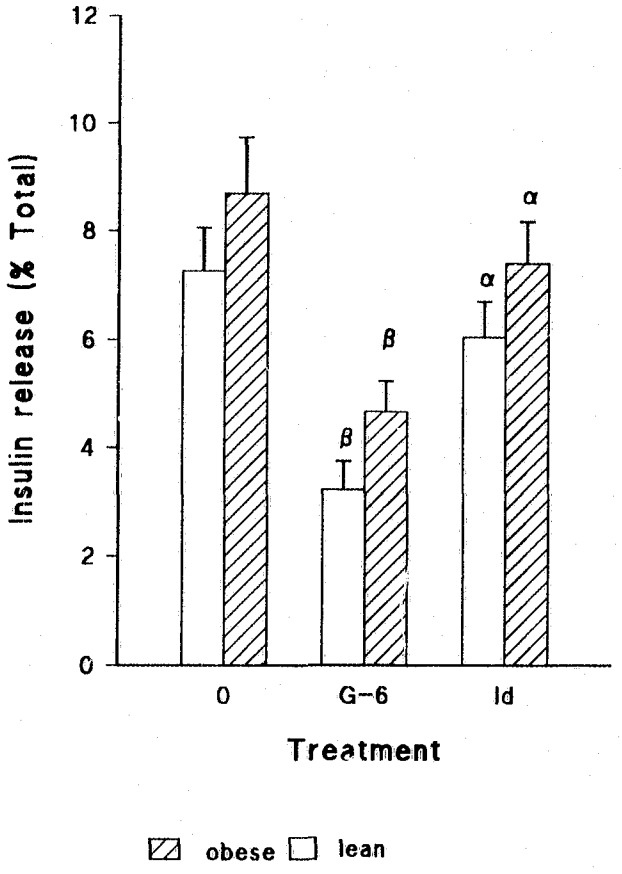

Figure 5. Effect of an α_2- adrenoceptor antagonist on insulin secretion from isolated islets of adult lean and fa/fa rats. Glucose (15 mM) stimulated insulin secretion in the absence (0, n = 8 lean and 9 fa/fa rats) or in the presence of 10^{-6} M guanabenz (G-6, n = 7 lean and 8 fa/fa rats) (agonist) was measured. Addition of 10^{-5} M idazoxan (Id, n = 7 lean and 8 fa/fa rats) antagonized the response to guanabenz. Data are expressed as Means ± SEM. $^{\beta}$ $p < 0.05$ compared to control (0). $^{\alpha}$ $p < 0.05$ compared to guanabenz inhibited values within phenotype using SNK.

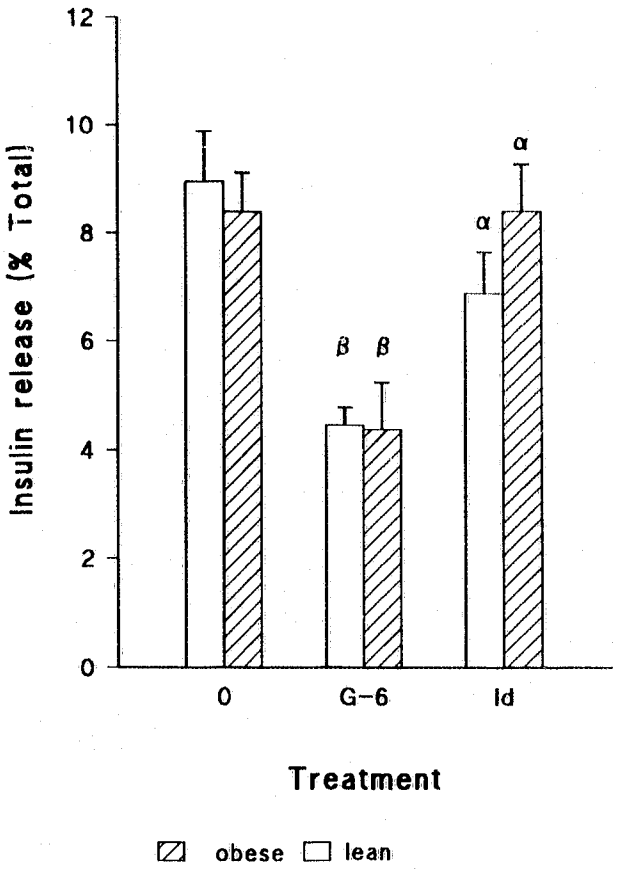

Figure 6. Effect of an α_2- adrenoceptor antagonist on insulin secretion from isolated islets of 5 wk old lean and fa/fa rats. Glucose (15 mM) stimulated insulin secretion in the absence (0, n = 9 lean and 12 fa/fa rats or presence of 10^{-6} M guanabenz (G-6, n = 7 lean and 12 fa/fa rats) was measured. Addition of 10^{-5} M idazoxan (Id, n= 9 lean and 12 fa/fa rats) antagonized the response to guanabenz. Data are expressed as Means ± SEM. $^{\beta}$ $p < 0.05$ compared to the control (0), $^{\alpha}$ $p < 0.05$ compared to guanabenz inhibited values within phenotype using SNK.

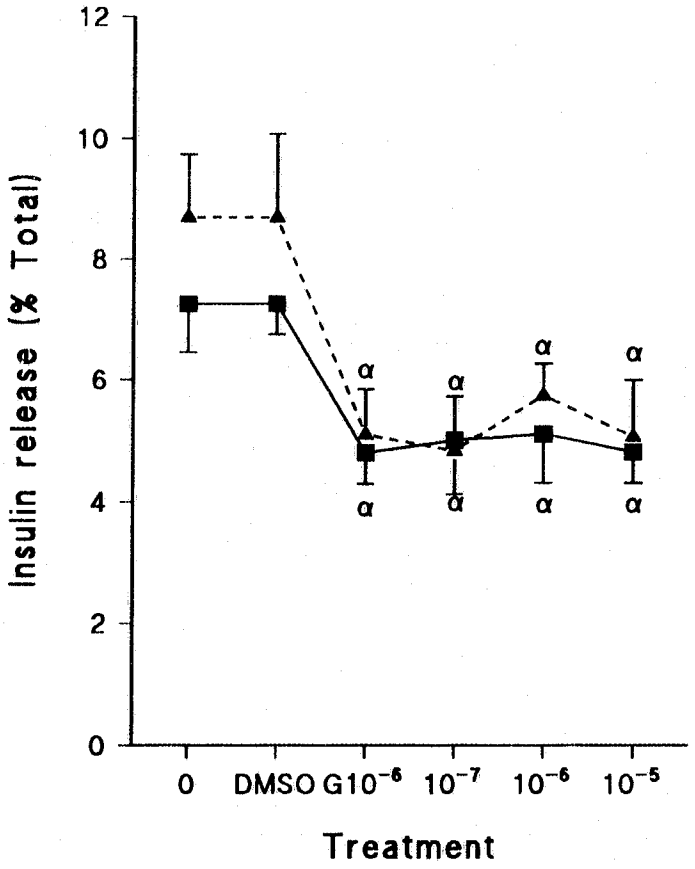

Figure 7. Effect of an α_1- antagonist on insulin secretion in isolated islets from adult lean and fa/fa rats. Insulin secretion was measured in the presence of 15 mM glucose alone (0, n = 8 lean, 9 fa/fa rats; glucose/ DMSO (DMSO), n = 9 for both lean and fa/fa rats; glucose/DMSO plus 10^{-6} M guanabenz (G-6), n = 9 for both lean and fa/fa rats); or 10^{-6} M guanabenz plus graded doses of prazosin (10^{-7} M, 10-6 M), n = 9 for both lean and fa/fa rats and 10^{-5} M, n = 10 lean and 9 fa/fa rats) as described in section 2.2.2. Values represent Means ± SEM for (■) lean and (▲) fa/fa rats.

$^{\alpha}$ $p<0.05$ compared to control (DMSO) within phenotype using SNK.

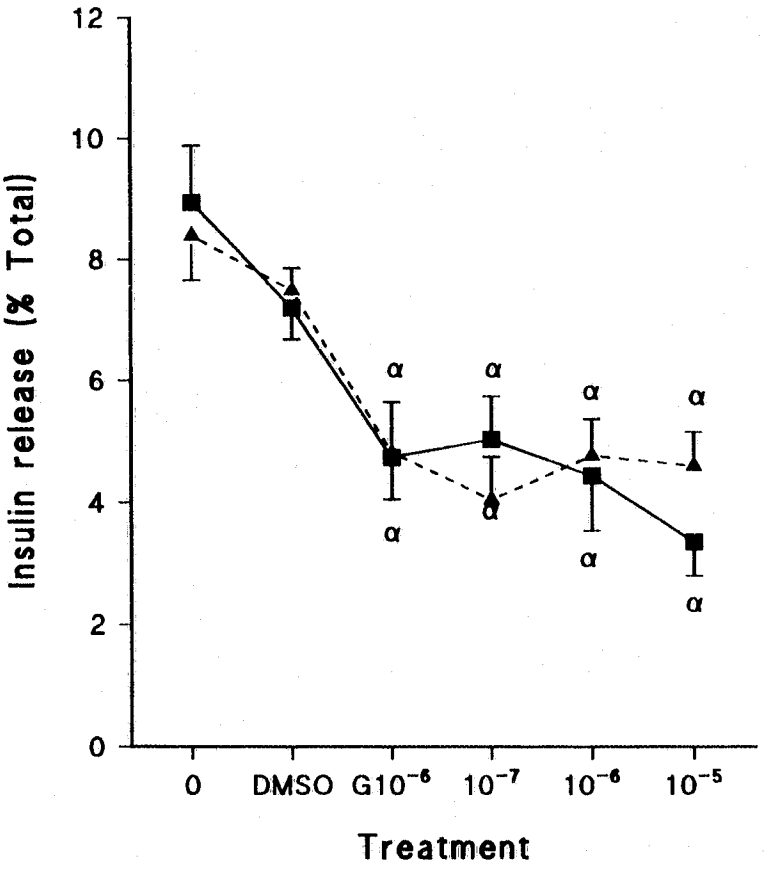

Figure 8. Effect of an α_1-antagonist on insulin secretion in isolated islets from 5 wk old lean and fa/fa rats. Insulin secretion was measured in the presence of 15 mM glucose alone (0, n = 9 lean and 12 fa/fa rats; glucose/DMSO (DMSO), n = 7 lean and 8 fa/fa rats; glucose/DMSO plus 10^{-6} M guanabenz (G-6), n = 8 lean and 9 fa/fa rats; or 10^{-6} M guanabenz plus graded doses of prazosin (10^{-7} M), n = 7 lean and 10 fa/fa rats; 10^{-6} M, n = 7 lean and 10 fa/fa rats and 10^{-5} M, n = 7 lean and 10 fa/fa rats) as described in the section 2.2.2. Values represent Means ± SEM for lean (■) and fa/fa (▲) rats. $^{\alpha}$ $p<0.05$ compared to control (DMSO) within phenotype using SNK.

3.3.3.3. Development of glycolysis regulation defects in isolated islets of fa/fa rats. Glucose (15 mM)-induced insulin release from isolated islets of adult, weanling and suckling lean and fa/fa rats was measured in the presence of various doses of mannoheptulose (MH) (10^{-4} - $3x10^{-2}$ M). Treatments, age alone and phenotype in combination with age had statistically significant effect on insulin release ($F(5,321) = 23.72$, $p < 0.00001$; $F(2,321) = 51.08$, $p < 0.00001$; $F(2,321) = 21.55$, $p < 0.00001$; respectively). Comparison of the means showed that the 15 mM glucose induced-insulin release percentage was not significantly different among all six groups ($p > 0.05$). However comparison of insulin release between 3 wk lean (8.4 ± 0.83; $n = 15$) and 3 wk preobese (5.36 ± 0.74; $n = 7$) rats showed a statistically significant difference (unpaired t-test, $p < 0.05$).

The effect of MH was affected by age and phenotype. MH reduced % glucose-induced insulin release in a dose dependent manner at all ages in the lean rat islets ($p<0.05$), (Figures 9-11). However, in the pancreatic islets of adult lean rats 15 mM glucose stimulated insulin was significantly reduced at a higher MH concentration (10^{-2} M or higher) compared to 10^{-4} M in 5wk and 3 wk old rats ($p < 0.05$), Figure 12). In the fa/fa rats, the glucokinase response to MH was lost in the adult stage, where all MH concentrations used did not produce a statistically significant reduction in 15 mM glucose-induced insulin release ($p > 0.05$). In 5 wk old fa/fa rats, islet glucokinase activity was sensitive to MH concentration of 10^{-2} M or higher, while in 3 wk old rat islets, MH concentration of $3x10^{-3}$ M or higher produced a significant reduction in 15 mM glucose-induced insulin release ($p <0.05$), (Figures 9-11 and 13).

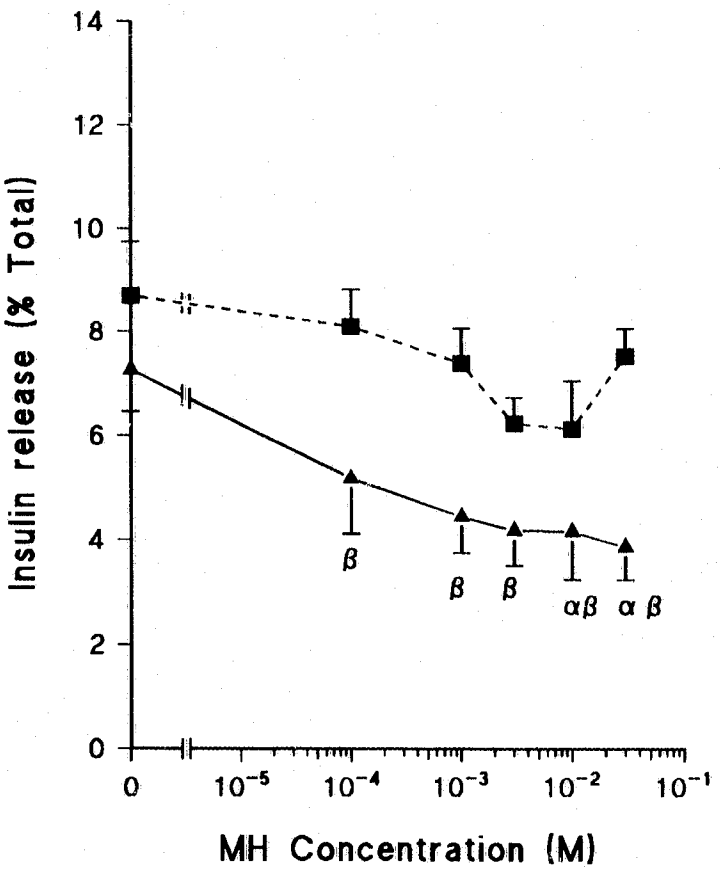

Figure 9. Effect of MH on insulin secretion from isolated islets from adult lean and fa/fa rats. Insulin release was measured in presence of 15 mM glucose (0, n = 8 lean and 9 fa/fa rats) or graded doses of MH 10^{-4} M - 3 x 10^{-2} M (n = 8 lean rats except 3 x 10^{-2} M with 7 rats, and n = 10 for fa/fa rats) as described in the section 2.2.2. Data is expressed an Means ± SEM (▲,lean and ■, fa/fa rats). $^{\alpha}$ $p < 0.05$ compared to control (o) within phenotype by SNK. $^{\beta}$ $p< 0.05$ comparison between lean and fa/fa rats, unpaired t-test.

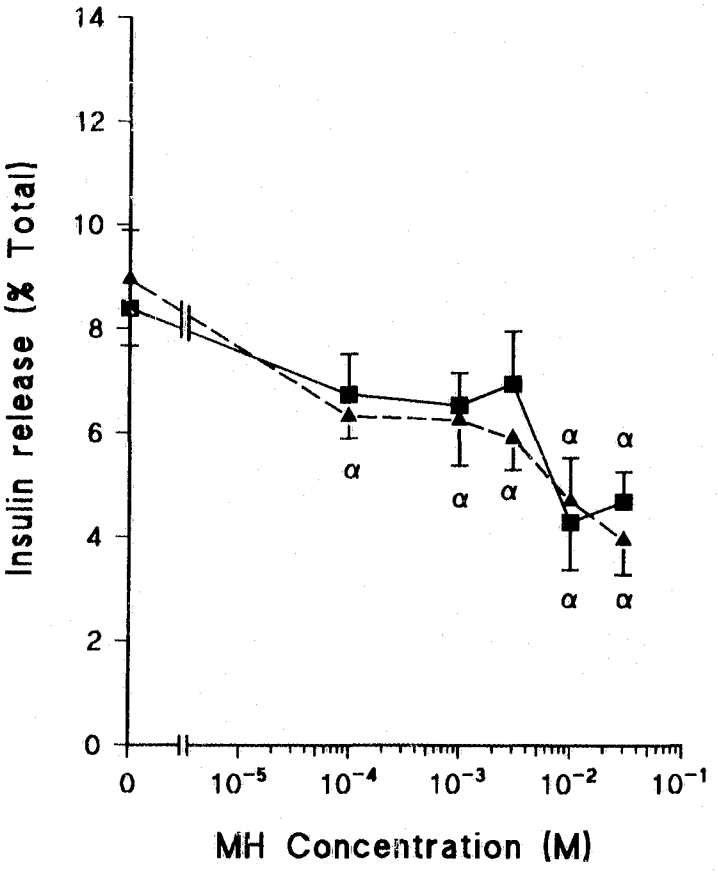

Figure 10. Effect of MH on insulin secretion from isolated islets of 5 wk old lean and fa/fa rats. Insulin release was measured in the presence of 15 mM glucose (0) and graded doses of MH as described in section 2.2.2. Data are expressed as Means ± SEM of 9 lean animals except for MH 10^{-3} M where n = 8 lean rats (▲), and 12 fa/fa animals except for MH 3 x 10^{-3} M where n = 11 rats (■). $^{\alpha}$ $p < 0.05$ compared to the control (0) within phenotype.

