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The Effects of Exercise Training Prior to, and During Pregnancy on Maternal, Fetal and Neonatal Outcomes and Glucose Homeostasis in Streptozotocin-Induced Diabetic Rats.

By

Jaci Lynn VanHeest

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ABSTRACT

THE EFFECTS OF EXERCISE TRAINING PRIOR TO, AND DURING PREGNANCY ON MATERNAL, FETAL AND NEONATAL OUTCOMES AND GLUCOSE HOMEOSTASIS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Chronic endurance training is known to positively impact the Type I diabetic condition. The influence of exercise on pregnancy outcomes in insulin-dependent diabetics remains unclear. The purpose of this project was to evaluate the effects of exercise prior to, and during gestation in Type I diabetic animals on offspring outcomes. Two sequential experiments were performed. The first was designed to determine the most beneficial training time course to optimize conception, maintain pregnancy and enhance offspring outcome. Animals were rendered diabetic (>20 mmol/l) by the drug streptozotocin and were maintained in a non-insulin treated state throughout the exercise and pregnancy periods. Three training treatments were used: exercise before and during gestation, sedentary prior to, and during gestation and exercise before gestation with cessation of exercise upon conception. Glucose and insulin concentrations and response to a glucose challenge were determined at various timepoints throughout the study. Litter size,

pup number, viability, pup weight and sex of pups were compared across the groups. In the second experiment, development and glucose homeostasis of the pups from the first experiment were assessed. Animals from diabetic mothers were fostered to euglycemic dams and observed in comparison to offspring originally born to the foster mothers. All animals were allowed to mature until 28 days of age at which time a glucose tolerance test (GTT) was performed. Tissue and blood collection was performed upon sacrifice. The results from this series of experiments indicate that (a) exercise prior to, and during gestation in a Type I diabetic animals does not appear to negatively influence the viability of offspring, (b) factors impacting the maternal system during the early portion of pregnancy may negatively influence the viability of offspring, (c) chronic exercise training in diabetic females prior to, and during pregnancy provides some protection for offspring relative to growth and glucose handling compared to pups from diabetic sedentary mothers, and (d) a potential sex specific response pattern is evident in offspring from diabetic dams. This scientific research provides support for previous work while generating numerous additional questions regarding the mechanisms controlling the outcomes seen in the present work.

This work is dedicated to two groups of individuals; in honour of my grandparents, **Mr. and Mrs. Cornelius DeJongh** and **Mr. and Mrs. William VanHeest** and in eager anticipation for my niece and nephews, **Martha, Daniel and Thomas Bouwens, Jason and Kevin Santefort** and **Joshua and Christopher VandenBerg**.

My grandparents instilled within my parents, **Rev. and Mrs. Jack VanHeest,** honour, sacrifice and persistence through all life's challenges. Their model for my parents and myself has left an indelible impression. I hope you can be proud.

I hope that the completion of this work can be a lighthouse to Martha, Dan, Tom, Jason, Kevin, Joshua and Christopher. It is proof of the following passage from a song by James Taylor.

> *" Hold tight to your heart's desire.*
> *Never, ever let it go.*
> *Let nobody fool you into giving it up too soon.*
> *Tend your own fire, lay low and be strong.*
> *Wait it out. . .*
> *It will come along."*

I hope you will always remember these words -- especially when people tell you that your dreams may not be possible. Remember only **you** can decide, you can be anything you truly desire.

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ABBREVIATIONS

acetonuria - The presence of acetone in the urine typical of ketosis in diabetes or starvation. Condition is due to the incomplete oxidation of fats.

C-peptide - A polypeptide (31 amino acid residues) which serves to connect the A and B chains of insulin when the hormone is in the proinsulin form. C-peptide will be secreted with insulin in a 1:1 ratio and has approximately 10% of the biological activity of insulin.

conceptus - The placenta and fetus expelled at birth.

corpus luteum - A small yellow body which develops within the ruptured ovarian follicle.

EE - Diabetic exercising prior to and during pregnancy.

ES - Diabetic exercising prior to pregnancy sedentary during pregnancy.