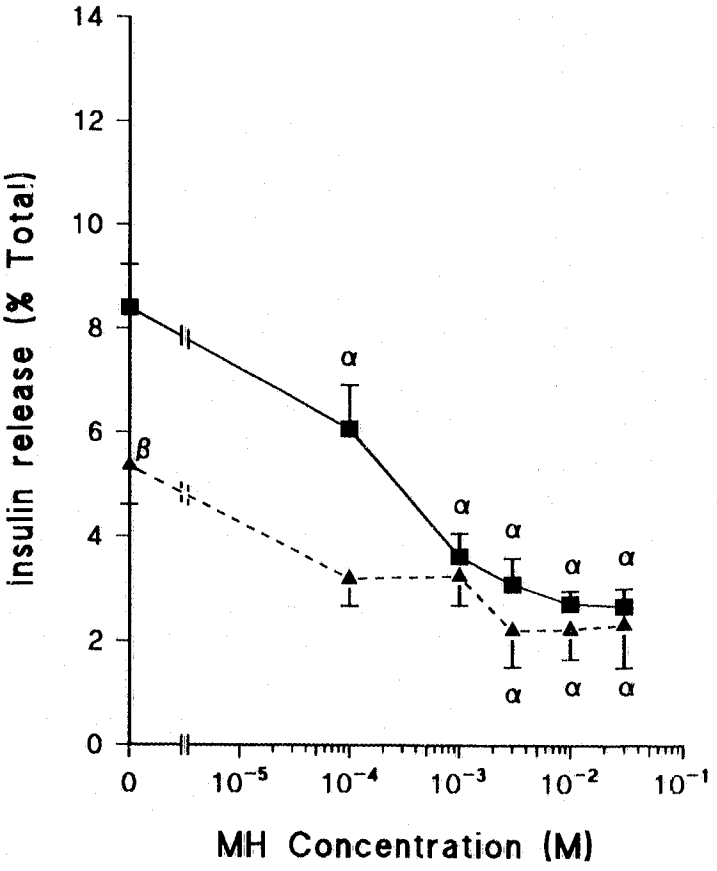

Figure 11. Effect of MH on insulin secretion from isolated islets of 3 wk old lean and preobese fa/fa rats. Insulin release was measured in the presence of 15 mM glucose (0) and graded doses of MH as described in section 2.2.2. Data are expressed as Means ± SEM of 15 animals except for MH 3 x 10^{-3} M and 10^{-2} M each with 14 lean animals (■), and 7 fa/fa animals for 15 mM glucose, MH 10^{-4} M, and 3 x 10^{-3} M and 6 animals for MH 10^{-3} M, 10^{-2} M and 3 x 10^{-2} M (▲). $^{\alpha}$ $p < 0.05$ compared to the control (0) within phenotype, using SNK test, following analysis of variance (see text). $^{\beta}$ $p < 0.05$ lean compared to fa/fa rats

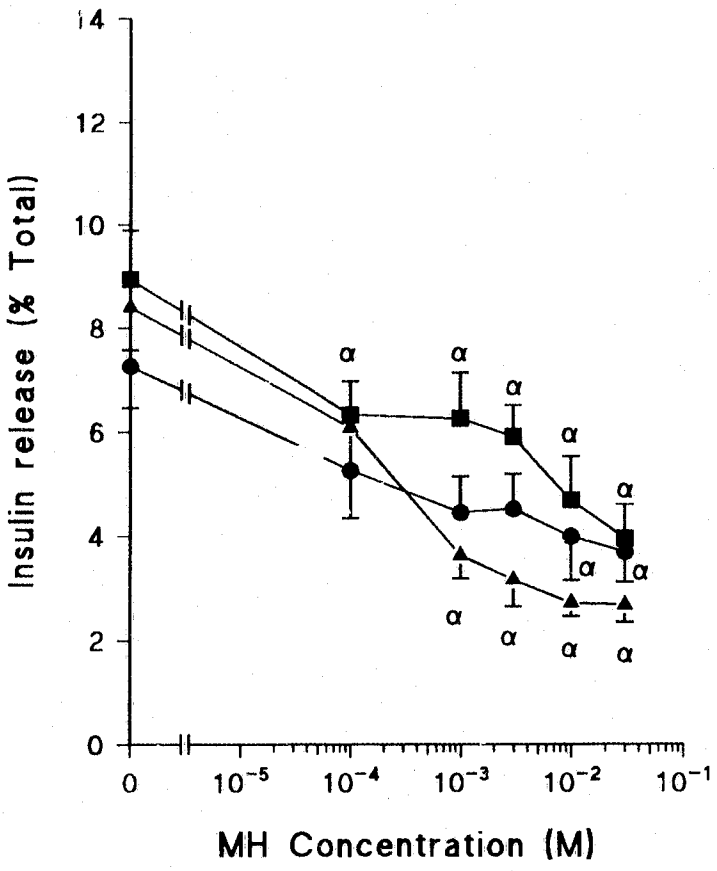

Figure 12. The effect of age on MH action in isolated pancreatic islets from adult, 5 wk and 3 wk old lean rats. 15mM glucose-induced insulin secretion was measured in the absence (0) or presence of different MH doses as indicated in section 2.2.2. Data are expressed as Mean ± SEM of lean animals as in Figures 9, 10 and 11; adult (●), 5 wk old (▄), 3wk old rats (▲).

α = statistically significant from the control (0) within the same age group.

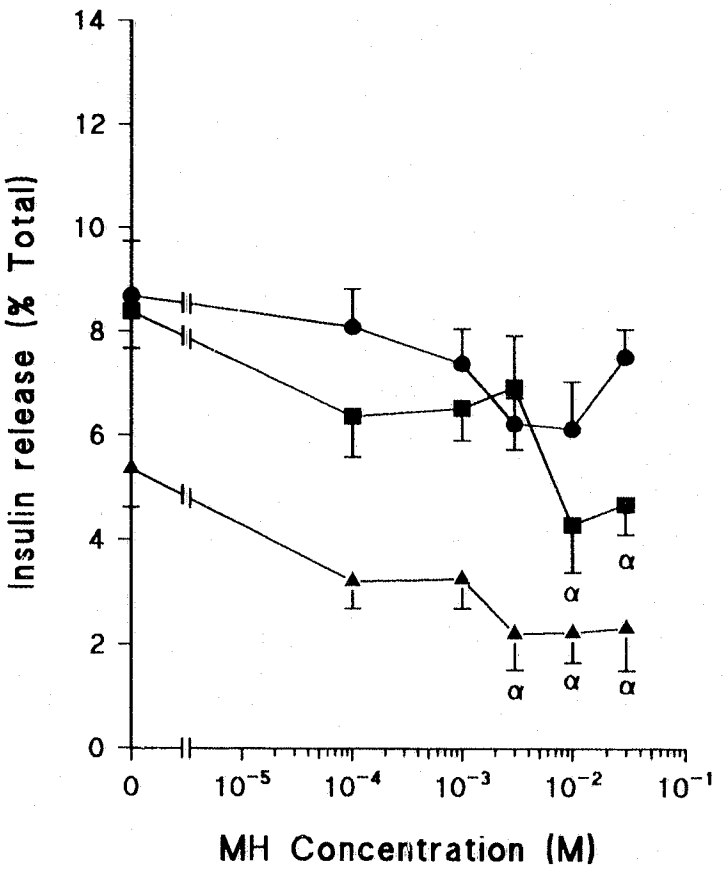

Figure 13. Effect of age on the MH action in isolated pancreatic islets from adult, 5 wk and 3 wk preobese fa/fa rats. 15 mM glucose-induced insulin secretion was measured in the absence (0) or the presence of different MH doses as indicated in section 2.2.2. Data are expressed as Mean ± SEM for fa/fa animals as in Figures 9, 10 and 11; adult (●), 5 wk old (▄), 3wk old (▲). α = significant from the control (0) within the same age group ($p < 0.05$, SNK)

3.4. Discussion.

The purpose of this study was to identify the time of onset of two pancreatic islet defects in fa/fa rats, specifically changes in catecholamine antagonist actions and abnormal glycolysis regulation in connection with glucose induced insulin secretion. Both of these defects were documented previously in adult fa/fa rat islets (Chan and MacPhail, 1992; Chan *et al.*, 1993).

Isolated islets from adult fa/fa rats were reported to possess an α_{2B}-adrenoceptor subtype, which is sensitive to prazosin (an α_1-adrenoceptor antagonist) as well as yohimbine (an α_2-adrenoceptor antagonist) (Chan and MacPhail, 1992). In the current study, using a glucose concentration (15 mM) which is near the physiological range, guanabenz inhibited insulin secretion with similar potency in adult and 5 wk old lean and fa/fa rats. Phenylephrine, an α_1-adrenergic agonist, did not have any effect on glucose-stimulated insulin secretion from isolated islets of either adult or 5 wk old rats in either phenotype. The data confirms that the sympathetic nervous system regulates glucose-induced insulin secretion through α_2-adrenoceptors on the pancreatic islet B-cell membrane (Efendic *et al.*,1991, Rorsman *et al.*, 1991; Chan and MacPhail, 1992).

The inhibition of insulin secretion by 10^{-6} M guanabenz was antagonized in both phenotypes by addition of 10^{-5} M idazoxan, an α_2-antagonist that is more selective than yohimbine. Contrary to the previous findings prazosin, an α_1-adrenoceptor antagonist, did not have a significant effect on guanabenz inhibited glucose-induced insulin release in either adult or 5 wk old lean or fa/fa islets. The

results of this study are similar to those of a study by Östenson *et al.* (1989) using isolated islets from streptozotocin (STZ) induced NIDDM and normal rats. Using glucose concentrations in the range of 3-16.5 mM, prazosin (10^{-7}-10^{-5} M) had no effect on UK 14304 (an α_2- agonist) inhibited insulin release in either normal or STZ Sprague-Dawley rats. Although they also reported that isolated islets from STZ diabetic rats were more sensitive to α_2- adrenoceptor agonist inhibition, this was not the case in fa/fa isolated islets in this study or elsewhere (Chan and MacPhail, 1992). Increased sensitivity to an α_2- adrenoceptor agonist may be caused by STZ since similar results were observed with stimulation of the splanchnic nerve in a perfused pancreas preparation of STZ diabetic and normal Wistar rats (Kurose *et al.*, 1992). The current study differed from the previous report (Chan and MacPhail, 1992) in the glucose concentrations used to stimulate insulin release, and the agonist used. Perhaps B-cell α_2- adrenoceptors in fa/fa rats behave normally when insulin release is measured at glucose concentration in the physiological range but their function is altered if higher glucose concentrations are used. Further studies using a full range of glucose concentrations would be required to test this hypothesis.

The importance of glucose as the main physiological insulin secretagogue is well known (Zawalich, 1979; Ashcroft *et al.*, 1970). Glucose induced-insulin release occurs after glucose is metabolized by the B-cell through the glycolytic pathway and Kreb's cycle. Glucokinase, a member of the hexokinase enzyme family, catalyses the first rate limiting reaction of glycolysis when plasma glucose concentrations are raised above basal levels. Pancreatic islet glucokinase activity is increased in the presence

of high glucose concentrations and reduced by low levels of glucose (Liang *et al.*, 1990; 1992). Conditions that lower glucose concentration or block glucokinase activity result in a reduction of insulin secretion by pancreatic B-cells. In the current study 15 mM glucose-induced insulin release was measured in the presence of MH, an inhibitor of glucokinase. In the lean rat islets the activity of glucokinase was found to be dose dependently inhibited by MH at all ages tested. In isolated islets from fa/fa rats, there was a progressive loss of response with age. In adult fa/fa rats there was a complete loss of sensitivity to MH inhibition even at the highest dose used (3 x 10^{-2} M). In 5 wk old fa/fa islets, significant inhibition was observed at a considerably higher dose (10^{-2} M) compared with the inhibitory dose (10^{-4} M) in the lean rats. In the 3 wk old lean group glucokinase sensitivity to MH inhibition was similar to 5 wk old lean rats (10^{-4} M), but in 3 wk old preobese rats the glucokinase sensitivity to MH inhibition was lower than in the lean 3 wk old rats. Glucose-induced insulin release was significantly reduced by a MH concentration of $3x10^{-3}$ M or higher in preobese rat islets compared to 10^{-4} M in the 3 wk old lean rat islets. Although not significant ($p > 0.05$), a MH concentration of 10^{-4} M produced a higher percent reduction in glucose induced insulin release in preobese islets (40%) compared to the 3 wk old lean rats islets (27%). This might be due to the small number of animals used in the preobese compared to lean group (6-7 and 14-15 rats; Figure 11, respectively). The data suggest that 3 wk old preobese pancreatic glucokinase sensitivity to MH inhibition is still intact, but this needs more investigation using a higher number of animals. If the statistically non significant effect of the lower concentration of MH

(10^{-4} M and 10^{-3} M) observed in 3 wk old rat islets is genuine, then the glucose-induced hyperinsulinemia (Chan *et al.*, 1985) could reflect the increased activity of glucokinase in preobese pancreatic islets. The data obtained from isolated islets in this study are in agreement with those of previous studies which showed that glucokinase in adult fa/fa rat islets did not respond to MH inhibition in the same manner as in lean control islets (Chan *et al.*, 1993; Chan, 1993). Reduced sensitivity of glucokinase to MH inhibition may be secondary to development of obesity since a similar defect has been observed in other strains of genetically obese rats, hyperglycemic SHR/N-cp and normoglycemic obese LA/N-cp rats (Timmers *et al.*, 1992).

Pancreatic islet glucokinase sensitivity to MH inhibition in 5 wk old fa/fa rats is still present, but is reduced compared to the 3 wk old preobese rats. Zucker fa/fa rats are mildly obese at this stage but as their obese syndrome worsens with age a complete loss of glucokinase response to MH results. Three wk old preobese pups exhibit glucose-stimulated hyperinsulinemia compared to lean animals but they do not have fasting hyperinsulinemia (Turkenkopf *et al.*, 1982; Chan *et al.*, 1985; Fletcher *et al.*, 1986). Also at 3 wk there is no difference in the body weights between preobese and lean pups (42.4 ± 0.9 g, n = 15 lean and 39.4 ± 0.1 g, n = 7 preobese rats), while 5 wk old (154.2 ± 6.1, n = 12 compare to 86.9 ± 3.4 gm n = 14 for 5wk old lean rats) and adult fa/fa rats are heavier (243.7 ± 9.2 gm, n = 6) than the lean rats (153 ± 3.0 gm, n = 7) and have excess intraabdominal fat accumulation. Abdominal fat accumulation has also been implicated in some abnormalities such as impaired

glucose tolerance test observed in human beings (Kohrt *et al.*, 1993). If pancreatic islet glucokinase activities were influenced by intraabdominal adipose accumulation then this could partially explain the slightly higher activity of the glucokinase enzyme in the 3 wk old preobese pups. In addition to the reduced glucokinase sensitivity to MH inhibition observed in this study, other defects become significant in fa/fa rats at 5 wk of age. These defects include increased body weight, fasting hyperinsulinemia (Zucker and Zucker, 1961; Bray and York, 1971; 1979; Chan *et al.*, 1985), increased response to GIP at low glucose levels (Chan *et al.*, 1985) and loss of the diurnal rhythm for glucocorticoid secretion from the adrenal glands (Fletcher *et al.*, 1986). The increased response of fa/fa rat pancreatic islet B-cells to stimulants such as GIP on glucose induced-insulin secretion might be secondary to increased glucokinase activity in the cells. Glucokinase has been shown to determine the rate of glucose metabolism by the B-cell at physiological plasma glucose levels (Matschinsky, 1990; German, 1993). Changes in glucokinase affinity for glucose would increase the ability of B-cells to respond to low levels of plasma glucose. It was suggested by Meglasson and Matschinsky (1984) that even a small reduction in pancreatic islet glucokinase activity might significantly shift (to the right) the concentration of glucose needed to initiate insulin secretion in conditions such as NIDDM. If glucokinase activity was increased, then a left shift in glucose concentration would be observed leading to induction of insulin release at a lower plasma glucose levels. Such a left-shift has been observed in fa/fa rats (Chan *et al.*, 1993), leading to the hypothesis that changes in glucokinase function results in fasting hyperinsulinemia as observed in fa/fa rats

(Chan *et al.*, 1984).

This defect in glucokinase function has not yet developed in 3 wk old preobese rats. It is possible that the loss of MH inhibitory action on pancreatic islet glucokinase activity is dependent on the extent of obesity in Zucker fa/fa rats and develops secondary to other metabolic disturbances. Also the changes in MH inhibition of glucose induced insulin secretion could be due to decreased entry of MH into B-cells of fa/fa islets. However, this is unlikely since measurements of MH effe on glucokinase in disrupted islets yielded similar results (Chan 1993). Since MH sensitivity was lost concurrently with the diurnal rhythm of glucocorticoid secretion (Fletcher *et al.*, 1986) and the obligatory role of adrenal glucocorticoids in maintaining obesity is well documented (Castonguay *et al.*, 1986; Fletcher, 1986a; Yukimura *et al.*, 1978), it seems reasonable to hypothesize a link between corticosterone and regulation of glucokinase function.

4. THE EFFECT OF ADRENALECTOMY ON DEVELOPMENT OF PANCREATIC ISLET LESIONS IN fa/fa RATS.

4.1. Introduction.

The adult genetically obese Zucker rat is hyperinsulinemic, hyperphagic, hyperlipidemic, normoglycemic and has a mild form of insulin resistance in both liver and skeletal muscle (Bray *et al.*, 1989). The precise location of the genetic defect is still not known. Bray and York (1971; 1979) suggested that this defect could be expressed in one of several tissues including the adrenal glands, pancreatic B-cells, adipose tissue or the hypothalamus.

Much attention has been focused on the hypothalamo-pituitary-adrenal (HPA) axis because glucocorticoid dependency of development and maintenance of many obese syndromes including that of fa/fa rats has been demonstrated (Yukimura and Bray, 1978; Bray and York, 1979; Bruce *et al.*, 1982; King and Smith, 1985; Bray *et al.*, 1989). When adrenalectomy (ADX) is performed in fa/fa rats before weaning, all metabolic, behavioral and endocrine defects are prevented (Fletcher, 1986b; Fletcher and McKenzie, 1988). However when ADX is performed in adult rats, the severity of the defects is reduced but not eliminated entirely (Yukimura and Bray , 1978; Yukimura *et al.*, 1978; Holt and York, 1982; Castonguay *et al.*, 1986). Administration of glucocorticoids reverses the effect of ADX whether done before weaning or in adult fa/fa rats (Castonguay *et al.*, 1986; Freedman *et al.*, 1986; Fletcher and McKenzie, 1988). The normalization or reduction of insulin secretion observed after ADX in fa/fa rats (Fletcher, 1986a; 1986b; Fletcher and Mckenzie, 1988) might

be a result of reduced food intake in fa/fa rats (Yukimura *et al.*, 1978; Turkenkopf *et al.*, 1991) or the removal of the inhibitory effect of corticosterone on the autonomic nervous system activity in the endocrine pancreas (Fletcher and Mckenzie, 1988; Bray *et al.*, 1989).

The effects of ADX in fa/fa rats are thought to be mediated mainly by the removal of corticosterone inhibition of hypothalamic corticotropin releasing factor (CRF) secretion. Dexamethasone was shown to decrease CRF mRNA in a mouse anterior pituitary cell line (AtT-20) (Rosen *et al.*, 1992). Also glucocorticoid receptor II binding is increased in the hypothalam and hippocampus of fa/fa rats, giving evidence of increased corticosterone activity in fa/fa rat brain (Langley and York, 1992). In addition to reducing food intake and increasing sympathetic activity in the periphery and brown adipose tissue (Arase *et al.*,1988), ADX was observed to increase CRF concentration in the hypophysial-portal circulation in fa/fa rats (Plotsky *et al.*, 1992). Acute or chronic intracerebroventricular (ICV) infusion of CRF in fa/fa rats mimics the effects of ADX in these rats (Arase *et al.*, 1989a; Holt and York, 1989; Rohner-Jeanrenaud *et al.*, 1989).

Adrenalectomy may also prevent hyperinsulinemia in fa/fa rats by reducing the synthesis or release of insulin in the pancreatic B-cells. Because several biochemical changes in B-cells of fa/fa rats have been identified, it is possible that improvement after ADX in fa/fa rats is due to normalization of those biochemical lesions. Of major interest is the enzyme glucokinase, which plays a pivotal role in determining insulin secretory rates.

Glucokinase phosphorylates glucose to glucose-6-phosphate and this is the first step in B-cell glucose metabolism that leads to insulin secretion. Because it is a rate-limiting enzyme, glucokinase is considered to be the primary glucose sensor for the pancreatic B- cells (Matschinsky, 1990). Evidence that glucokinase plays a crucial role in glucose-induced insulin release was provided by Burch et al (1981) using albino Wistar rats. They found that pancreatic glucose usage, glucose-induced insulin secretion and glucokinase activity in islets were reduced in rats starved for 24 h while other glycolytic enzymes were only affected after 120 h starvation. Refeeding restored glucokinase activity and glucose usage by the islets (Burch *et al.*, 1981). Glucokinase activity in the B-cell is correlated with the glucose level to which the B-cells are exposed (Liang *et al.*, 1990; 1992).

In adult fa/fa rats, glucokinase activity may be abnormally regulated by glucose. Islets from starved lean rats exhibited reduced glucose-induced insulin secretion but no decrease was observed in islets from starved adult fa/fa rats, suggesting failure to adapt to chronically low plasma glucose levels (Chan *et al.*, 1993). Furthermore, fa/fa rats exhibit fasting hyperinsulinemia despite having basal glucose levels similar to lean rats (Chan *et al.*, 1984). Glucokinase from islets of fa/fa rats has been shown to have increased sensitivity to glucose (Chan, 1993) and this increased responsiveness might contribute to the fasting hyperinsulinemia observed in fa/fa rats.

Mannoheptulose (MH), which competitively inhibits glucokinase, normally blocks glucose-induced insulin release (Zawalich, 1979) but did not have any effect on insulin secretion or glucokinase activity from adult fa/fa rats (Chan, 1993; Chan

et al., 1993; Chapter 3). The lack of glucokinase sensitivity to MH inhibition has also been identified in other rodents like cp/cp rats (Timmers *et al.*, 1992) and ob/ob mice (Hoshi and Shreeve, 1969) which have genetically transmitted obese syndromes.

In the initial developmental studies (Chapter 3) the time of onset for measurable glucokinase insensitivity to MH was identified as 5 wk. In these studies, the dependence on the presence of an intact HPA axis on the development of MH insensitivity was investigated by adrenalectomizing 5 wk old lean and fa/fa rats. The presence or absence of the biochemical lesion was investigated 2 wk post surgery.

4.2. Methods

4.2.1. Adrenalectomy.

To study the effect of the HPA axis on the development of pancreatic islets in obese rats, 5 wk old rats of both fa/fa and Fa/? phenotypes were bilaterally adrenalectomized. Thirty (14 lean and 16 obese) 4 wk old animals were obtained from Charles River Laboratories. The animals of the same phenotype were housed 4 animals per cage for 5 d prior to surgery, then divided in two groups 2 d before surgery. Adrenalectomies were performed twice a week. The animals (two at a time) were fasted overnight and then weighed. They were anaesthetized using a mixture of sodium pentobarbital (65 mg/ml) and diazepam (5 mg/ml) in saline (0.9%) i.p at a dose of 0.1 to 0.15 ml/100 g body weight. The hair was clipped on the ventral abdomen, the skin cleaned and the abdominal cavity exposed by midline incision from the umbilicus to the xiphoid cartilage. This approach is normally used in surgery of companion animals during exploratory surgery (Scavelli *et al.*, 1986) and has been used for ADX in rats (Castonguay *et al.*, 1986). Both adrenal glands were removed by ligating the adrenal vasculature then gently freeing the adrenal glands with the aid of forceps. The abdominal incision was closed in two steps: first the muscles were sutured using absorbable suture material (4-0 Dexon), and then the skin was closed using nonabsorbable suture (4-0 Nylon). The animals recovered from anaesthesia before they were returned to the rat housing area. The sham operated control rats had the abdominal cavity opened and the adrenal glands teased but left in place. The wound was then closed as described above. Surgery on the ADX and sham rats

was done on the same day and those animals were put in the same cage. In addition to the normal diet of rat chow (see general methods for diet composition) and tap water, they were given saline/glucose (0.9% NaCl, 40 mg/L glucose) for drinking to maintain sodium balance in the absence of mineralocorticoid.

After 15 d, before the pancreas was removed, the animals were weighed and blood samples were collected in heparized tubes from the tail vein of free moving conscious rats. Serum was collected by centrifugation (1854 g at 4° C for 10 min) and stored at - 20° C for corticosterone determination. Animals that survived the surgery having no residual adrenal gland pieces and decreased serum corticosterone levels were considered completely adrenalectomized and were included in further experiments. The mortality rate was very low. Of 30 operations performed, only one obese rat died during surgery. This was determined to be due to reaction to the anaesthetic as the animal had seizures immediately after it was anaesthetized. The rest of the animals recovered well. Only 28 animals were used in further experiments. The only observed complication at the time of pancreas removal was an occasional adhesion of the liver to the left kidney.

4.2.2. Pancreatic islet preparation.

Pancreatic islet isolation, insulin release, and insulin measurement experiments and statistical analysis were done as described in sections 2.2, 2.3 and 2.4 respectively. 26 animals were used in insulin release experiment (7 ADX, 7 sham-operated lean, 6 ADX and 6 sham-operated fa/fa rats, respectively)

4.2.3. Corticosterone radioimmunoassay.

Corticosterone assay measuring the total serum corticosterone levels was carried out according to the kit manufacturer's manual (ICN Biomedical Inc.). The assay employed a highly specific antiserum to corticosterone-3-carboxymethyloxime (< 0.1% cross reactivity with related compounds) and corticosterone ^{125}-I as the tracer. All 28 samples were assayed in one run.

4.3. Results

4.3.1. Effect of ADX on plasma corticosterone.

In sham operated rats there was no phenotype difference in the serum levels of corticosterone. Adrenalectomy reduced the measurable hormone by over 85% in both lean and fa/fa rats (Table 4). Corticosterone levels of 0.05 to 0.4 μg/ml (Shimizu *et al.*, 1983) and 0.02 to 0. 48 μg/ml (Bruckdorfer *et al.*, 1974) are considered as basal levels in rats, depending on the time of day. Shargill *et al* (1987) reported total corticosterone levels of 0.64 ± 0.055 μg/ml and 0.647 ± 0.082 μg/ml in adult lean and fa/fa rats respectively.

Some investigators have used a corticosterone concentration of 0.021 μg/ml as an indicator of successful ADX (Fletcher and McKenzie, 1988). In this study the 85% reduction in corticosterone concentration combined with absence of adrenal tissue in the surviving rats was taken as a successful operation and all rats were used in subsequent experiments. Lack of total corticosterone clearance after adrenalectomy depends on the amount of hormone in circulation at the time of surgery and the amount of adipose tissue in the animal tissues. The latter will reduce clearance of the hormone and 15 d may not be enough time for the body to rid itself of the residual hormone. The high corticosterone levels observed in sham rats of both phenotypes is probably due to stress during handling before collection of blood (overnight fasting and clipping the tips of the tails).

4.3.2. Pancreatic islet insulin content.

Islet insulin content was calculated as described in section 3.3.1. Adrenalectomy did not have any significant effect on the islet insulin content in either phenotype ($p > 0.05$). Zucker fa/fa ADX and sham rats had more than 2 fold greater insulin content compared to the lean groups before and 2 wk post surgery. Sham fa/fa rats tended to have higher pancreatic insulin content compared to ADX fa/fa rats, although the difference was not significant ($p > 0.05$) (Table 5).

Table 4. Total serum corticosterone (μg/ml) levels in ADX and sham lean and obese Zucker rats 2 weeks post surgery

Phenotype	ADX	Sham
lean	0.071 ± 0.025 (7)	0.983 ± 0.052 (7)[§]
obese	0.135 ± 0.040 (7)	0.910 ± 0.133 (7)[§]

Data are means ± SEM of the number of animals (n)

[§] = significant effect of ADX compared to sham of the same phenotype, $p < 0.05$, unpaired t-test.

Table 5. Effect of ADX on pancreatic islet insulin content (pmol/islet) of fa/fa and lean rats.

Phenotype	ADX	Sham
lean	7.2 ± 0.8 (7)	6.5 ± 0.5 (7)
obese	14.7 ± 2.8 (6)[§]	17.9 ± 5.5 (6)[§]

Data are means ± SEM of number of animals used (n).

[§] = significantly different from lean rats, $p < 0.05$, unpaired t-test.

4.3.3. Effect of ADX on weight gain in fa/fa rats.

The effect of ADX on weight gain is shown in Figure 14. Obese rats weighed more than lean rats at 5 wk (before ADX) and at 7 wk (15 d post surgery). Multiple comparison of both sham and ADX groups at 5 and 7wk of age showed a significant effect due to both ADX and age. However within phenotype comparison showed that while ADX did not affect the weight gain pattern in lean rats, it slowed weight gain in fa/fa rats. There was no significant difference between weight of fa/fa rats at 5 wk (147.5 ± 3.3 g vs 151.5 ± 7.3 g, n = 6) for ADX and sham rats, respectively. Two wk post surgery, ADX fa/fa rats weighed 11% less than sham fa/fa rats (219.5 ± 4.6 g and 243.7 ± 9.2 g, respectively, n = 6). Adrenalectomy and sham lean rats did not show any significant difference in weight before or 2 wk post surgery (Figure 14).

4.3.4. Effect of ADX on regulation of glycolysis.

Glucose (15 mM)-induced insulin secretion was measured in the presence of different concentrations of MH as described in section 2.2.2. The amount of insulin released was affected by MH ($F(5,126) = 14.28$; $p < 0.0001$), phenotype ($F(1,126) = 7.50$; $p = 0.007$), by ADX/sham ($F(1,126) = 33.64$; $p < 0.0001$) and phenotype and ADX interaction ($F(1,126) = 49.55$; $p < 0.0001$). Further analysis of the data by SNK showed that a MH concentration of 10^{-3} M or higher, significantly reduced insulin secretion by pancreatic islets of both lean ADX, sham-operated lean (Figure 15) and fa/fa ADX (Figure 17) from their respective controls ($p < 0.05$).

Adrenatectomy affected MH inhibition of glucose-induced insulin release only in fa/fa rats. In isolated islets from sham-operated fa/fa rats different MH concentrations used did not produce a significant reduction in glucose-induced insulin release ($p > 0.05$), (Figure 16). In isolated islets from ADX fa/fa rats MH inhibited insulin secretion in a dose-dependent manner (21.8% to 53.2%) (Figure 17). The MH 10^{-3} M concentration produced only a 5% reduction in insulin secretion by pancreatic islets from sham operated fa/fa rats compared to 48% in sham operated lean rats. The observed difference in the islets from fa/fa rats was caused by adrenalectomy (Figure 17). Adrenalectomy increased the sensitivity of B-cell glucokinase to MH inhibitory actions within 2 wk post operation in fa/fa rats but did not have any effect in the lean rats.

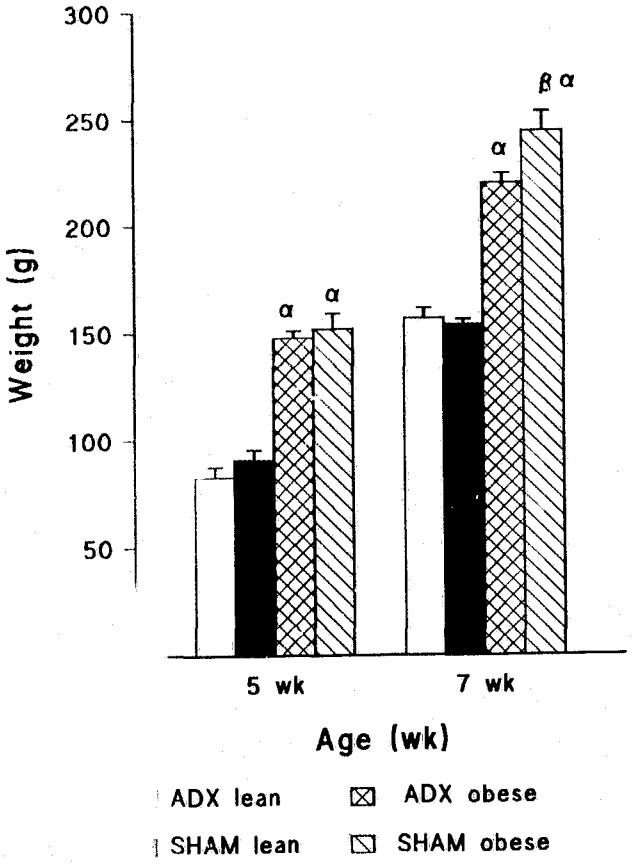

Figure 14. Effect of ADX on body weight gain in Zucker lean and fa/fa rats ADX or sham-operated rats were weight ADX and 2 wk post surgery. Values represent Mean ± SEM of 7 lean and 6 fa/fa rats.

$^{\beta}$ $p< 0.05$ ADX compared to sham-operated within phenotype.

$^{\alpha}$ $p<0.05$ comparison between phenotypes.

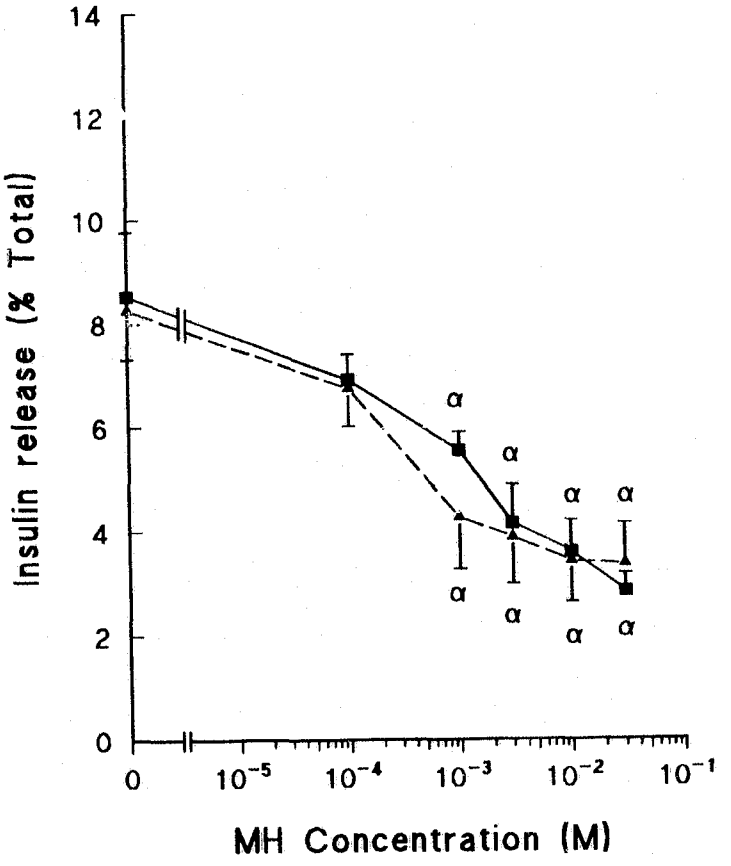

Figure 15. Effect of ADX on glucokinase sensitivty to MH in lean rats. Insulin secretion from isolated islets from ADX and Sham-operated lean rats was measured in presence of 15 mM glucose and different concentrations of MH as described in section 2.2.2. MH inhibited insulin release similarly in (■) ADX and (▲) sham rats. Values are means ± SEM of 7 rats. $^{\alpha}$ $p< 0.05$ compared with the phenotype control (0), SNK test after analysis of variance.

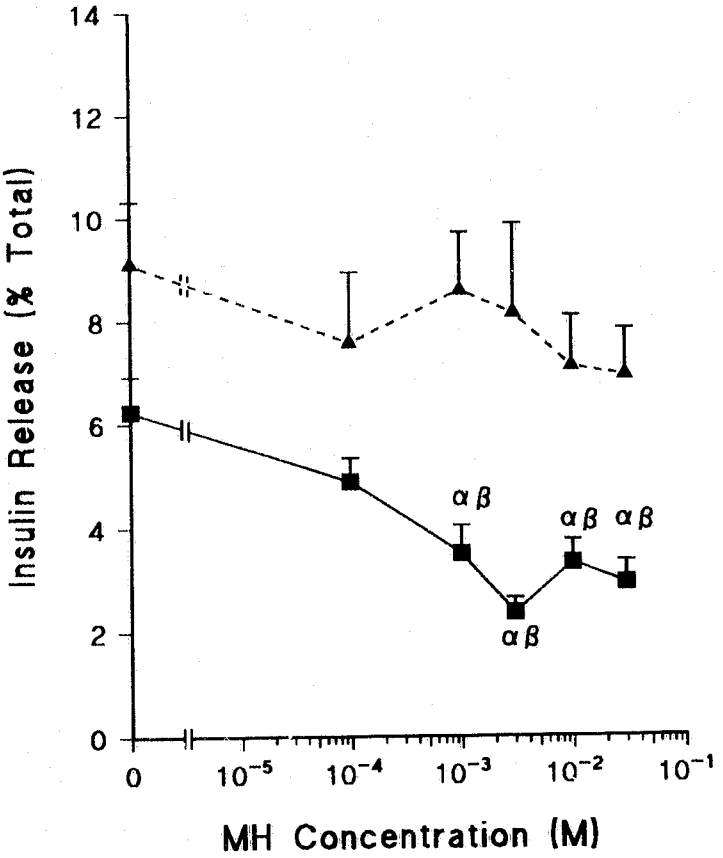

Figure 16. Effect of ADX on glucokinase sensitivity to MH inhibition in obese rats. Insulin secretion of isolated islets of ADX and sham operated fa/fa rats was measured in the presence of 15 mM glucose and graded concentrations of MH as described in the Methods section. Values are Means ± SEM (n = 6 sham fa/fa rats ▲, except for MH 3x 10^{-2} M with 5 rats, and n = 6 for ADX fa/fa rats ■). $^{\alpha}$ $p < 0.05$ compared with the phenotype control (0), SNK test after analysis of variance. $^{\beta}$ $p < 0.05$ ADX compared to sham operated fa/fa rats, unpaired t-test.

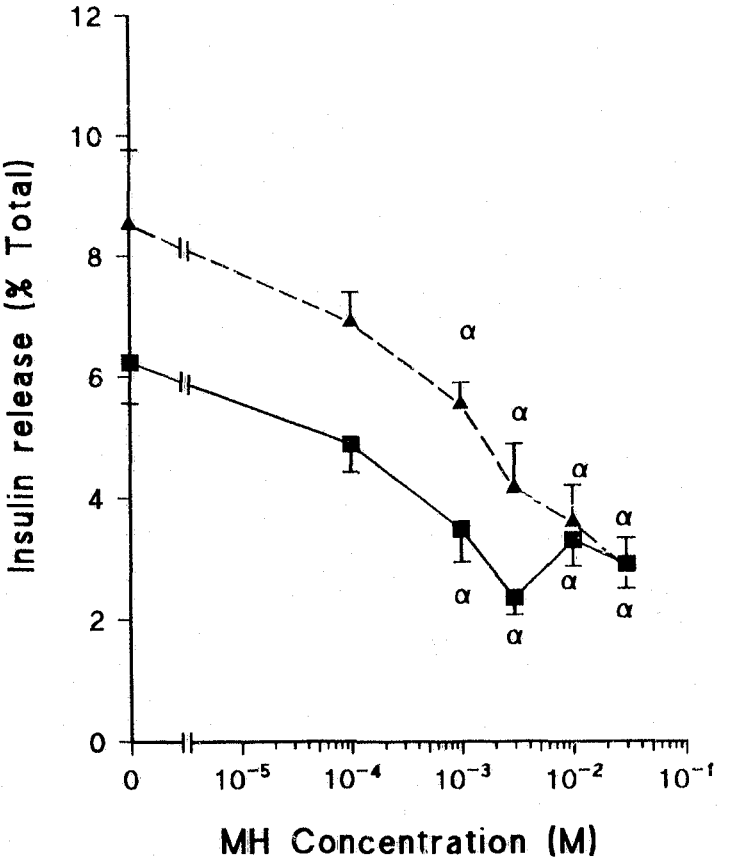

Figure 17. Effect of MH on 15 mM glucose in ADX fa/fa and ADX lean rats. Glucose stimulated insulin release was measured in absence (0) and in presence of various MH doses as described in the section 2.2.2. Insulin release is expressed as % of the total pancreatic islet insulin content and points represent the Mean ± SEM for 7 ADX lean (▲) and 6 ADX fa/fa (■) rats.