DM (diabetes mellitus) - A pathophysiological disorder of carbohydrate metabolism with either absolute or relative insulin deficiency. The symptoms include polyuria, polydipsia, weight loss despite polyphagia, hyperglycemia, glycosuria and acetonuria.

SS - Diabetic sedentary prior to and during pregnancy.

fetus - An organism in the later stages of development either within the uterus or egg.

GDM (gestational diabetes mellitus) - Classification of diabetes for women who are diagnosed with the disease during pregnancy.

glucagon - A catabolic pancreatic hormone released from alpha cells which functions in the mobilization of glucose, fatty acids and amino acids from tissue stores into the blood stream.

glucose - A primary form of carbohydrate fuel with the chemical formula $C_6H_{12}O_6$.

glucose intolerance - The condition where glucose levels are above the normal range in response to glucose ingestion yet the individual is not severe enough to be classified as diabetic.

glucosuria - An abnormal amount of glucose in the urine.

HADH (3-hydroxyacyl-CoA dehydrogenase) - An enzyme involved in fat metabolism

hyperglycemia - Elevated blood sugar for humans in excess of 300-2000 mg/dl of blood. In rats the serum level of glucose will exceed 22 mmol/l of blood.

*h*CS or *h*PL *(Human chorionic somatomammotropin or Human placental lactogen)* - A hormone produced by the placenta during gestation that has a primary action of stimulating glucose and fat metabolism.

IDDM (Type I diabetes mellitus) - The clinical condition often referred to as juvenile-onset diabetes; typically occurring before age 25. The primary pathophysiological abnormality is absolute insulin deficiency.

IPGTT (intraperitoneal glucose tolerance test) - A test performed by injecting a volume of glucose into the peritoneum. Blood samples are taken at specified intervals and evaluated for glucose and insulin levels. The ability of the subject to metabolize glucose is determined with this procedure.

insulin - An anabolic pancreatic hormone released from beta cells which functions at the tissues in up-regulating glucose, fatty acid and amino acid uptake.

insulin sensitivity - The ability of tissue to uptake glucose.

insulin resistance - A condition whereby the tissue has a reduced capacity to transfer glucose into the cell.

LHRH (lutenizing hormone releasing hormone) - A hormone produced in the hypothalamus which controls the release and synthesis of lutenizing hormone. Lutenizing hormone is secreted by the anterior lobe of the hypophysis and stimulates the development of the corpus luteum.

macrosomia - A condition of enlarged body size typical of infants born of diabetic mothers.

myofibrillar ATPase - An enzyme catalyzing the hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) within the skeletal muscle.

neonate - A newborn infant (rat pup).

NIDDM (Type II diabetes mellitus) - The disorder that typically manifests itself during times of stress such as pregnancy, infectious disease, obesity, inactivity or trauma. This disease is frequently called adult-onset or latent diabetes mellitus.

PFK (phosphofructokinase) - A rate limiting enzyme of glycolysis which catalyzes the reaction converting fructose-6-phosphate to fructose 1,6, diphosphate.

polydipsia - Excessive stimulus for thirst.

polyphagia - Increased food intake.

polyuria - An excessive urine production.

SDH (succinate dehydrogenase) - Kreb's cycle enzyme that converts succinate into fumerate.

Chapter 1

Introduction and Rationale

INTRODUCTION AND GENERAL REVIEW

Diabetes mellitus (DM) is one of several endocrine abnormalities which has been shown to negatively affect pregnancy outcome. Clinical accounts of anomalies characteristic of neonates born to diabetic women have been described for many years. Bennewitz reported the first account of clinical symptoms associated with the diabetic pregnancy in 1826. He described a patient who developed thirst polyuria, sweet-tasting urine with a saccharine like matter during three successive pregnancies (Chahal and Hawkins, 1967).