α $p < 0.05$ compared to control (0) within the group.

Differences between the phenotypes were not significant.

4.4. Discussion.

The purpose of this study was to investigate the influence of the HPA axis on development and persistence of pancreatic islet lesions. Although insulin secretion is known to be reduced by ADX, the effects on specific pancreatic defects have not been reported. In this study we investigated ADX effects on the activity of glucokinase in isolated islets from both lean and fa/fa rats. The sensitivity of glucokinase to MH inhibition was reduced in intact 5 wk fa/fa rats (Figure 10; Chapter 3) but by 15 d post ADX the glucokinase activity was inhibited similarly by MH in both phenotypes (Figure 17). Glucose (15 mM) induced-insulin release was significantly reduced by MH (10^{-3} M) in islets from both fa/fa and lean rats ($p< 0.05$). This study clearly demonstrated that the function of glucokinase is abnormally modulated by the HPA axis in hyperinsulinemic obese rats.

Glucokinase (ATP: D-glucose 6-phosphotranferase, EC 2.7.1.2) is a low affinity glucose phosphorylating enzyme found in liver cells, where it plays a regulatory role in glucose uptake and release, and in pancreatic islet B-cells where it acts as a primary glucose sensor in the regulation of glucose-induced insulin secretion (Meglasson and Matschinsky, 1984; Lenzen and Panten, 1988; Matschinsky, 1990 ; Epstein *et al.*, 1992; Magnuson, 1992). Differences in the regulation of this enzyme in the two tissues have only recently been studied. Molecular genetic studies show that there is only one glucokinase gene in man and rodents but tissue specific promoters result in expression of two closely related active enzyme isoforms (Magnuson and Shelton, 1989; Magnuson, 1990; Magnuson, 1992; Nishi *et al.*, 1992).

While the activities of both liver and pancreatic glucokinase isoforms are similar, gene expression of the isoforms is regulated differently (Bedoya *et al.*, 1986; Iynedjian *et al.*, 1989b).

Glucokinase in the liver is positively influenced by insulin, biotin and triiodothyronine and is inhibited by cAMP or any compound that will raise cellular cAMP (Sibrowski and Sietz, 1984; Hoppner and Seitz, 1989; Iynedjian *et al.*, 1989a; Narkewicz *et al.*, 1990; Chauhan and Dakshinamurti, 1991; Nouspikel and Iynedjian, 1992). On the other hand pancreatic islet B-cell glucokinase expression and activity is positively influenced by glucose and negatively regulated by exercise and starvation (Bedoya *et al.*, 1986; Liang *et al.*, 1990; ; Koranyi *et al.*, 1991; Magnuson, 1992). The onset of detectable glucokinase gene expression in liver and pancreatic islet B-cells also differs in rodents. In the pancreatic islet B-cells, glucokinase mRNA (2.8 kb) has been reported in 2 d old neonatal rodent pancreatic tissues (Tiedge and Lenzen, 1993) while the 4.4 kb mRNA species becomes abundant in the pancreatic tissue in the second wk (10 - 16 d), the same time liver glucokinase mRNA expression is reported (Tiedge and Lenzen, 1993; Jamdar and Greengard, 1970; Walker, 1974; Iynedjian *et al.*, 1987). Liver glucokinase activity can be prematurely induced in young rodents in response to carbohydrate feeding (Walker and Eaton, 1967 ; Girard *et al.*, 1992a). Carbohydrate feeding raises blood glucose which stimulates insulin secretion from the pancreatic B-cells and then insulin regulates glucokinase mRNA expression and glucokinase activity in the liver (Bedoya *et al.*, 1986; Narkewicz *et al.*, 1990). The data obtained in the current study are evidence that glucokinase activity

is regulated by the HPA axis but does not indicate whether glucocorticoids directly modulate glucokinase or exert their effects via their influence on the anterior pituitary or hypothalamic nuclei.

Glucocorticoids are essential in the development of hyperinsulinemia and other metabolic defects of fa/fa rats because ADX reduces or abolishes many of these defects, including hyperinsulinemia, and glucocorticoid replacement reverses ADX effects (Freedman *et al.*, 1985; Fletcher, 1986a; Freedman *et al.*, 1986; Fletcher and McKenzie, 1988). Results of studies investigating direct glucocorticoid action on insulin secretion or biosynthesis and on pancreatic glucokinase activity are not conclusive. However, glucocorticoids may increase pancreatic islet B-cell glucokinase activity indirectly through regulating total body glucose metabolism by increasing glucose production by the liver thruogh gluconeogenesis or by acting through the HPA axis.

Direct actions of the synthetic glucocorticoid, dexamethasone, on rodent B-cells or on insulin secreting cell lines *in vitro* include increased proinsulin mRNA and glucokinase mRNA, but reduced insulin secretion in response to varying stimulants including glucose (Chan and Lejeune, 1992; Fernandez-Meija and Davidson 1992; Philippe *et al.*, 1992). *In vivo*, glucocorticoids cause both enhanced biosynthesis and secretion of insulin in the pancreatic B-cells (Koranyi *et al.*, 1992; Ludvik *et al.*, 1993). Insulin and glucokinase gene expression have been reported to be regulated in a similar manner *in vivo* and *in vitro*. Both proinsulin mRNA and glucokinase mRNA are reduced by exercise (Koranyi *et al.*, 1991), fasting (Burch *et al.*, 1981), and

culturing pancreatic islets in low glucose concentrations (Liang *et al.*, 1990; 1992), but increased by refeeding (Burch *et al.*, 1981) and culturing islets in medium containing high glucose concentrations (Liang *et al.*, 1990; 1992). A study by Fiedorek and Permutt (1989) showed that dexamethasone was not essential for insulin biosynthesis in ADX rats if they received adequate sucrose replacement. This gave evidence that the actions of glucocorticoids on insulin biosynthesis and secretion are partly through regulation of glucose metabolism in normal animals. Effects of glucocorticoid replacement on glucokinase synthesis and activity remains to be investigated in both normal and obese animals. Dexamethasone was shown not to have any effect on liver glucokinase mRNA expression but did enhance insulin induced glucokinase mRNA (Narkewicz *et al.*, 1990).

Developmental studies show that glucokinase mRNA is present in pancreatic tissues of 2 d old pups, and in the liver of 16 d old rats (Tiedge and Lenzen, 1993). Glucocorticoids are detected at very low levels in 6 - 12 d old rats. However, the levels of corticosterone start to rise significantly from d 14 and reach a peak by d 24 (Henning, 1978). It is during this period, in 17 - 21 d old fa/fa rats, that enhanced parasympathetic nervous system stimulation of glucose induced insulin secretion in fa/fa rats was observed (Rohner-Jeanrenaud *et al.*, 1983). Adrenalectomy performed in 18 d old fa/fa rats abolished this parasympathetic nervous system effect in the fa/fa pancreas but it could be restored by corticosterone replacement within 24 h (Fletcher and McKenzie, 1988). Increased parasympathetic nervous system stimulation of glucose-induced insulin release in the fa/fa pancreatic tissue was even observed in 5

d old preobese pups (Atef *et al.*, 1991). Although not supported by experimentation to date, one could speculate that glucokinase activity is increased in fa/fa pups even at this early stage since the 2.8 kb glucokinase mRNA is detectable in pancreatic tissues of 2 d old neonatal rodents (Tiedge and Lenzen, 1993). The increased glucokinase activity is probably masked by the high fat diet during suckling.

Adrenalectomy or infusion of CRF into cerebral ventricles results in body weight loss, reduced food intake, increased sympathetic nervous activity in the periphery and reduced insulin secretion in fa/fa rats and other obese rodents (Brown and Fisher, 1985; Arase *et al.*,1988; Arase *et al.*,1989a; 1989b; Holt and York, 1989; Rohner-Jeanrenaud *et al.*, 1989). These observations have led to the hypothesis that the actions of corticosterone in obesity are due to an inhibitory action on central CRF, especially in the hypothalamic nuclei that are involved in the integration of both endocrine and autonomic nervous system function in relation to energy balance. (Bestetti *et al.*, 1990; Plotsky *et al.*, 1992). It then follows that the biochemical defects that develop in 5 wk old fa/fa rats might be under the control of an already abnormally regulated HPA axis (Fletcher *et al.*, 1986). How increasing hypothalamic CRF levels modulate peripheral events such as insulin secretion is not precisely known. However, ADX appears to increase sympathetic outflow relative to parasympathetic outflow (Holt and York, 1982; Freedman *et al.*, 1985; York *et al.*, 1985; Holt and York, 1989) and it is believed that CRF influences this balance independent of its effect on pituitary ACTH secretion (Holt *et al.*, 1988). Thus it is tantalizing to speculate that a deficient sympathetic nervous system influence on the

developing fa/fa rat pancreas causes reversible changes in glucokinase function. This idea is consistent with the data showing that exercise, which also alters sympathetic outflow, can decrease glucokinase activity (Koranyi *et al.*, 1992).

In this study, ADX did not return the body weight of fa/fa rats to the same level as lean rats. These results agree with those of other investigators showing that adrenalectomy performed at 5 wk or later does not completely normalize body weight (Yukimura and Bray, 1978; Yukimura *et al.*, 1978; Turkenkopf *et al.*, 1991) but reduces the rate of body fat accumulation after 15 d. Adrenalectomy fa/fa rats were 11% lighter than the sham operated fa/fa rats 2 wks post surgery while the weight of ADX and sham lean rats was not different either at 5 wk or 2 wk post surgery (age 7 wk).

Adrenalectomy did not affect pancreatic islet insulin content in either lean or fa/fa rats. These result are in agreement with those of Fiedorek and Permutt (1989) in which ADX did not affect proinsulin mRNA levels in 20% sucrose fed or dexamethasone injected ADX Sprague-Dawley rats, but was reduced in fasted ADX or fed ADX with out sucrose supplement. In our study both ADX and sham rats had unlimited access to saline/glucose (0.9% NaCL and 40 g/l glucose) solution throughout the 15 d post surgery. There was a slight decrease (18%) in pancreatic islet insulin content of the ADX fa/fa rats compared to the sham-operated fa/fa rats which may be due to removal of corticosterone in ADX fa/fa rats. However, the difference was not statistically significant ($p > 0.05$), we therefore reach the same conclusion, that glucocorticoids are not essential for insulin synthesis if the animals

have adequate plasma glucose.

In conclusion, changes in pancreatic glucokinase activity likely contribute to the development of hyperinsulinemia in the fa/fa rats. The development of this lesion is dependent on the presence of an intact HPA axis. Adrenalectomy in this study restored pancreatic islet glucokinase sensitivity to mannoheptulose inhibition in isolated islets of fa/fa rats. However, these results are not conclusive until corticosterone replacement experiments are carried out. Other ADX studies have shown that glucocorticoid replacement restores metabolic and behavioral defects abolished by bilateral adrenalectomy. These include increased parasympathetic activity in fa/fa pancreas (Fletcher and MacKenzie, 1988), decreased sympathetic activity in brown adipose tissues (Arase *et al.*, 1988), increased food intake or normalize feeding behavioral and insulin secretion (Yukimura *et al.*, 1978; King *et al.*, 1983; Castonguay *et al.*, 1986; Bray *et al.*, 1989) and suppression of hypophysial-portal CRF levels (Plotsky *et al.*, 1990). Also further studies are required to define precisely how the HPA axis influences glucokinase function in normal and obese animals.

5. GENERAL DISCUSSION.

The development of genetically transmitted obesity in Zucker fa/fa rats follows a predetermined timetable, with different metabolic, behavioral and hormonal abnormalities appearing at specific periods in the rat's life. Glucose stimulated insulin secretion is increased in fa/fa rats compared to lean rats at 3 wk of age, and after weaning to a high carbohydrate diet other metabolic and behavioral changes are manifested. Glucose induced insulin release by the pancreatic B-cells is regulated by a balance of inputs from the autonomic nervous system and the rate of glucose metabolism in the B-cell. Development of lesions involving the sympathetic nervous system and glycolysis were investigated using isolated pancreatic islet cells, in order to identify the time of onset of the lesions.

The inhibitory action of the sympathetic nervous system on glucose-stimulated insulin secretion is mediated through α_2-adrenoceptors. Using α_2- and α_1-adrenergic agonists and antagonists, a previously reported functional change in α_2-adrenoceptors (Chan and MacPhail, 1992) on the pancreatic islet B-cells was investigated using isolated islets from adult and weanling, lean and fa/fa rats. Functional changes in α_2-adrenoceptors in fa/fa pancreatic islets were not detected under the conditions used in this study. Glucose (15 mM)- induced insulin secretion was similarly inhibited by guanabenz (an α_2-adrenoceptor agonist) in adult and 5 wk lean and fa/fa rats and this was equally antagonized by idazoxan (an α_2-adrenoceptor antagonist) in both phenotypes. An α_1- adrenergic agonist (phenylephrine) and antagonist (prazosin) did not affect glucose induced insulin release in either phenotype. Thus, a prazosin-

sensitive α_2-adrenoceptor was not demonstrated and the earlier report (Chan and MacPhail, 1992) was not confirmed.

The possible role of unbalanced autonomic nervous system activity (reduced sympathetic and increased parasympathetic tone to the periphery, including the pancreas) in the pathogenesis of hyperinsulinemia and the development of obesity in fa/fa rats has been documented (Berthoud and Jeanrenaud, 1979; Rohner-Jeanrenaud *et al.*, 1983; Jeanrenaud, 1985; Greco *et al.*, 1987; Bray *et al.*, 1989). The results of studies using electrical stimulation of hypothalamic nuclei indicate that sympathetic nervous system innervation to the brown adipose tissue (BAT) and the pancreas is intact (Levin *et al.*, 1983a; 1984; Holt *et al.*, 1987). This implies that the defect in the ANS is centrally located. Although α_2- adrenergic agonists used in a previous study (Chan and MacPhail, 1992) and the current study are different, their actions show that the sympathetic agonist regulation of the pancreatic islet B-cell secretory activity is similar in both phenotypes. Whether the α_2-adrenoceptor functional change observed previously was due to the use of different glucose concentrations or different agonists needs to be further investigated.

Regulation of glycolysis in pancreatic islet B-cells was investigated using MH as a probe for glucokinase activity in the pancreatic B-cell. MH competitively inhibits glucokinase, a pancreatic islet B-cell glucose sensor. Isolated islets from adult, weanling and suckling lean and fa/fa rats were used. Glucokinase sensitivity to MH was completely lost in adult, and reduced in 5 wk old fa/fa rat pancreatic islets. The enzyme was responsive to MH inhibition in adult, 5 wk and 3 wk old lean rats and

3 wk old preobese fa/fa rats. The reduction in enzyme activity correlated with increasing weight in the rats. At 3 wk of age there was no difference in weight between fa/fa and lean rats but by 5 wk fa/fa rats were heavier than the lean rats (Zucker and Zucker, 1961; Bray, 1977; Chan *et al.*, 1985; Bray *et al.*, 1989). Other biochemical abnormalities observed in 5 wk old fa/ fa rats include fasting hyperinsulinemia (Bray and York, 1979; Chan *et al.*, 1985), loss of the glucose threshold for GIP stimulatory effects on the pancreas (Chan *et al.*, 1985) and loss of diurnal rhythm for corticosterone secretion (Fletcher *et al.*, 1986). All these abnormalities become more severe with age. Functional changes in glucokinase may contribute to the onset of fasting hyperinsulinemia and inappropriate GIP-stimulated insulin secretion.

In the second part of the study, the role played by the presence of an intact HPA axis on the pancreatic islet glucokinase response to MH inhibition was explored. The data obtained support the hypothesis that the pancreatic islet glucokinase abnormality of fa/fa rats is dependent on the presence of an intact HPA axis. Two wk post surgery, MH inhibited glucose-induced insulin release similarly in ADX fa/fa rats and in ADX and sham lean rats. In contrast, MH did not produce any significant reduction in glucose-induced insulin release from isolated islets of sham fa/fa rats. Together with evidence from other studies, the data obtained in these two experiments lead to a refinement of the initial hypothesis. It was previously proposed that altered HPA axis activity causes functional changes in B-cell glucokinase activity, which results in increased insulin secretion by fa/fa rats. It is

conceivable these defects are masked by the pre-weaning high fat diet, leading to lack of detectable MH insensitivity at 3 wk of age. As with several symptoms of obesity in fa/fa rats, weaning to a high-carbohydrate diet exacerbates the condition, allowing detection in changes of MH insensitivity by 5 wk of age. Furthermore, maturation of the HPA axis appears to occur within a similar timeframe (see below), leading to speculation that onset of pancreatic biochemical changes are dependent on primary defects in the HPA axis of fa/fa rats.

It seems apparent that the period between 21-35 d of age is critical to the development of many symptoms of obesity in fa/fa rats. Evidence in the literature documents the development during this timeframe of elements critical to our hypothesis: islet glucokinase, corticosterone secretion and insulin secretion. With respect to glucokinase, developmental molecular studies have shown that active pancreatic islet glucokinase mRNA (2.8 kb) is detectable in 2 d old pups while the 4.4 kb mRNA is not detected until d 16 (Tiedge and Lenzen, 1993). The possibility exists that the activity of pancreatic islet glucokinase might be increased in pancreatic islets of fa/fa rats at a young age. Parasympathetic nervous system stimulation produced increased insulin secretion in response to glucose in 5 d old (Atef *et al.*, 1991), in 17 d old (Rohner-Jeanrenaud *et al.*, 1983) and in 3 wk old fa/fa but not lean rats (Turkenkopf *et al.*, 1982). Whether there is a difference in the distribution of glucokinase or the amount available between fa/fa rats and the lean rats has still to be determined.

Heterogeneity has been reported among pancreatic B-cells where some of the

cells respond to low glucose concentrations while others need intermediate or high concentrations to induce insulin biosynthesis and secretion (Pipeleers, 1992). If the fa/fa rat B-cell population is composed of more of the low glucose responsive B-cells then a left-shift in the glucose response curve and fasting hyperinsulinemia fa/fa rats is expected. The low glucose sensitive cells are reported to have an increased proportion of pale granules which apparently reflects the active process of hormone synthesis and secretion (Orci, 1985; Kiekens *et al.*, 1992). This type of B-cells has been observed in rabbits treated with cortisone (Bencosme and Martinez-Palormo, 1968), fa/fa rats (Shino *et al.*, 1973), pregnant rats (Aerts and Van Assche, 1975) and in insulinoma cells (Nakangama *et al.*, 1971). Because glucokinase activity is regulated by glucose in a similar manner to insulin, similar heterogeneity and responsiveness might well apply to the glucokinase activity in the fa/fa rat B-cells. Heterogeneity in glucokinase expression among B-cells has been reported in Sprague-Dawley rats (Jetton and Magnuson, 1992). This heterogeneous expression of glucokinase and its dependence on corticosterone would be very interesting to investigate in both Zucker lean and fa/fa rats.

Both protein bound and total corticosterone levels in rodents reach a peak at the end of the third wk (Henning, 1978) which coincides with the onset of glucose induced hyperinsulinemia and worsening of hyperphagia in fa/fa rats (Bray, 1977). The onset of fasting hyperinsulinemia in 5 wk old fa/fa rats (Chan *et al.*, 1985) and changes in glucokinase function observed in this study seem to coincide with the loss of normal diurnal rhythm for corticosterone secretion from the adrenal in fa/fa rats

(Fletcher *et al.*, 1986). Although neither bound nor free corticosterone in weanling or adult fa/fa rats is different from the control (Shargill *et al.*, 1987; Langley and York, 1992), the presence of high morning corticosteroid levels means that fa/fa rat tissues are exposed to high circulating corticosterone longer than lean rats (Cunningham *et al.*, 1986; Fletcher *et al.*, 1986). This may cause altered feedback to hypothalamic nuclei, and not suprisingly, lesions in the HPA axis consistent with a hyperactive axis have been reported in fa/fa rats (Bestetti *et al.*, 1990; Guillaume-Gentil *et al.*, 1990). More recently the presence of increased glucocorticoid binding activity in the hypothalamic nuclei and hippocampus of fa/fa rats was reported (Langley and York, 1992), indicating that glucocorticoids act centrally in modulating obesity in fa/fa rats. That ADX and chronic ICV infusion of CRF produce similar effects in fa/fa rats by increasing sympathetic nervous system activity (Arase *et al.*, 1989a; Rohner-Jeanrenaud *et al.*, 1989) is enough evidence that glucocorticoids act centrally on a site from which CRF regulates ANS outflow to the periphery. This particular central site is probably responsible for energy balance and activity of various endocrine glands, including the endocrine pancreas. In future studies, it would be of interest to study how ANS influence on the B-cells regulates glucokinase function in normal and obese rats.

6. SUMMARY AND CONCLUSIONS.

This study was carried out to determine the time of onset of pancreatic islet lesions that accompany development of hyperinsulinemia and obesity in fa/fa rats, and the lesions' dependence on an intact HPA axis. Functional changes in the α_2-adrenoceptors and abnormal regulation of glycolysis in the pancreatic islet B-cells of fa/fa rats were investigated. If changes in adrenoceptor activity and/or glycolysis regulation were observed in islet B-cells of fa/fa rats before the onset of hyperinsulinemia or obesity, then the pancreas could be considered one of the main sites for "fa" gene action. This means that other factors would then be modifying the activity of an already abnormally regulated pancreatic activity.

Guanabenz, idazoxan, phenylephrine and prazosin were used to test the inhibitory actions of catecholamine on glucose- induced insulin release from isolated pancreatic islets of 5 wk old and adult fa/fa and lean rats. Mannoheptulose was used to investigate glucokinase activity in isolated islets from intact 3 wk old, 5 wk old and intact and ADX adult rats of both phenotypes. Results from the developmental study failed to reveal the presence a functional change in the α_2-adrenoceptors in either 5 wk old or adult rats fa/fa rats. Further studies using a range of glucose concentrations with different α_2-adrenoceptor agonists may be warranted to rule out the possible confounding effects of glucose concentration.

The glucokinase response to MH was reduced in islets from 5 wk old fa/fa rats and completely lost in islets from adult fa/fa rats. Adrenalectomy performed in 5 wk old lean rats had no effect on the regulation of pancreatic islet glucokinase, weight

gain or pancreatic islet insulin content 2 wk post surgery. However, ADX normalized the pancreatic islet glucokinase sensitivity to MH inhibition and reduced the rate of body weight gain in fa/fa rats without affecting pancreatic islet insulin content. Glucocorticoid actions on the activity of pancreatic islet glucokinase appear to be mediated differently from actions on the pancreatic islet insulin content in rats. More studies investigating the direct effects of glucocorticoids on glucokinase expression and activity in Zucker lean and fa/fa pancreatic B-cells are needed.

From the data obtained in this study, it is concluded that changes that occur in pancreatic islet glucokinase activity in fa/fa rats are dependent on the presence of an intact HPA axis. These changes contribute to the development and maintenance of hyperinsulinemia in fa/fa rats.

APPENDIX 1

Calculations for gamma counter

Radioimmunoassays

Insulin and corticosterone were measured using a Packard Riastar gamma counter. The curve set up for insulin consisted of duplicate of total counts, and triplicates of nonspecific binding, zero counts and the standards ranging from 37.5 - 1200 pMol. For corticosterone, all standards were done in duplicate with the standards ranging from 25 to 1000 ng/ml.

1.1 The spline curve fit.

The following curve fit parameters are printed after the fitting algorithm is performed.

Iterations.

The maximum number of iterations is six. If a suitable curve is not produced after six iterations, it is assumed that the data do not describe a typical RIA curve.

Smoothing Factor (Smoothed spline and MASS)

This factor is defined as

$$S_i \leq \left(\frac{g(x_i) - y_i}{\Delta y_i} \right)^2$$

▲ y_i = standard deviation (counting error + pipetting error) of response value yi (response on y-axis)

x_i = concentration value on x-axis.

$g(x_i)$ = cubic spline segment function.

Root Mean Square Residuals (RMS) (smoothed spline and MASS)

Average deviation between the standard points and calculated curve, expressed in CPM. (Residual error fitting process).

$$RMSR = \sqrt{\frac{\sum_{i=O}^{n} (g(x_i - y_i)^2)}{n}}$$

1.2. **Noncurve specific statistics.**

% error of CPM (% ERR)

The statistical error of each standard point and patient sample is reported in the % ERR column of the print out, expressed as percentage of the average number of counts.

$$\% \text{ ERR} = \frac{\text{Sigma (CPM)}}{\text{Avg (CPM)}} \times 100$$

Where sigma (CPM) value used in the equation is the larger of A) or B) below.

A) Sigma (CPM)= Replicates standard deviation of CPM =

$$\sqrt{\frac{\sum^{n} (CPM_i - \overline{CPM})^2}{n}}$$

B) Sigma (CPM) = Statistical Error =

$$\frac{\sqrt{\frac{Avg(CMP)}{time(\text{min})}}}{\sqrt{No\ of\ replicate}}$$

1.3. % NSB /Total

Nonspecific bound concentration (NSB) expressed as percentage of total concentration (T).

$$\frac{\%\ NSB}{T} = \frac{NSB}{T} \times 100 \quad \text{(for a bound assay)}$$

$$= \frac{T - NSF}{T} \times 100 \quad \text{(for a free assay)}$$

Where NSF = nonspecific free.

APPENDIX 2.

2.1. Materials and sources

Materials	Sources
Animals	Charles River Laboratories, St Constant, Quebec
Acetic acid	Baxter
Albumin fraction 5	Boehringer
Antibiotic/antimycotic solution	Sigma
Bouin's fluid	BDH
Calf serum	Gibco
Carbon decolorizing neutral	Fisher
Collagenase Type XI	Sigma
Corticosterone assay kit	Immunocorp
4.0 Dexon	Cyanamid Canada Inc
4.0 Dermalon	Cyanamid Canada Inc
Dextran Industrial grade	Sigma
Dextran Clinical grade	Sigma
Dextran T70	Pharmacia
Diazepam	Sabex Inc, Quebec.
Dimethyl sulfoxide	Sigma
Dulbecco's modified Eagle's medium	Gibco
Ethanol 95%	Consolidated Alcohol Ltd
Gelatin	Difco
Glucose	Sigma
L-Glutamine	Gibco
Guanabenz	Sigma
Hank's Balanced salt solution	Gibco
HEPES	Sigma
Idazoxan	Sigma
Iodine ^{125}	Amersham (Canada)
Insulin (rat, standard)	Novo Biolabs
Insulin antibody	Dr. R.A Pederson, Vancouver, Canada
Insulin (porcine, tracer)	Novo Biolabs
Mannoheptulose	Sigma
Nylon mesh screen nitex (800 μm)	B&SH Thompson Bros
Prazosin	Sigma
(Somnotol) Sodium Pentobarbital	MTC Pharmaceuticals
Perchloric acid	Mallinckrodt
Phenylephrine	Sigma

Polyethylene tubing (50 and 10)	Fisher
Sodium chloride	Fisher
Sodium bicarbonate	Fisher
Sodium iodide	Fisher
Sodium phosphate diphasic	Fisher

2.2. Adresses of material sources.

Amersham Canada Ltd, 1166 South Service Road West, Oakville, Ont, L6L 5T7 Canada.

Baxter Diagnostic Corporation, 2390 Argentia Road, Mississauga, Ont, L5N 3P1 Canada.

Beckman Instruments Inc, Diagnostic System Group, 200 South Kraemer Blvd, Brea, CA 92621 USA.

BDH, 10 Morris Drive, Ste 20, Dartmouth NS B3B 1K8 Canada.

Boehringer Mannheim, 11450 Cote De Liesse, Dorval, Quebec, H9P 1A9 Canada.

B& SH Thompson Bros, 8148 Devonshire Road, Town of Mt Royal PQ, H9P 1A1 Canada.

Charles River Laboratory, Canada, P.O. Box 300, St Constant, Quebec J5A 2G3 Canada.

Consolidated Alcohols Ltd, P.O. Box 372 Station A Toronto, Ont, M5W 1C8 Canada.

Cyanamid Canada Inc, P.O. Box 4000, IND, PK, Markham, Ont, L3R 8G7 Canada.

Difco Laboratories, P.O. Box 1058, Detroit, MI, USA.

Dr. R.A. Pederson, Dept of Physiology, University of British Colombia, Vancouver, V6T 1W5 Canada.

Fisher Scientific, 8505 Devonshire Rd, Montreal, Quebec, H4P 2L4 Canada.

Gibco/BRL Canada Inc, 2270 A Industrial St, Burlington Ont, L7P 1 A1 Canada.

Immunocorp 5800 Royalmount Ave, Montreal, Quebec, H4P 1K5 Canada.

Mallinckrodt Inc., 600 Delmar Ave, Pointe Claire, Quebec, H9R 4A8 Canada.

MTC, 5100 Timberlea Blvd, Mississauga, Ont, L4W 2S4 Canada

Novo Biolabs, 1755 Steeles Ave West, Willowdale, Ont, M2R 3T4 Canada

Pharmacia Canada Ltd., 2044 St. Regis Blvd, Dorval, Quebec, H9P 1H6 Canada.

Sabex Inc, Boucherville, Quebec, J4B, 7K8 Canada.

Sigma Chemical Co, P.O. Box 18817B, St. Louis, Missouri 63160 USA.

APPENDIX 3. STATISTICAL ANALYSIS.

Anova, general linear model for unbalanced data was used to analyze data in all groups treatments. Two sets of experiments were done, one to study the development of defects in regulation of insulin secretion and one to investigate the effects of adrenalectomy on glucose induced insulin release in response to mannoheptulose. Effects of treatments, age, phenotype or adrenalectomy/sham-operation were tested using two way analysis of variance (GLM). One example of the statistical process is given below:

To test for the effect of agonists, 4 doses of guanabenz were used (10^{-9} M, 10^{-8} M, 10^{-7} M and 10^{-6} M) in the presence of 15 mM glucose thus giving 5 different treatment groups and 4 different animal groups (adult lean, adult fa/fa rats; 5 wk lean and 5 wk fa/fa rats).

The equation for testing for interaction between treatment, age and phenotype is

$Y = A + B + C$

Where Y = data,

A = treatments

which were coded 1- 5, 1 = glucose response

2 to 5 = response in presence of guanabenz doses (10^{-9} to 10^{-6})

B = age coded as 1 = adults and 2 = 5 wk old rats.

C = phenotype coded as 1 = lean and 2 = obese.

Total data points in all groups of animals and treatments make up the degrees of freedom.

Treatment 1 (glucose) had n = 38: 8 lean adults, 9 fa/fa adult rats, 9 lean 5 wk and 12 fa/fa 5 wk rats.

Treatment 2 (guanabenz 10^{-9} M) had n = 35: 6 lean adult, 9 fa/fa adult rats, 9 lean 5 wk and 11 fa/fa 5 wk rats.

Treatment 3 (guanabenz 10^{-8} M) had n = 37: 8 for both lean and fa/fa adult rats and 9 for 5 wk lean and 12 fa/fa rats.

Treatment 4 (guanabenz 10^{-7} M) had n = 35: 7 lean, 9 fa/fa adult rats, 9 lean and 10 fa/fa 5 wk rats.

Treatment 5 (guanabenz 10^{-6} M) had n = 36: 7 lean, 8 fa/fa adult rats, 9 lean and 12 fa/fa 5 wk rats.

Total n = 38 + 35 + 37 + 35 + 34 = 180.

Error = 180 - 20 = 160 (5 treatments and 4 groups of animals 4 x 5 = 20)

For treatments, degrees of freedom is 5 - 1 = 4

The degrees of freedom for treatment response = 4,160.

For the second study the model included adrenalactomy/sham-operated variables.

The equation was Y = A + B + C + D (Y, A, B and C are as above while D is for adrenalectomized (coded 1) or sham-operation (coded 2)

APPENDIX 4. Islet distribution according to diameter size (μm) for individual 3 wk old rats

Rat ID	0 - 50	51 - 100	101 - 150	151-200	≥ 201	PHENO
18/1/93A	6	9	1	-	-	FA/?
18/1/93B	10	4	-	-	-	FA/?
19/1/93A	6	2	2	-	-	FA/?
19/1/93B	6	2	2	-	-	FA/?
01/2/93A	6	7	1	-	-	FA/?
01/2/92B	5	3	1	-	-	FA/?
01/2/93C	3	1	-	-	-	?
02/2/93A	8	1	5	-	-	?
02/2/93B	11	3	-	-	-	FA/?
02/2/93C	12	9	-	-	-	FA/?
08/2/93A	1	6	-	-	-	?
08/2/93B	1	7	1	1	-	fa/fa
08/2/93C	16	4	-	-	-	FA/?
08/2/93D	10	7	3	-	-	FA/?
08/2/93E	12	4	-	-	-	FA/?
22/3/93A	9	5	-	-	-	FA/?
22/3/93B	6	8	3	-	1	fa/fa
05/4/93A	10	10	-	-	-	FA/?
05/4/93B	7	7	-	3	1	fa/fa
05/4/93C	12	2	-	-	-	FA/?
05/4/93D	4	11	6	1	1	fa/fa
06/4/93A	15	11	-	1	-	fa/fa
06/4/93B	17	19	-	2	-	fa/fa
06/4/93C	6	12	2	2	-	fa/fa
07/4/93A	15	16	4	2	-	fa/fa
07/4/93B	13	12	6	2	1	fa/fa
07/4/93C	9	8	1	-	-	FA/?

PHENO = phenotype

Rat ID = rat identification .

7. REFERENCES

AERTS L, VAN ASSCHE FA. Ultrastructural changes in the endocrine pancreas in pregnant rat. Diabetologia 1975; 11: 285-289.

ARASE K, SHARGILL NS, BRAY GA. Effects of intraventricular infusion of corticotropin-releasing factor on VHM lesioned obese rats. Am J Physiol 1989b; 256: R751-R756.

ARASE K, SHARGILL NS, BRAY GA. Effects of corticotropin releasing factor on genetically obese (fatty) rats. Physiol Behav 1989a; 45(3): 565-570.

ARASE K, YORK DA, SHIMAZU H, SHARGILL NS, BRAY GA. Effects of corticotropin releasing factor on food intake and brown adipose tissue thermogenesis in rats. Am J Physiol 1988; 255: E255-E259.

ARGILÉS MJ. The obese Zucker rat: A choice for fat metabolism 1968-1988. Twenty years of research on the insights of Zucker mutation. Prog Lipid Res 1989; 28: 53-66.

ASCROFT SJH, BASSETT JM, RANDLE PJ. Glucose metabolism in mouse pancreatic islets. Biochem J 1970; 118: 143-154.

ATEF N, BRULÉ C, BIHOREAU M-T, KTORZA A, PICON L, PÉNICAUD L. Enhanced insulin secretory response to acetylcholine by perifused pancreas of 5-day old preobese Zucker rats. Endocrinology 1991; 129: 2219-2223.

BABINSKI MJ. Tumeur du corps pituitaire sans acromegalie et avec de developement des organes genitaux. Rev Neurol 1900; 8: 531-533.

BAILEY CJ, FLATT PR. The enteroinsular axis in animals with hyperinsulinemic and hypoinsulinemic diabetes. In: Frontier in diabetes Research: Lessons from Animals II, Shafrir E, Renold AE, eds. John Libby, London 1988; p 217-224.

BARAKAT HA, McLENDON VD, MARKS R, PORIES W, HEATH J, CARPENTER JW. Influence of morbid obesity and non-insulin-dependent diabetes mellitus on high-density lipoprotein composition and subpopulation distribution. Metabolism 1992; 41: 37-41.

BAZIN R, ETÉVE D, LAVAU M. Evidence for decreased GDP binding to brown-adipose-tissue mitochondria of obese Zucker (fa/fa) rats in the very first days of life. Biochem J 1984; 221: 241-245.

BEARD CM, ORENCIA A, KNOTTE T, BALLARD DJ. Body mass index and initial manifestation of coronary heart disease in women aged 40-59 years. Int J Epidemiol 1992; 21: 656-664.

BECK B, STRICKER-KRONGRAD A, NICOLAS J-P, BURLET C. Chronic and continuous intracerebroventricular infusion of neuropeptide Y in Long-Evans rats mimics the feeding behaviour of obese Zucker rats. Int J Obes 1992; 16: 295-302.

BEDOYA FJ, MATSCHINSKY FM, SHIMIZU T, O'NEIL JJ, APPEL MC. Differential regulation of glucokinase activity in pancreatic islets and liver of the rat. J Biol Chem 1986; 23: 10760-10764.

BELL G, STERN JS. Evaluation of body composition of young obese and lean Zucker rats. Growth 1977; 41: 63-80.

BELOFF-CHAIN A. Hyperinsulinemia in obesity. In: Recent Advances in Obesity Research V: Berry EM, Blondheim SH, Eliahou HE, Shafrir E, eds; John Libbey Ltd, London, England. 1987; 240-245.

BENCOSME SA, MARTINEZ-PALORMO A. Formation of secretory granules in pancreatic islet B cells of cortisone-treated rabbits. Lab Invest 1968; 18: 746-756.

BENCOSME SA, TSUTSUMI V, MARTIN JM, AKERBLOM HK. Ultrastructural changes in B cells of pancreatic islets from rats with elevated levels of circulating growth hormone secreted by Mt-WI5 turnor. Diabetologia 1971; 20: 15-20.

BERTHOUD H-R, JEANRENAUD B. Acute hyperinsulinemia and its reversal by vagotomy after lesions of the ventromedial hypothalamus in anesthetized rats. Endocrinology (Baltimore) 1979; 105: 146-151.

BESTETTI GE, ABRAMO F, GUILLAUME-GENTIL C, ROHNER-JEANRENAUD F, JEANRENAUD B, ROSSI GL. Changes in the hypothalamo-pituitary-adrenal axis of genetically obese fa/fa rats: A structural, immunocytochemial, and morphometrical study. Endocrinology 1990; 126: 1880-1887.

BLACK M, HEICK HM, BÉGIN-HEICK N. Abnormal regulation of cAMP accumulation in pancreatic islets of obese mice. Am J Physiol 1988; E833-E838.

BLAIR SC, CATERSON ID, COONEY GJ. Insulin response to an

intravenous glucose load during development of obesity in gold thioglucose-injected mice. Diabetes 1993; 42: 1153-1158.

BLUNDELL JE, BURLEY VJ. Evaluation of the satiating power of dietary fat in man. In: Progress in Obesity Research Oomura Y *et al.* eds, John Libby 1990: p 453-457.

BONORA E, BONADONNA RC, PRATO SD, GULLI G, SOLINI A, MATSUDA M, DEFRONZO RA. In vivo glucose metabolism in obese and type II diabetic subjects with or without hypertension. Diabetes 1993; 42: 764-772.

BOUCHARD C. Current understanding of etiology of obesity: Genetic and nongenetic factors. Am J Clin Nutr 1991; 53: 1561S-1565S.

BOUCHARD C, PÉRUSSE L, LEBLANC C, TREMBLAY A, THÉRIAULT G. Inheritance of the amount of human body fat. Int J Obes 1988; 12: 205-215.