Almost fifty years later, J. Matthews Duncan presented a paper entitled, "*Puerperal diabetes*" to the London Obstetrical Society. In this paper he cited only 22 pregnancies in 15 women with diabetes. Four of the mothers died during pregnancy with seven stillbirth or perinatal deaths (Duncan, 1882). Duncan reported the following passages in his oration,

> *"Diabetes may come on during pregnancy.*
> *Diabetes may occur only during pregnancy,*
> *being absent at other times.*
> *Diabetes may cease with the termination of pregnancy*
> *recurring sometime afterwards.*
> *Pregnancy is ... liable to be interrupted ... by the death*
> *of the foetus ... the dead foetus is sometimes described*
> *as enormous"* (Duncan, 1882).

Shortly after the report by Duncan, individuals at the London Hospital documented information on the fertility rates and pregnancy outcomes of diabetic women. During the years from 1893 through 1922 physicians such as Lecorche and Skipper reported fertility rates ranging from 2-6% of all female diabetics seen at the

hospital (Skipper, 1933; Drury, 1961). Elliot Joslin indicated that only 10 out of 1300 (0.01%) female diabetics seen at the Joslin Clinic became pregnant. Of these ten women, three mothers and two fetuses died during gestation (Hare, 1985). With the introduction of insulin as a treatment for diabetes in the early 1920's, maternal mortality however, perinatal mortality remained high (White, 1978).

Jorgan Pedersen attempted to more closely examine this elevated level of perinatal mortality and through his efforts he developed a theory hypothesizing the events responsible for neonatal outcomes in diabetic pregnancy.

His theoretical framework is summarized as follows: maternal hyperglycemia results in fetal hyperglycemia which causes hypertrophy of fetal islet cells. The hypertrophy of fetal beta cells causes hypersecretion of insulin with concomitant hyperinsulinism in the fetus. The increased levels of insulin ultimately resulting in enhanced glucose utilization by the developing fetus. Finally, one notes enhanced fetal growth or macrosomia (large bodies) (Pedersen, 1967). Although the theory was first developed in the early 1920's following the advent of insulin, the Pedersen hypothesis was not put into print until 1967. At that time he wrote a book entitled *The Pregnant Diabetic and Her Newborn* (Pedersen, 1967). This book provided the theoretical framework for many of the present concepts regarding the impact of maternal diabetes on offspring outcome.

The role of the diabetic state in the overall development of the fetus has continued to received considerable attention. The literature has indicated that offspring of diabetic women typically have a greater risk for developing diabetes than offspring from non-

diabetic counterparts and that the associated genetic factor is a primary component in the development of diabetes in the offspring of diabetic mothers (Steinberg, 1961; Freinkel, 1980). Furthermore, recent data has provided additional insight into the role of the environment, specifically the altered fuel metabolism which naturally accompanies the maternal diabetic state, as a primary contributor to the subsequent diabetic characteristics of the fetus. It is this area of research which is of pertinent interest to the present series of experiments.

Investigations which have simulated a moderate hyperglycemic state in normal rats during the later portion of pregnancy have shown that the newborns from the hyperglycemic dams showed the main features of newborns from diabetic mothers (hyperglycemia, hyperinsulinemia and macrosomia) (Gauguier et al., 1990). Moreover, this altered glucose metabolism persisted into adulthood in these animals. These data emphasize the role of the maternal uterine environment on fetal outcome and lead one to speculate that improvements in the maternal environment may produce concomitant improvements in the fetal state.

Review of the diabetic pregnancy literature suggests that maternal hyperglycemia causes a reflexive hyperglycemia in the developing fetus. The fetal response to elevated glucose is hypersecretion of insulin associated with pancreatic islet hypertrophy. Ultimately the hyperinsulinism causes increased uptake of nutrients into the fetal system. This enriched fuel milieu provides exaggerated levels of molecular 'building blocks' which the developing organism utilizes, resulting in macrosomia. It seems

logical then, that any intervention (such as a reduction in maternal hyperglycemia or alteration in placental transport of glucose) which would impact on the rather linear response pattern seen in diabetic pregnancy could reduce the negative outcomes typical in diabetic offspring. Furthermore, even if the effects were not evident in gestational outcomes (enhanced maternal fertility, increased viability of pups, or reduction in macrosomia), the altered maternal environment may reduce the potential for abnormal glucose homeostasis during the adult life of mature offspring. One potential intervention is chronic endurance training.