BOUCHARD C, TREMBLAY A, DESPRES JP, NADEAU A, LUPIEN PJ, THÉRIAULT G, DUSSAULT J, MOORJANI S, PINAULT S, FOURNIER G. The response to long-term overfeeding in identical twins. N Engl J Med 1990; 322: 1477-1482.

BRAY GA. Hypothalamic and genetic obesity: An appraisal of the autonomic hypothesis and endocrine hypothesis. Int J Obes 1984; 8: 119-137

BRAY GA. The Zucker-fatty rat: a review. Fed Proc 1977; 36: 148-153.

BRAY GA, YORK DA. Hypothalamic and genetic obesity in experimental animals: An autonomic and endocrine hypothesis. Physiol Rev 1979; 59: 719-809

BRAY GA, YORK DA. Genetically transmitted obesity in rodents. Physiol Rev 1971; 51: 598-646.

BRAY GA, YORK DA, FISLER JS. Experimental obesity: A homeostatic failure due to defective nutrient stimulation of sympathetic nervous system. Vit and Horm 1989; 45: Aurbach GB, McCormic DB eds, Academic Press San Diego 1-125.

BRAY GA, YORK DA, SWERDLOFF RS. Genetic obesity in rats. 1. The effect of food restriction on body composition and hypothalamic function. Metabolism 1973; 22: 435-442.

BROOK CGD, HUNTLEY RMS, SLARK J. Influence of heredity and environment in determination of skinfold thickness in children. Br Med J 1975; 21: 716-721.

BROWN MR, FISHER LA. Corticotropin-releasing factor: effects on autonomic nervous system and visceral systems. Fed Proc 1985; 44: 249-249.

BROWN MR, FISHER LA, SPIESS J, RIVIER J, RIVIER C, VALE W. Corticotropin releasing factor: Actions on the sympathetic nervous system and metabolism. Endocrinology (Baltimore) 1982; 11: 928-931.

BRUCE BK, KING BM, PHELPS GR, VIETIA MC. Effects of adrenalectomy and corticosterone administration on hypothalamic obesity in rats. Am J Physiol 1982; 243: E152-E157.

BRUCKDORFER KR, KANG SS, KHAN BOURNE AR, YUDKIN J. Diurnal changes in the concentration of plasma lipids, sugars, insulin and corticosterone in rats fed diets containing various carbohydrates. Horm Metabol Res 1974; 6: 99-106.

BRUNZELL JD. Obesity and risk for cardiovascular disease. In: Contemporary isues in Clinical Nutrition 4: Obesity. Greenwood MRC, ed, Churchhill Livingstone, New York, 1983; 3-16.

BURCH PT, TRUS MD, BERNER DK, LEONTIRE A, ZAWALICH KC, MATSCHINSKY FM. Adaptation of glycolytic enzymes: Glucose use and insulin release in rat pancreatic islets during fasting and refeeding. Diabetes 1981; 30: 923-928.

CASTONGUAY TW, DALLMAN MF, STERN J. Some metabolic and behaviour effects of adrenalectomy on obese Zucker rats. Am J Physiol 1986; 251: R923-R933.

CAWTHORN EG, CHAN CB. Effect of pertussis toxin on islet insulin secretion in obese (fa/fa) Zucker rats. Mol Cell Endocrinol 1991; 75: 197-204.

CHAN CB. Glucokinase activity in isolated islets from obese fa/fa Zucker rats. Biochem J 1993; 295: 673-677.

CHAN CB. On the of the enteroinsular axis in obesity. PhD thesis 1985; University of British Columbia.

CHAN CB, BUCHAN AMJ, GREEN KA, PEDERSON RA. The effect of jejunoileal bypass (JIB) in the obese Zucker on a sub-group of

enteroendocrine cells. Int J Obes 1987; 11: 285-293.

CHAN CB, LEJEUNE J. Reduced sensitivity to dexamethasone of pancreatic islets from obese (fa/fa) rats. Can J Physiol Pharmacol 1992; 70: 1518-1522.

CHAN CB, MACPHAIL RM. Functional characterization of α-adrenoceptors on pancreatic islets of fa/fa Zucker rats. Mol Cell Endocrinol 1992; 84: 33-37

CHAN CB, MACPHAIL RM, MITTON K. Evidence for defective glucose sensing by islets of fa/fa obese Zucker rats. Can J Physiol Pharmacol 1993; 71: 34-39.

CHAN CB, PEDERSON RA, BUCHAN AMJ, TUBESING KB, BROWN JC. Gastric inhibitory polypeptide and hyperinsulinemia in Zucker (fa/fa) rat: A developmental study. Int J Obes 1985; 9: 137-146.

CHAN CB, PEDERSON RA, BUCHAN AMJ, TUBESING KB, BROWN JC. Gastric inhibitory polypeptide (GIP) and insulin release in the obese Zucker rat. Diabetes 1984; 33: 536-542.

CHAUHAN J, DAKSHINAMURTI K. Transcriptional regulation of the glucokinase gene by biotin in starved rats. J Biol Chem 1991; 266: 10035-10038.

CHONG PKK, JUNG RT, BARLTETT WA, BROWNING MCK. The acute effects of corticotropin-releasing factor on energy expenditure in lean and obese women. Int J Obes 1992; 16: 529-634.

CHUI KC, PROVINCE MA, PERMUTT MA. Glucokinase gene is a genetic marker for NIDDM in American Blacks. Diabetes 1992a; 41: 843-849.

CHUI KC, PROVINCE MA, DOWSE GK, ZIMMET PZ, WAGNER G, SERJEANTSON S, PERMUTT MA. A genetic marker at the glucokinase gene locus for type 2 (non-insulin-dependent) diabetes mellitus in Mauritian Creoles. Diabetologia 1992b; 35: 632-638.

CLEARY MP, VASSELLI JR, GREENWOOD MRC. Development of obesity in Zucker obese (fafa) rats in absence of hyperphagia. Am J Physiol 1980; 238: E284-E292.

COLEMAN DL, HUMMEL KP. Studies with the mutation, diabetes, in the mouse. Diabetologia 1967; 3: 238-248.

COPPACK SW, EVANS RD, FISHER RM, FRAYN KN, GIBBONS GF,

HUMPHREYS SM, KIRK ML, POTTS JL, HOCKADAY TDR. Adipose tissue metabolism: Lipase action in vivo before and after a mixed meal. Metabolism 1992; 41: 264-272.

CRANE SW. Occurrence and management of obesity in companion animals. J Small Anim Pract 1991; 32: 275-282.

CUNNINGHAM JJ, CALLES-ESCANDON J, GARRIDO F, CARR DB, BODE HH. Hypercorticosteronuria and diminished pituitary responsiveness to corticotropin releasing factor in obese Zucker rats. Horm Metab Res 1986; 118: 98-101.

CUSIN I, TERRETTAZ J, ROHNER-JEANRENAUD F, JEANRENAUD B. Metabolic consequences of hyperinsulinemia imposed on normal rats on glucose handling by white adipose tissue, muscles and liver. Biochem J 1990; 267:99-103.

CZECH MP, RICHARDSON DK, BECKER SG, WALTERS CG, GITOMER W, HEINRICH J. Insulin response in skeletal muscle and fat cells of the genetically obese Zucker rat. Metabolism 1978; 27: 1967-1981.

DARNELL J, LODISH H, BALTIMORE D. Molecular cell biology 2nd ed, Scientific American Books, New York 1991; Chapter 19

DEBANT A, GUERRE-MILLO M, LE MARCHAND-BRUSTEL Y, FREYCHET P LAVAU M, VAN OBBERAGHEN E. Insulin receptor kinase is hyperresponsive in adipocytes of young obese Zucker rats. Am J Physiol 1987; 252: E273-E278.

DEBONS AF, KRIMSKY I, MAAYAN ML, FANI K, JIMENEZ A. Gold thioglucose obesity syndrome. Fed Proc 1977; 36: 143-147

DEFRONZO RA. Pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus : A balanced overview. Diabetologia 1992; 35: 389-397.

DEL PRATO S, CASTELLINO P, SIMINSON DC, DEFRONZO RA. Hyperinsulinemia and insulin mediated glucose metabolism. J Clin Invest 1987; 79: 547-556.

DOCHERTY JR. The pharmacology of α_1- and α_2-adrenoceptors: Evidence for and against a further subdivision. Pharmac Ther 1989; 44: 241-284.

DOLAN PL, TAPSCOTT EB, DORTON PJ,DOHM L. Contractile activity restores insulin responsiveness in skeletal muscles of obese Zucker rats.

Biochem J 1993; 298: 423-426.

DUNBAR JC, ERGENE E, BARRACO RA. Neuropeptide-Y stimulation of insulin secretion is mediated via Nucleus Tractus Solitarius. Horm Metab Res 1992; 24: 103-105.

DUNN LJ, BRADBURY LJ. Endocrine factors in endometrial carcinoma. Am J Obstet Gynecol 1967; 97: 465-471.

DWYER JT, FELDMAN JJ. The social psychology of dieting. In: Weight and Obesity: Causes, Fallacies, Treatment. Hafen BQ, ed. Brigham Young University Press, Provo, Utah 1975; 112-130.

ECK LH, KLESGES RC, HANSON CL, SLAWSON D. Children at familial risk for obesity: an examination of diet intake, physical activity and weight status. Int J Obes 1992; 16: 71-78.

EFENDIC S, KINDMARK H, GERGGREN P-O. Mechanisms involved in the regulation of insulin secretory process. J Int Med 1991; 229: 9-22.

EPSTEIN PN, BOSCHERO AC, ATWATER I, CAI X, OVERBEEK PA. Expression of yeast hexokinase in pancreatic B cells of transgenic mice reduces blood glucose, enhances insulin secretion, and decreases diabetes. Proc Natl Sci USA 1992; 89: 12038-12042.

FELBER J-P. From obesity to diabetes. Pathophysiological considerations. Int J Obes 1992; 16: 937-952.

FERNANDEZ-MEJIA C, DAVIDSON MB. Regulation of glucokinase and proinsulin gene expression and insulin secretion in RIN-m5F cells by dexamethasone, retinoic acid and thyroid hormone. Endocrinology 1992; 130: 1660-1668.

FERRANNINI E, HAFFNER SM, MITCHELL BD, STERN MP. Hyperinsulinemia: The key feature of a cardiovascular and metabolic syndrome. Diabetologia 1991; 34: 416-422.

FIEDORECK FT, PERMUTT MA. Proinsulin mRNA levels in fasting and fed ADX rats: evidence for indirect effect of glucocorticoids. Am J Physiol 1989; 256: E303-E308

FINKELSTEIN JA, STEGGLES A-W, MARTINEZ P, PRUISSMAN M. Cholecystokinin receptor binding levels in the genetically obese rat brain. Peptides 1984; 5: 11-14.

FLATT PR, BAILEY CJ. Dietary components and plasma insulin response to fasting and refeeding in genetically obese hyperglycaemic mice. Br J Nutr 1984; 51: 403-413.

FLATT PR, BAILEY CJ. Role of dietary factors in the hyperinsulinemia of genetically obese hyperglycaemic (ob/ob) mice. J Nutr 1982; 112:2212-2216.

FLATT PR, BAILEY CJ. Importance of the enteroinsular axis for the insulin secretory response to glucose in obese hyperglyceamic (ob/ob) mice. Biochem Soc Trans 1981; 9: 221-222.

FLATT PR, BAILEY CJ, KWASOWSKI P, PAGE T, MARKS V. Plasma immunoreactive gastric inhibitory polypeptide in obese hyperglycaemic (ob/ob) mice. J Endocrinol 1984; 101: 249-256.

FLATT PR, BAILEY CJ, KWASOWSKI P, SWANSON-FLATT SK, MARKS V. Abnormalities of GIP in spontaneous syndrome of obesity and diabetes in mice. Diabetes 1983; 32: 433-435.

FLETCHER JM. Effects of adrenalectomy before weaning in the genetically obese Zucker rat (fatty). Br J Nutr 1986a; 56: 141-151.

FLETCHER JM. Effects of adrenalectomy before weaning and short-or long-term glucocorticoid administration on the genetically obese Zucker rat. Biochem J 1986b; 238: 459-463.

FLETCHER JM, HAGGARTY P, WAHLE KWJ, REEDS PJ. Hormonal studies of young lean and obese Zucker rats. Horm Metabol Res 1986; 18: 290-295.

FLETCHER JM, McKENZIE N. The parasympathetic system and glucocorticoid-mediated hyperinsulinemia in genetically obese fa/fa Zucker rat. Endocrinology 1988; 118: 87-92.

FOLSOM AR, BURKE GL, BYERS L, HUTCHINSON RG, HEISS G, FLACK JM. JACOBS JR DR, CAAN B. Implications of obesity for cardiovascular diseases in blacks: the CARDIA and ARIC studies. Am J Clin Nutr 1991; 53: 1604S-1611S.

FOLSOM AR, KAYE SA, POTTER JD, PRINEAS RJ. Association of incident carcinoma of endometrium with body weight and fat distribution in older women: Early findings of the Iowa women's health study. Cancer Res 1989; 49: 6828-6831.

FOURNIER LA, HEICK HM, BEGIN-HEICK N. The influence of K^+-induced membrane depolarization on insulin secretion in islets of lean and obese (ob/ob) mice. Biochem Cell Biol 1990; 68: 242-248.

FREEDMAN MR, CASTONGUAY TW, STERN JS. Effect of adrenalectomy and corticosterone replacement on meal patterns of Zucker rats. Am J Physiol 1985; 249: R584-R594.

FREEDMAN MR, HORWITZ BA, STERN JS. Effect of adrenalectomy and glucocorticoid replacement on development of obesity. Am J Physiol 1986; 250: R595-R607.

FRIEDMAN JE, DE VENTÉ JE, PETERSON RG, DOHM GL. Altered expression of muscle glucose transporter GLUT-4 in diabetic fatty Zucker rats (ZDF/Drt-fa). Am J Physiol 1991; 261: E782-E788.

FRIEDMAN JE, SHERMAN WM, REED MJ, ELTON CW, DOHM GL. Exercise training increases glucose transporter proteins GLUT4 in skeletal muscles of obese Zucker (fa/fa) rats. FEBS Lett 1990; 268: 13-16.

FRIEDMAN MI. Body fat and metabolic control of food intake. Int J Obes 1990; 14 (suppl 3): 53-57.

FROGUEL PH, VAXILLAIRE M, SUN F, VELHO G, ZOUSLI H, BUTEL MO, LESAGE S, VOINNET N, LATHROP GM, PASSA Ph, TANIZAWA Y, PERMUTT MA. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin dependent mellitus. Nature 1992; 356: 162-164.

FROHLICH A. Ein fall von tumor der hypophysis cerebri ohne akromegalie. Wien.Klin Rundsch 1901; 15: 883-886.

FÜRNSINN C, KOMJATI M, MADSEN OD, SCHNEIDER B, WALDHÄUSL W. Lifelong sequential changes in glucose tolerance and insulin secretion in genetically obese Zucker rats (fa/fa) fed a diabetogenic diet. Endocrinol 1991; 128: 1093-1099.

GARVEY WT, HUECKSTEADT TP, BIRNBAUM MJ. Pretranslational suppression of an insulin-responsive glucose transporter in rats with diabetes. Science 1989; 245: 60-65.

GERMAN MS. Glucose sensing in pancreatic islet beta cell: The key role of glucokinase and glycolytic intermediates. Proc Natl Acad Sci USA 1993; 90: 1781-1785.

GIBSON MJ, LIOTTA AS, KRIGER DT. The Zucker fa/fa rat: Absent circadian corticosterone periodicity and elevated B-endorphin concentrations in the brain and neurointermediate pituitary. Neuropeptides 1981;1: 349-362.

GIDH-JAIN M, TAKEDA J, XU LZ, LANGE AJ, VIONNET N, STOFFEL M, FROGUEL P, VELHO G, COHEN D, PATEL P, LO Y-MD, HATTERSLEY AT, LUTHMAN H, WEDELL A, CHARLES R ST, HARRISON RW, WEBER IT, BELL GI, PILKIS SJ. Glucokinase mutations associated with non-insulin-dependent (type 2) diabetes mellitus have decreased enzymatic activity: Implications for structure/function relationships. Proc Natl Acad Sci USA 1993; 90: 1932-1936.

GIRARD J, DECAUX JF, BOSSARD P. Regulation of the initial expression of hepatic phosphoenolpyruvate carboxylase and glucokinase genes during development. Diabete & Metabolisme (Paris) 1992a; 18: 74-80.

GIRARD J, FERRÉ P, PÉGORIER JEAN-PAUL, DUÉE PIERRE-HENRI. Adaptations of glucose and fatty acid metabolism during perinatal and suckling transition. Physiol Rev 1992b; 72: 507-562.

GLANTZ SL. Primer of biostatistics. 2nd ed, McGraw-Hill Book company, 1987.

GLOWA JR, BARRET JE, RUSSELL J, GOLD PW. Effect of corticotropin releasing hormone on appetite behaviors. Peptides 1992; 13: 609-621.

GODBOLE V, YORK DA, BLOXHAM DP. Developmental changes in fatty (fa/fa) rat, evidence for defective thermogenesis preceding hyperlipogenesis and hyperinsulinemia. Diabetologia 1978; 14: 41-44.

GRECO R, ZANINETTI D, ASSIMACOPOULOS-JEANNET F, JEANRENAUD B. Stimulatory effect of cold adaptation on glucose utilization by brown adipose tissue: Relationship with changes of the glucose transporter system. J Biol Chem 1987; 262: 7732-7736.

GREENWOOD MRC, TURKENKOPF IJ. Genetic and metabolic aspects. In: Contemporary issues in Clinical Nutrition 4: Obesity. Greenwood MRC Ed, Churchill Livingstone, New York, 1983; 193-208.

GUILLAUME-GENTIL C, ROHNER-JEANRENAUD F, ABRAMO F, BESTETTI GE, ROSSI GL, JEANRENAUD B. Abnormal regulation of hypothalamo-pituitary-adrenal axis in genetically obese (fa/fa) rat. Endocrinology 1990; 126: 1873-1879.

HAINAULT M, GUERRE-MILLO M, GUICHARD C, LAVAU M. Differential regulation of adipose tissue glucose transporters in genetic obesity (fatty rat). J Clin Invest 1991; 87: 1127-1131.

HAMILTON CL, BROBECK JR. Hypothalamic hyperphagia in monkeys. J Comp Physiol Psychol 1964; 57: 271- 278.

HAMPTON SM, MORGAN LM, TREDGER JA, CRAMB R, MARKS V. Insulin and C-peptide levels after oral and intranevous glucose. Contribution of enteroinsular axis to insulin secretion. Diabetes 1986; 35: 612-616.

HANDBERG A, VAAG A, VINTEN J, BECK-NIELSEN H. Decreased tyrosine kinase activity in partially purified insulin receptors from muscles of young, non-obese first degree relatives with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1993; 36: 668-674.

HATTERSLEY AT, TURNER RC, PERMUTT MA, PATEL P, TANIZAWA Y, CHIU KC, O'RAHILLY S, WATKINS PJ, WAINSCOAT JS. Linkage of type 2 diabetes to glucokinase gene. Lancet 1992; 339: 1307-1310.

HAYS SE, GOODMAN FK, PAUL SM. Cholecystokinin receptors in brain: Effects of obesity, drug treatment and lesions. Peptides 1981; 2: 1-26.

HENNING SJ. Plasma concentrations of total and free corticosterone during development in the rat. Am J Physiol 1978; 235: E451-E456.