Although research examining the triangular relationship between exercise, diabetes and pregnancy is limited, pairwise evaluation of these three parameters has been reported in the literature. The following section will provide a general overview of the literature relating to the various pairwise interactions.

Various studies have attempted to evaluate the effect of exercise on glucose homeostasis in Type I diabetics. A recent review summarized the beneficial effects of exercise as follows:

(1) increases insulin sensitivity
(2) decrease blood glucose concentration both during following exercise
(3) increases cardiorespiratory fitness
(4) enhances high density lipoproteins and decreases total blood cholesterol concentration
(5) decreases blood pressure in hypertensive patients
(6) improves psychological variables typically affected with chronic endurance training (Franz, 1992).

The data provide clear evidence that chronic endurance training results in beneficial adaptations typically seen in euglycemic

individuals. Therefore, it is apparent that endurance training is a stimulus producing positive effects in diabetic patients.

The physiological responses of women to both exercise and pregnancy allow for alterations in many organ systems. Research focusing on the effects of exercise during normal gestation has produced controversial results. The positive effects include shorter hospitalization time and reduced cesarian births (Hall and Kaufman, 1987). However, other researchers have suggested failure to progress in pregnancy, higher incidence of cesarian sections, reduced weight gain in both mother and infant, and early labor onset (Dale, Mullinax and Bryan, 1982; Clapp and Dickstein, 1984) as a few of the negative outcomes associated with exercise during pregnancy. Other research reported neither significant negative or positive effects associated with endurance training during gestation in both human (Collings, Curet and Mullin, 1983; Kulpa, White and Visscher, 1987; Lutter, Lee and Cushman, 1984) and animal studies (Mottola, Bagnall and McFadden, 1983; Mottola, Bagnell, Belcastro, Foster and Secord, 1986; Mottola and Christopher, 1991; Rodgers, Mottola, Corbett and Taylor, 1991).

Summarizing the review by Wolfe and Mottola (1993), the authors suggested that exercise during pregnancy, if prescribed carefully, can promote positive effects both in the maternal and fetal systems. These authors caution, however, that highly intense training regimes and/or exercise coupled with nutritional, environmental or pathological conditions may cause negative outcomes to the fetus (Wolfe and Mottola, 1993).

The effects of exercise during pregnancy coupled with diabetes (Type I, Type II or GDM) has been studied primarily in a clinical setting. In the past, physicians have been reticent to prescribe exercise for pregnant diabetics. This was due to the fear of potential fetal risks although maternal benefits may occur (Bung et al., 1991). Research has clearly described the beneficial effects of endurance exercise on glucose homeostasis and skeletal muscle metabolism in non-pregnant diabetic animals (Noble and Ianuzzo, 1985). The changes associated with endurance exercise have recently been evaluated in pregnant diabetics (Artal, Wiswell and Romem, 1985; Jovanovic-Peterson, Durak and Peterson, 1989). These studies have utilized human subjects (GDM) engaged in mild to moderate physical exercise and reported physiological and metabolic responses like those of non-diabetic patients. Exercise as a therapeutic modality seemed to have greater potential versus insulin treatment due to the reduced peripheral insulin sensitivity which evolves from the hormonal alterations during pregnancy (Hjollund, Pedersen, Espersen and Klebe, 1986).

It appears that the typical increases in free fatty acid and lipoprotein concentrations which occur in late pregnancy are attenuated with diabetes (Hollingsworth, 1983). Results from studies utilizing exercise in diabetic female rats suggest reductions in circulating free fatty acids and triglycerides in trained diabetic animals (Goodyear et al., 1988). The potential beneficial effects in lipid metabolism could impact the fetal outcome. Although data suggests potential in mild diabetic conditions, controlled studies in more severe levels of diabetes remains untested.