HOEBEL BG, HERNANDEZ L. Basic neural mechanism of feeding and weight regulation. In: Obesity: Theory and Therapy, 2nd edition. Stunkard AJ, Wadden TA., eds, Raven Press, Ltd., New York, 1993; 43-62.

HOFFMANS MDAF, KROMHOUT D. Changes in body mass index in relation to myocardial infarcation (the Zutphen study). Int J Obes 1989; 13 Suppl 1: A25.

HOLMÄNG A, BRZEZINSKA Z, BJÖRNTORP P. Effect of hyperinsulinemia on muscle fiber composition and capillarization in rats. Diabetes 1993; 42: 1073-1081.

HOLT SJ, ROTHWELL NJ, STOCK MJ, YORK DA. Changes in energy balance, thermogenesis and brown adipose tissue activity following hypophysectomy in obese Zucker rats. Am J Physiol 1988; 254: E162-E166.

HOLT SJ, YORK DA. The effects of adrenalectomy, corticotropin releasing

factor and vasopressin on the sympathetic firing rate of nerves to interscapular brown adipose tissue in Zucker rat. Physiol Behav 1989; 45: 1123-1129.

HOLT SJ, WHEAL HJ, YORK DA. Hypothalamic control of brown adipose tissue in Zucker lean and obese rats. Effect of electrical stimulation of the ventromedial nucleus and other hypothalamic centres. Brain Res 1987; 405: 227-233.

HOLT SJ, YORK DA. The effect of adrenalectomy on GDP binding to brown adipose tissue mitochondria of obese rats. Biochem J 1982; 208: 819-822.

HOPPNER W, SEITZ H-J. Effect of thyroid hormones on glucokinase gene transcription in the rat liver. J Biol Chem 1989; 264: 20643-20647.

HORTON ED, KING PA, HIRSHMAN MF, HORTON ES. Failure of insulin to stimulate glucose transporter translocation in skeletal muscles from obese (fa/fa) Zucker rat. Diabetes 1990; 39: 83A.

HOSHI M, SHREEVE WW. Chronic effects of mannoheptulose in hyperglycemic-obese mice. Metabolism 1969; 18: 422-426.

ILYÉS I, YAHATA T, STOCK M. Brown tissue cell respiration in hypo-and hyperthyroidism after stimulation with selective and non selective ß-adrenergic agonists. Acta Biol Hungaria 1991; 42: 345-355.

INOUE S, BRAY GA. The effects of subdiaphragmatic vagotomy in rats with ventromedial hypothalamic obesity. Endocrinology (Baltimore) 1977; 100: 108-114.

ISAAD T, COUPÉ C, FERRÉ P, GIRARD J. Insulin resistance during suckling period in rats. Am J Physiol 1987; 253: E142-E148.

IYNEDJIAN PB, JOTTERAND D, NOUSPIKEL T, ASFERI M, PILOT P-R. Transcriptional induction of glucokinase gene by insulin in cultured liver cells and its repression by the glucagon-cAMP system. J Biol Chem 1989a; 264: 21824-21829.

IYNEDJIAN PB, PILOT P-R, NOUSPIKEL T, MILBURN JL, QUAADE C, HUGHES S, UCLA C, NEWGARD CB. Differential expression and regulation of glucokinase gene in liver and islets of Langerhans. Proc Natl Acad Sci USA 1989b; 86: 7838-7842.

IYNEDJIAN PB, UCLA C, MACH B. Molecular cloning of glucokinase

cDNA: Developmental and dietary regulation of glucokinase mRNA in rat liver. J Biol Chem 1987; 262: 6032-6038.

JAMDAR SC, GREENGARD O. Premature formation of glucokinase in developing rat liver. J Biol Chem 1970; 245: 2779-2783.

JAMES DE, JENKINS AB, KRAEGEN EW. Heterogeneity of insulin action in individual muscles in vivo: Euglycemic clamp studies in the rat. Am J Physiol 1985; 248: E567-E574.

JEANRENAUD B. An hypothesis on the aetiology of obesity: Dysfunction of the central nervous system as a primary cause. Diabetologia 1985; 28: 502-513.

JEANRENAUD B. Insulin and obesity. Diabetologia 1979; 17: 133-138.

JEFFERSON LS, KIMBALL SC. Insulin regulation of protein synthesis. In: Molecular and Cellular Biology of Diabetes Mellitus vol II: Insulin Action, Drazinin B, Melmed S, LeRoith D, eds, Alan R. Liss, Inc., New York 1989; 133-142.

JETTON TL, MAGNUSON MA. Heterogeneous expression of glucokinase among pancreatic B-cells. Proc Natl Acad Sci USA 1992; 89: 2619-2623.

JOHNSON JH, OGAWA A, CHEN L, ORCI L, NEWGARD CB, ALAM T, UNGER RH. Under expression of B cell K_m glucose transporters in non-insulin-dependent diabetes. Science 1990; 250: 546-549.

JORDAN RE. Voluntary intragastic feeding : Oral and gastric contribution to food and hunger in man. J Comp Physiol Pyschol 1969; 68: 498

KAHN BB. Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. J Clin Invest 1992a; 89: 1397-1374.

KAHN BB. Alteration in glucose transporter expression and function in diabetes: mechanism for insulin resistance. J Cellular Biochem 1992b; 48: 122-128.

KAHN BB, PEDERSEN O. Suppression of GLUT4 expression in skeletal muscle of rats that are obese from high fat feeding but not from high carbohydrate feeding or genetic obesity. Endocrinology 1993; 132: 13-22.

KAHN BB, ROSSETTI L, LODISH HF, CHARRON MJ. Decreased in vivo glucose uptake but normal expression of GLUT1 and GLUT4 in skeletal

muscles of diabetic rats. J Clin Invest 1991; 87: 2197-2209.

KAMILARIS TC, JOHNSON EO, CALOGERO AE, KALOGERAS KT, BERNARDINI R, CHROUSOS GP, GOLD PW. Cholecystokinin-octapeptide stimulates hypothamic-pituitary-adrenal function in rats: Role of corticotropin releasing hormone. Endocrinology 1992; 130: 1764-1774.

KARAKASH C, HUSTVEDT BE, LOVO A, LE MARCHAND Y, JEANRENAUD B. Consequences of ventromedial lesions on metabolism of perfused rat liver. Am J Physiol 1977; 232: E286-E293.

KATSUURA G, IBII N, MATSUSHITA A. Activation of CCK-A receptors induces elevation of plasma corticosterone in rats. Peptides 1992; 13: 203-205.

KEESEY RE. Physiological regulation of body energy implications for obesity. In: Obesity: Theory and Therapy 2nd Edition, Stunkard AJ and Wadden TA., eds, Raven press, New York 1993; 77-95.

KEMMER FW, BERGER M, HERBERG L, GRIES FA, WIRDEIER A, BECKER K. Glucose metabolism in perfused skeletal muscle: Demonstration of insulin resistance in obese Zucker rat. Biochem 1979; 178: 733-741.

KIEKENS R, IN'T VELD P, SCHUIT F, VAN DE WINKEL M, PIPELEERS D. Differences in glucose recognition by individual pancreatic B-cells predispose to intracellular differences in glucose-inducible synthetic activity. J Clin Invest 1992; 88: 117-125.

KING AP, BETTS JJ, HORTON ED, HORTON ES. Exercise, unlike insulin, promotes glucose transporter translocation in obese Zucker rat muscle. Am J Physiol 1993; 265: R447-R452.

KING AP, HIRSHMAN M, HORTON ED, HORTON ES. Insulin resistance in obese Zucker rats fa/fa skeletal muscle is associated with a failure of glucose transporter translocation. J Clin Invest 1992; 90: 1568-1575.

KING BM, BANTA AR, THAREI GN, BRUCE BK, FROHMAN LA. Hypothalamic hyperinsulinemia and obesity: Role of adrenal glucocorticoids. Am J Physiol 1983; 245: E194-E199.

KING BM, SMITH RL. Hypothalamic obesity after hypophysectomy or adrenalectomy: Dependence on corticosterone. Am J Physiol 1985; 249: R522-R526.

KISSEBAH AH, PEIRIS A, EVANS DJ. Mechanisms associating body fat

distribution with the abnormal metabolic profile in obesity. In: Recent Advances in Obesity Research, Vol V, Berry EM, Blondheim SH Eliahou HE, Shafrir E., eds, John Libby Ltd, London, England, 1987; 54-59.

KLINK D, ESTRICH D. Plasma insulin concentration in Cushing's syndrome and thyrotoxicosis. Clin Res 1964; 12: 354

KNITTLE JL. Obesity in childhood: A problem in adipose tissue cellular development. In: Weight and Obesity: Causes, Fallacies, Treatment. Hafen BQ Ed, Brigham Young University Press, Provo, Utah 1975; 216-227

KNITTLE JL, FELLNER FG, BROWN RE. Adipose tissue development in man. Am J Clin Nutr 1977; 30: 762-766.

KNOTT RM, HESKETH JE. Insulin receptor and glucose mRNA expression in skeletal muscles of genetically obese Zucker rats. FEBS Lett 1992; 309: 153-156.

KOHRT WM, KIRWAN JP, STATEN MA, BOUREY RE. Insulin resistance in aging is related to abdominal obesity. Diabetes 1993; 42: 273-281.

KORANYI LI, BOUREY RE, SLENTZ CA, HOLLOSZY JO, PERMUTT MA. Coordinate reduction of rat pancreatic islet glucokinase and proinsulin mRNA by exercise training. Diabetes 1991; 40: 401-404.

KORANYI LI, BOUREY R, TURK J, MUECKLER M, PERMUTT MA. Differential expression of rat pancreatic islet beta-cell glucose transporter (GLUT 2), proinsulin and islet amyloid polypeptide gene after prolonged fasting, insulin-induced hypoglycaemia and dexamethasone treatment. Diabetologia 1992; 35: 1125-1132.

KRIEF S, BAZIN R. Genetic obesity: Is the defect in the sympathetic nervous system? A review through developmental studies in the preobese Zucker rat. Pro Soc Exp Biol Med 1991; 198: 528-538.

KRIEF S, BAZIN R, DUPUY F, LAVAU M. Role of brown adipose tissue in glucose utilization in conscious pre-obese rats. Biochem J 1989; 263: 305-308.

KROTKIEWSKI M, BJORNTORP P, SJÖSTROM L, SMITH U. Impact of obesity on metabolism in men and women -importance of regional adipose tissue distribution. J Clin Invest 1983; 72: 1152-1162.

KROTKIEWSKI M, SJÖSTROM L, BJORNTORP P, CARLGREN G,

GARELLIC G, SMITH U. Adipose tissue cellularity in relation to prognosis for weight reduction. Int J Obes 1977; 1: 395-416.

KUROSE T, TSUDA K, ISHIDA H, TSUJI K, OKAMOTO Y, TSUURA Y, KATO S, USAMI M, IMURA H, SEINO Y. Glucagon, insulin and somatostatin secretion in response to sympathetic neural activation in streptozotocin-induced diabetic rats. A study with the isolated perfused rat pancreas in vitro. Diabetologia 1992; 35: 1035-1041.

LANGLEY SC, YORK DA. Glucocorticoid receptor numbers in the brain and liver of the obese Zucker rat. Int J Obes 1992; 16: 135-143.

LANGLEY SC, YORK DA. Effect of antiglucocorticoid RU 486 on development of obesity in obese fa/fa Zucker rats. Am J Physiol 1990a; 259: R539-R544.

LANGLEY SC, YORK DA. Increased type II glucocorticoid receptor numbers and glucocorticoid-sensitive enzyme activity in brain of obese Zucker rat. Brain Res 1990b; 533: 268-274.

LARA-PANTIN E. Obesity in developing countries. In Recent advances in Obesity Research V, Berry EM, Blondheim SH, Eliahou HE and Shafrir E, eds. John Libby London, England 1987, 5-15.

LARSSON B, BJÖRNTORP P, TIBBLIN G. The health consquences of moderate obesity. Int J Obes 1981; 5: 97-116.

LAURIER D, GUIGUET M, CHAU NP, WELLS JA, ALLAIN-JACQUES VALLERON A-J. Prevalence of obesity : a comparative survey in France, the United Kingdom and the United States. Int J Obes 1992; 16: 565-572.

LAVAU M, BAZIN R, GUERRE-MILLO M. Increased capacity for fatty acid synthesis in white and brown adipose tissue from 7-day obese Zucker pups. Int J Obes 1985; 9: 61-66.

LE MARCHAND-BRUSTEL Y, GRÉMEAUX T, BALLOTTI R, VAN OBBERGHEN E. Insulin receptor tyrosine kinase is defective in skeletal muscles of insulin-resistant obese mice. Nature 1985; 315: 676-679.

LEMONNIER D, ALEXIEU A. Nutritional, genetic and hormonal aspects of adipose tissue cellularity. In: Regulation of the Adipose Tissue Mass. Vogue F, Boyer J eds. Amsterdam, Excerpta Med 1974: 154-173.

LENZEN S, PANTEN U. Signal recognition by pancreatic B-cells. Biochem

Pharmacol 1988; 37: 371-378.

LEVIN BE, STODDARD-APTER S, SULLIVAN AC. Central activation and peripheral function of sympatho-adrenal and cardiovascular systems in Zucker rat. Physiol Behav 1984; 32: 295-299.

LEVIN BE, TRISCARI J, SULLIVAN AC. Studies of origins of abnormal sympathetic function in obese Zucker rats. Am J Physiol 1983a; 245: E87-E93.

LEVIN BE, TRISCARI J, SULLIVAN AC. Altered sympathetic activity during development of diet-induced obesity in rat. Am J Physiol 1983b; 244: R347-R355.

LEVIN BE, TRISCARI J, SULLIVAN AC. Defective catecholamine metabolism in peripheral organs of genetically obese Zucker rats. Brain Res 1981; 224: 353-366.

LEWIS LD. Obesity: A commentary on nutrition management of small animals. Morris Associates, Topeka 1987; 1-34.

LIANG Y, JETTON TL, ZIMMERMAN EC, NAJAFI H, MATSCHINSKY FM, MAGNUSON MA. Effects of alternate RNA splicing on glucokinase isoform activities in the pancreatic islet, liver, and pituitary. J Biol Chem 1991; 266: 6999-7007.

LIANG Y, NAFAJI H, MATSCHINSKY FM. Glucose regulates glucokinase activity in cultured islets from rat pancreas. J Biol Chem 1990; 265: 16863-16866.

LIANG Y, NAJAFI H, SMITH RM, ZIMMERMAN EC, MAGNUSON MA, TAL M, MATSCHINSKY FM. Concordant glucose induction of glucokinase, glucose usage, and glucose-stimulated insulin release in pancreatic islets maintained in organ culture. Diabetes 1992; 41: 792-806.

LISSNER L, ODELL PM, D'AGOSTINO RB, STOKES J, KREGER BE, BELANGER AJ, BROWNELL KD. Variability of body weight and health outcomes in the Framingham population. N Engl J Med 1991; 324: 1839-1844.

LUDVIK B, CLIDI M, KAUTZKY-WILLER A, CAPEK M, HARTTER E, PACINI G, PRAGER R. Effect of dexamethasone on insulin sensitivity, islet amyloid polypeptide and insulin secretion in humans. Diabelotogia 1993; 36: 84-87.

MAGNUSON MA. Tissue-specific regulation of glucokinase gene. J Cellular Biochem 1992; 48: 115-121

MAGNUSON MA. Glucokinase gene structure: Functional implications of molecular genetic studies. Diabetes 1990; 39: 523-527.

MAGNUSON MA, SHELTON KD. An alternative promoter in the glucokinase gene is active in the pancreatic B-cell. J Biol Chem 1989; 264: 15936-15942.

MAKOTO O, JOHNSON JH, INMAN LI, CHEN L, SUZUKI K-I, GOTO Y, ALAM T, RAVAZZOLA M, ORCI L, UNGER RH. GLUT2 expression and function in ß-cells of GK rats with NIDDM. Diabetes 1993; 42: 1065-1072.

MANOLIO TA, SAVAGE PJ, BURKE GL, HINER JE, LIU K, ORCHARD TJ, SIDNEY S, OBERMAN A. Correlates of fasting insulin levels in young adults: The cardia study. J Clin Epidemiol 1991; 44: 571-578.

MANSON JE, COLDITZ GA, STAMPFER MJ, WILLETT WC, ROSNER B, MONSON RR, SPEIZER FE, HENNEKENS CH. A prospective study of obesity and risk of coronary heart disease in women. N Engl J Med 1990; 322: 882-889.

MARCHINGTON D, ROTHWELL NJ, STOCK MJ, YORK DA. Energy balance, diet-induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy. J Nutr 1983; 113: 1395-1402.

MARETTE A, ATGIÉ C, LIU Z, BUKOWIECKI LJ, KLIP A. Differential regulation of GLUTI and GLUT4 glucose transporters in skeletal muscle of a new model of type II diabetes. The obese SHR/N-cp rat. Diabetes 1993; 42: 1195-1201.

MARKS V, MORGAN L. The entero-insular axis. In: Recent Advances in Diabetes 1. Nattras M, Santiago AJ, eds, Edinburg, Churchhill Livingstone 1984; 55-71.

MATSCHINSKY FM. Glucokinase as glucose sensor and metabolic signal in pancreatic B-cells and hepatocytes. Diabetes 1990; 39: 647-652.

McCALEB M, SREDY J. Metabolic abnormalities of hyperglycemic obese Zucker rat. Metabolism 1992; 41: 522-525.

McLAUGHLIN CL, BAILE CA. Feeding responses of weanling Zucker

obese rats to cholecystokinin and bombesin. Physiol Behav 1980; 25: 341-346.

McLAUGHLIN CL, PEIKIN SR, BAILE CA. Decreased pancreatic CCK receptor binding and CCK stimulated amylase in Zucker obese rats. Physiol Behav 1984; 32: 961-965.

MEGLASSON MD, MATSCHINSKY FM. New perspective on pancreatic islet glucokinase. Am J Physiol 1984;246: E1-E13.

MEYER JM, STUNKARD AJ. Genetics and human obesity. In: Obesity: Theory and Therapy; 2nd Ed. Stunkard AJ and Wadden TA., eds, Raven Press, New York 1993; 137-149.

MOHR B. Hypertrophie der hypophysis cerebri und dadurchbedingter druck auf die hirngrundflache, insbesndere auf die schnerven, das chiasma deselben und den linkseitigen. Hirnschenkel Wschr Ges Heilk 1840; 6: 565-671.

MOLTZ JH, McDONALD JK. Neuropeptide Y: Direct and indirect action on insulin secretion in the rat. Peptides 1986; 6: 1155-1159.

MOORE BJ, ARMBRUSTER SJ, HORWITZ BA, STERN J. Energy expenditure is reduced in preobese 2-day Zucker fa/fa rats. Am J Physiol 1985; 249: R262-R265.

MORRISON SD. The hypothalamic syndrome in rats. Fed Proc 1977; 36: 139-142.

MOSTHAF L, ERIKSSON J, HARING H-U, GROOP L, WIDEN E, ULLRICH A. Insulin receptor isotype expression correlates with risk of non-insulin-dependent diabetes. Proc Natl Acad Sci USA 1993;90: 2633-2635.

NADIV O, COHEN O, ZICK Y. Defects in insulin's signal transduction in old rat livers. Endocrinology 1992; 130: 1515-1524.

NAKANGAMA I, TAKAHUZO O, TSUCHIYAMA H. An ultrastructural study of synthesis and release of beta granules in human pancreas. Acta Pathol Jpn 1971; 21: 329-347.

NARKEWICZ MR, IYNEDJIAN PB, FERRE P, GIRARD J. Insulin and triiodothyronine induce glucokinase mRNA in primary culture of neonatal rat hepatocytes. Biochem J 1990; 271: 585-589.

NISHI S, STOFFEL M, XIANG K, SHOWS TB, BELL GI, TAKEDA J. Human pancreatic Beta-cell glucokinase: cDNA sequence and localization of

the polymorphic gene to chromosome 7, and p 13. Diabetologia 1992; 35: 743-747.

NODA K, MASTSUTANI A, TANIZAWA Y, NEUMAN R, KANEKO T, PERMUTT MA, KAKU K. Polymorphic microsatellite repeat markers at the glucokinase gene locus are positively associated with NIDDM in Japanese. Diabetes 1993; 42: 1147-1152.

NOMURA A, HEILBRUN LK, STEMMERMANN GN. Body mass index as a predictor of cancer in men. J Natl Cancer Inst 1985; 74: 319-323.

NOUSPIKEL T, IYNEDJIAN PB. Insulin signalling and regulation of glucokinase gene expression in cultured hepatocytes. Eur J Biochem 1992; 210: 365-373.

OLNEY JW. Brain lesions, obesity, and disturbances in mice with monosodium glutamate. Science 1969; 164: 719.

OPSAHL CA, POWLEY TL. Failure of vagotomy to reverse obesity in the genetically obese Zucker rat. Am J Physiol 1974; 226: 34-38.

ORCI L. The insulin factory: A tour of the plant surroundings and a visit to the assembly line. Diabetologia 1985; 28: 528-546.

ORCI L, RAVAZZOLA M, BAETENS D, INMAN L, AMHERDT M, PETERSON RG, NEWGARD CB, JOHNSON JH, UNGER RH. Evidence that down-regulation of ß-cell glucose transporters in non-insulin-dependent diabetes may be the cause of diabetic hyperglycemia. Proc Natl Acad Sci USA 1990; 87: 9953-9957.

ÖSTENSON C-G, CATTANEO AG, DOXEY JC, EFFENDIC S. α-Adrenoceptor and insulin release from pancreatic islets of normal and diabetic rats. Am J Physiol 1989; 257: E439-E443.

PEIRIS AN, MUELLER RA, SMITH GA, STRUVE MF, KISSEBAH AH. Splanchnic insulin metabolism in obesity; influence of body fat distribution. J Clin Invest 1986; 78: 1648-1657.

PEIRIS AN, STAGNER JI, VOGEL RL, NAKAGAWA A, SAMOLS E. Body fat distribution and peripheral insulin sensitivity in healthy men: Role of insulin pulsatility. J Clin Endocrinol Metabol 1992; 75: 290-294.

PÉNICAUD L, FERRÉ , ASSIMACOPOULOS-JEANNET F, PERDEREAU D, LETURQUE A, JEANRENAUD B, PICON L, GIRARD

J. Increased gene expression of lipogenic enzymes and glucose transporter in white adipose tissue of suckling and weaned obese Zucker rats. Biochem J 1991; 279: 303-308.

PÉNICAUD L, ISSAD T, FERRE P, PUJOL A, PICON L, GIRARD J. Changes in insulin sensitivity during suckling-weaning transition in normal Wistar and obese Zucker rats. Frontier in Diabetes Research: Lesson from Animals Diabetes II. Shafrir E, Renold AE., eds, John Libby 1988; 438-443.

PERLEY M, KIPNIS DM. Effect of glucocorticoids on plasma insulin. N Engl J Med 1966; 274: 1237- 1241.

PERMUTT MA, CHIU KC, TANIZAWA Y. Glucokinase and NIDDM: A candidate gene that paid off. Diabetes 1992; 41: 1367-1372.

PHILIPPE J, GIORDNO E, GJINOVCI A, MEDA P. Cyclic adenosine monophosphate prevents the glucocorticoid-mediated inhibition of insulin gene expression in rodent islet cells. J Clin Invest 1992; 90: 2228-2233.

PIPELEERS DG. Heterogeneity in pancreatic B-cell population. Diabetes 1992; 41: 777-781.

PIPELEERS DG, MARICHAL M, MALAISSE WJ. The *stimulus-secretion* coupling of glucose-induced insulin secretion. XV. Participation of cations in the regulation of glucose by the B-cell. Endocrinology 1973; 93: 1012-1018.

PI-SUNYER FX. Health implications of obesity. Am J Clin Nutr 1991; 53: 1595S-1603S.

PLANCHE E, JOLIFF M, DE GASQUET P, LELIEPVRE X. Evidence of a defect in energy expenditure in 7-day old Zucker rat (fa/fa). Am J Physiol 1983; 245: E107-E113.

PLOTSKY PM, THRIVIKRAMAN KV, WATTS AG. Hypothalamic-pituitary -adrenal axis function in the Zucker obese rat. Endocrinology 1992; 130: 1931-1941.

POWLEY TL, OPSAHL CA. Ventromedial hypothamic obesity abolished by subdiaphragmatic vagotomy. Am J Physiol 1974; 226: 25-33.

PRAISSMAN M, IZZO RS. A reduced pancreatic protein secretion in response to cholecystokinin in the obese rat correlates with a reduced receptor capacity for CCK. Endocrinology (Baltimore) 1986; 119: 546-553.

PRICE RA, STUNKARD AB, NESS R, WADDEN T, HESHKA S, KANDERS B, CORMILLO A. Childhood onset (age <10) obesity has a high familial risk. Int J Obes 1989; 14: 185-195.

RADCLIFFE JD, WEBSTER AJF. Regulation of food intake during growth in fatty and lean female Zucker rats given diets of different protein content. Br J Nutr 1976; 36: 457-469.

ROHNER-JEANRENAUD F, HOSHSTRASSER A-C, JEANRENAUD B. Hyperinsulinemia of preobese and obese fafa rats is partly vagus-mediated. Am J Physiol 1983; 244: E317-E322.

ROHNER-JEANRENAUD F, JEANRENAUD B. Beneficial effect of intravenous bolus of corticotropin-releasing factor on glucose intolerance of genetically obese (fa/fa) rats. Diabetes 1992; 41: 493-498.

ROHNER-JEANRENAUD F, WALKER C-D, GRECO-PEROTTO R, JEANRENAUD B. Central corticotropin-releasing factor administration prevents the excessive body weight gain of genetically obese (fa/fa) rats. Endocrinology 1989; 124: 733-739.

RORSMAN P, BOKVISK K, AMMÄLÄ C, ARKAMMAR P, BERGGREN PO, LARSSON O, WÄHLANDER K. Adrenaline activity a low conductance G-protein-regulated K^+ channel in mouse pancreatic B-cells. Nature 1991; 349: 77-79.

ROSARIO LM, ATWATER I, ROJAS E. Membrane potential measurements in islets of Langerhans from ob/ob obese mice suggest an alteration in $[Ca^{2+}]_i$-activated K^+ permeability. Q J Exp Physiol 1985; 70: 137-150.

ROSEN LB, MAJZOUB JA, ADLER AK. Effects of glucocorticoid on corticotropin-releasing hormone gene regulation by second messenger pathways in NPLC and AtT-20 cells. Endocrinology 1992; 130: 2237-2244.

RUTMAN RJ, LEWIS FS, BLOOMER WD. Bipiperidyl mustard, a new obesifying agent in the mouse. Science 1966; 153; 1000-1002.

RYAN BF, JOINER BL, RYAN TA. Mintab handbook 2nd ed, PWS- Kent Publishing Company, Boston 1985.

SAITO M, BRAY GA. Adrenalectomy and food restriction in the genetically obese (ob/ob) mouse. Am J Physiol 1984; 246: R20-R25.

SALANS BC. Obesity and non-insulin-dependent diabetes mellitus. In: Recent Advances in Obesity Research V: Berry EM, Blondheim SH, Eliahou HE, Shafrir E. eds; John Libby, London, 1987; 26-32.

SALTIEL AR. Transmembrane signalling in insulin action. In: Molecular and Cellular Biology of Diabetes Mellitus Vol II: Insulin Action, Drazinin B, Melmed S, LeRoith D., eds, Alan R. Liss, New York 1989; 107-116.

SANACORA G, KERSHAW M, FINKELSTEIN JA, WHITE JD. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 1990; 127: 730-737.

SCAVELLI TD, PETERSON ME, MATTHIESEN DT. Results of surgical treatment for hyperadrenocorticism caused by adrenocortical neoplasia in the dog: 25 cases (1980-1984). J Am Vet Med Assoc 1986; 189: 1360-1364.

SCHAPIRA DV, KUMAR NB, LYMAN GH, COX CE. Obesity and body fat distribution and breast cancer prognosis. Cancer 1991; 67: 523-528.

SCHEMMEL R, MICKELSON O, GILL JL. Dietary obesity in rats: Body weight and body fat accretion in seven strains of rats. J Nutr 1970; 100: 1041-1048.

SCHMIDT I, KAUL R, CARLISLE HJ. Body temperature of huddling newborn Zucker rats. Pflugers Arch 1984; 401: 418-420.

SCLAFANI A. Dietary obesity. In: Obesity: Theory and Therapy, 2nd edition, Stunkard AJ, Wadden TA., eds, Raven Press, New York 1993; 125-135.

SCLAFANI A, GORMAN AN. Effects of age, sex, and prior body weight on the development of dietary obesity in adult rats. Physiol Behav 1977; 18: 1021-1026.

SCLAFANI A, KIRCHGESSNER AL. The role of the medial hypothalamus in the control of food intake; an update. In: Feeding behavior: Neural and Humoral Control. Ritter S, Barnes CD, eds. Academic Press, Orlando, Florida 1986; 27-66.

SCLAFANI A, SPRINGER D. Dietary obesity in adult rats: Similarities to hypothalamic and human obesity syndromes. Physiol Behav 1976; 17: 461-471.

SEIDELL JC, BATEX JC, DEKOER E, DEURENBERG P, HAUTRAST JGAJ. Fat distribution of overweight persons in relation to morbidity and

subjective health. Int J Obes 1985; 9: 363-374.

SEINO Y, YAMAMOTO T, KOH G. Insulin and glucose transporter gene expression in obesity and diabetes. Proc Soc Exp Biol Med 1992; 200: 210-213.

SHARGILL NS, AL-BAKER I, YORK DA. Normal levels of serum corticosterone and hepatic glucocorticoid receptors in obese (fa/fa) rats. Bioscience Reports 1987; 7: 843-851.

SHIMIZU K, AMAGAYA S, OGIHARA Y. Analysis of corticosterone in serum of mice and rats, using high performance liquid chromatography. J Chromatog 1983; 272: 170.

SHINO A, MATSMO T, IWATSUKA H, SUZUOKI Z. Structural changes of pancreatic islets in genetically obese rats. Diabetologia 1973; 9: 413-421.

SIBROWSKI W, SEITZ HJ. Rapid action of insulin and cyclic AMP in the regulation of functional messenger RNA for glucokinase in rat liver. J Biol Chem 1984; 259: 343-346.

SJÖSTRÖM L. Impacts of body weight, body composition, and adipose tissue distribution on morbidity and mortality. In Obesity: Theory and therapy 2nd Ed, Stunkard AJ and Wadden TA., eds, Raven Press, New York 1993; 13-41.

SJÖSTRÖM LV. Morbidity of severely obese subjects. Am J Clin Nutr 1992a; 55: 508S-515S.

SJÖSTRÖM LV. Mortality of severely obese subjects. Am J Clin Nutr 1992b; 55: 516S-523S.

SORENSEN TIA, HOLST C, STUNKARD AJ, SKOVGAARD. Correlation of body mass index of adult adoptees and their biological and adoptive relatives. Int J Obes 1992; 16: 227-239.

SORENSEN TIA, PRICE RA, STUNKARD AJ, SCHULSINGER F. Genetics of obesity in adult adoptees and their biological siblings. Br Med J 1989; 298: 87-90.

STEVENS J, LISSNER L. Body weight variability in the Charleston Heart study. Int J Obes 1990; 14: 385-386.

STONE M, SCHEMMEL R, CZAJKA-NARINS DM. Growth and development of kidneys, heart and liver in S5B/P1 and Osborne-Mendel rats

fed high or low-fat diets. Int J Obes 1980; 4: 65-78.

STUNKARD AJ, HARRIS JR, PEDERSEN NL, McCLEARN GE. The body-mass index of twins who have been reared apart. N Engl J Med 1990; 322: 1483-1487.

STUNKARD AJ, PENICK SB. Behavior modification in the treatment of obesity: The problem of maintaining weight loss. Arch Gen Psychiatry 1979; 36: 801-806.

STUNKARD AJ, SORENSEN TIA, HANIS C. An adoption study of human obesity. N Engl J Med 1986; 314: 193-198

TAI T-Y, CHUANG L-M, WU H-P, CHEN C-J. Association of body build with non-insulin-dependent diabetes mellitus and hypertension among Chinese adults: A 4-year follow-up study. Int J Epidemiol 1992; 21: 511-517.

TAKASAKI Y. Studies on brain lesion by administration of monosodium L-glutamate to mice. 1. Brain lesions in intact mice caused by administration of monosodium L-glutamate. Toxicology 1978; 9: 293-305.

TERRETTAZ J, JEANRENAUD B. In vivo hepatic and peripheral insulin resistance in genetically obese (fa/fa) rats. Endocrinology 1983; 112: 1346-1351.

TERRY RB, PAGE WF, HASKELL WL. Waist/hip ratio body mass index and premature cardiovascular disease mortality in US army veterans during a twenty three year follow-up study. Int J Obes 1992; 16: 417-423.

TIEDGE M, LENZEN S. Differential regulation of glucokinase and GLUT-2 glucose transporter gene expression in the pancreas and liver from neonatal and 16 day old rats. Biochem Mol Biol Int 1993; 29: 161-166.

TIMMERS KI, VOYLES NR, RECANT L. Genetically obese rats with (SHR/N-cp) and without diabetes (LA/N-cp) share abnormal islet response to glucose. Metabolism 1992; 41: 1125-1133.

TURKENKOPF IJ, JOHNSON PR, GREENWOOD MR. Development of pancreatic and plasma insulin in prenatal and suckling Zucker rats. Am J Physiol 1982; 242: E220-E225.

TURKENKOPF IJ, KAVA RA, FELDWEG A, HOROWITZ C, GREENWOOD MR, JOHNSON PR. Zucker and Wistar diabetic fatty rats show different response to adrenalectomy. Am J Physiol 1991; 261: R912-

R919.

UNGER RH. Diabetic hyperglycemia: Link to impaired glucose transport in pancreatic ß-cells. Science 1991; 251: 1200-1205.

VAN ITALLIE TB, HASHIM SA. Obesity in age of caloric anxiety. Weight and Obesity: Causes, Fallacies, Treatment. Hafen BQ, ed, Brigham Young University Press, Provo Utah, 1975; 40-47.

VANDER TIUG JG, KNEHANS AW, ROMSOS DR. Reduced sympathetic nervous activity in rats with ventromedial hypothalamic lesions. Life Sci 1982; 30: 913-920.

VAN DER VLIET JA, MELOCHE RM, FIELD MJ, CHEN D, KAUFMAN DB, SUTHERLAND DER. Pancreatic islet isolation in rats with ductal collagenase distension, stationary digestion and dextran separation. Transplantation 1988; 45: 493-495.

VIONNET S, STOFFEL M, TAKEDA J, BELL GI, ZOUALI H, LESAGE S, VELHO G, IRIS F, PASSA P, FROGUEL PDC. Nonsense mutation in the glucokinase gene causes early-onset non-insulin dependent diabetes mellitus. Nature 1992; 356: 721-722.

VOGT B, MÜHLBACHER J, OBERMAIER-KUSSER B, SEFFER E, MUSHACK J, PONGRAZ D, HÄRING HU. Subcellular distribution of GLUT 4 in the skeletal muscles of lean type 2 (non-insulin-dependent) diabetic patients in the basal state. Diabetologia 1992; 35: 456-463.

WAGER-PAGE SA, GHAZALI B, ANDERSON W, VEALE WL, DAVISON JS. The peripheral modulation of duodenal and pancreatic polypeptide-fold family: Neuropeptide Y, peptide YY, pancreatic polypeptide. Peptides 1993; 14: 153-160.

WALKER DG, EATON SW. Regulation of development of hepatic glucokinase in the neonatal rat by the diet. J Biochem 1967; 105: 771-777.

WALKER RP. Effects of controlled feeding on the isoenzymes of pyruvate kinase hexokinase and aldolase during development. Life Sci 1974; 15: 1507-1514.

WEINGARTEN HP, CHANG PK, McDONALD TJ. Comparison of metabolic and behavioral disturbances following paraventricular and ventromedial hypothalamic lesions. Brain Res Bull 1985; 14: 551-559.

WHITE F, PERIERA L, GARNER JB. Associations of body mass index and waist-hip ratio with hypertension. Can Med Assoc J 1986; 135: 313-320.

WIDÉN E, EKSTRAND A, SALRANTA C, FRANSSILA-KALLUNKI A, ERIKSSON J, SCHALIN-JANTTI C, GROOP L. Insulin resistance in type 2 (non-insulin-dependent) diabetic patients with hypertriglyceridaemia. Diabetologia 1992; 35: 1140-1145.

WILLIAMS G, SHELLARD L, LEWIS DE, McKIBBIN PF, McCARTHY DH, KOESLAG DG, RUSSELL JC. Hypothalamic neuropeptide Y disturbances in the obese (cp/cp) JCR:LA corpulent rat. Peptides 1992; 13: 537-540.

WILSON JD, FOSTER DW. Williams Textbook of Endocrinology 7th ed, WB Saunders Company, Philadelphia (PA) 1985; Chapter 17 and Chapter 22.

WOLLHEIM CB, SHARP GWG. Regulation of insulin release by calcium. Physiol Rev 1981; 61: 914-973.

YORK DA, BRAY GA. Genetic obesity in rat. II. The effect of food restriction on the metabolism of adipose tissue. Metabolism 1973; 22: 443-453.

YORK DA, MARCHINGTON D, HOLT SJ, ALLARS J. Regulation of sympathetic activity in lean and obese Zucker (fa/fa) rats. Am J Physiol 1985; 249: E299-E305.

YORK DA, SHARGILL NS, GODBOLE V. Serum and lipogenesis in the suckling 'fatty' fa/fa rat. Diabetologia 1981; 21: 143-148.

YOSHIDA Y. The significant role of brown adipose tissue in the development of obesity. In Obesity: Dietary Factors and Control, Ramsos DR *et al* eds; Japan Sci. Soc. Press Tokyo/ S. Karger, Basel 1991; 97-106.

YOUNG JB, LANDSBERG L. Impaired suppression of sympathetic activity during fasting in gold thioglucose treated mouse. J Clin Invest 1980; 65: 1086-1094.

YUKIMURA Y, BRAY GA. Effects of adrenalectomy on body weight and the size and number of fat cells in the Zucker (fatty) rats. Endocr Res Commun 1978; 5: 189-198.

YUKIMURA Y, BRAY GA, WOLFSEN AR. Some effects of adrenalectomy in fatty rats. Endocrinology 1978; 103: 1924-1928.

ZARJEVSKI N, DOYLE P, JEANRENAUD B. Muscle insulin resistance may not be a primary etiological factor in the genetically obese fa/fa rat. Endocrinology 1992; 130: 1564-1570.

ZAWALICH WS. Intermediary metabolism and insulin secretion from isolated rat islets of Langerhans. Diabetes 1979; 28: 252-260.

ZUCKER LM. Some effects of caloric restriction and deprivation on the obese hyperlipemic rat. J Nutr 1967; 91: 247-254.

ZUCKER LM, ZUCKER TF. Fatty, a new mutation in the rat. J Hered 1961; 52: 275-278.