To date, studies which have evaluated exercise effects on the diabetic condition in female rats have initiated the exercise regime either prior to adjustment to streptozotocin (STZ) (Goodyear et al., 1988; Goodyear et al., 1991) or following conception (Jovanovic-Peterson, Durak and Peterson, 1989). Research which initiates exercise prior to complete adjustment to STZ could be assessing the interactive affects of the two processes. It is necessary to allow for the animals to develop a new homeostasis under the diabetic condition prior to initiation of training. The impact of endurance training on the diabetic condition can then be evaluated. Additionally, training protocols initiated prior to conception must also be of sufficient duration to enable a training effect in the animals. By training the animals prior to pregnancy, the data can be evaluated for the effects of exercise on the animals' ability to conceive, maintain pregnancy, and improve offspring outcome. If the exercise stimulus in inadequate prior to gestation, it is difficult to interpret the effects of training, gestation or the combination on various outcome parameters. Therefore, one purpose of the present work was to allow for adjustment to the diabetic condition prior to exercise as well as to provide the exercise stimulus for a period which would maximize the potential for typical training effects to occur. The training treatments included: (a) sedentary both prior to, and during gestation, (b) training prior to gestation with cessation upon conception, and (c) training both prior to and during pregnancy. The three training time courses selected were designed to provide adequate time for adjustment to both the diabetic condition and the training program before inducing pregnancy. These controls were

utilized to allow for clear delineation of both maternal and neonatal responses to the treatment.

GENERAL PURPOSE OF THE PROJECT

The present series of studies has been developed to evaluate the effects of exercise training prior to, and during gestation on maternal and neonatal glucose homeostasis in experimentally induced Type I diabetic rats. To date, research is limited regarding the time course of both maternal and neonatal blood parameter alterations under the aforementioned conditions. Moreover, the type of exercise protocol which has the greatest potential for beneficial effects on both the mother and the fetus has yet to be established. Clinical situations such as termination of exercise early into pregnancy are typical especially in diabetic individuals. Therefore, the exercise time courses (exercise then stop at conception) attempt to mimic conditions which are typical in human pregnancy. The proposed series of experiments will not only extend the scope of research available on maternal and fetal alterations associated with diabetes but, more importantly, it will provide new information regarding the role of exercise in affecting the maternal uterine environment and the subsequent effect of this alteration on neonatal outcome.

SPECIFIC AIMS

In a global sense, the series of research experiments was developed to assess the effects of exercise prior to, and during

gestation in Type I diabetic animals on offspring outcomes. Additionally, the following two specific research questions were evaluated.

1. **Does the training time course order have an effect on pregnancy outcome in a STZ diabetic rat?**

Rationale.

The literature is limited as to the effects of chronic endurance training on diabetic pregnant animals. Previous work with Type I diabetic animals utilized a three week training program prior to gestation plus training during pregnancy as the intervention (Urie-Hare et al., 1989). The three week training may not have provided a sufficient stimulus to attain optimal changes associated with longer duration training programs. Therefore, the training program utilized in the present work was ten weeks in duration (accommodation plus eight weeks at training intensity) which has been used by other investigators. The three training time courses were selected to mimic those common in clinical setting for human diabetic. Women who train generally follow one of two routes once pregnant: terminate exercise totally or continue either at equal intensity or at a lowered intensity level. Furthermore, diabetic women who are sedentary are generally not recommended to initiate a training program once pregnant. These three treatments allowed for evaluation of the conditions generally seen in diabetic pregnancies.

Most studies evaluate either a glucose intolerant or Type II model in humans and suggest benefits from the training on both

maternal and neonatal outcomes (Jovanovic-Peterson, Durak and Peterson, 1989; Durak, Jovanovic-Peterson, and Peterson, 1990; Jovanovic-Peterson, and Peterson, 1991). Since these studies evaluated only GDM and NIDDM, the present work served as a novel approach to determine the impact on the more severe Type I (non-insulin treated) pathophysiological condition. Assessment of the maternal diabetic state throughout both the training program and gestation was critical for this component of the work. Specific parameters assessed include plasma glucose and insulin concentrations at specific timepoints throughout the study, glucose response to a glucose challenge, anthropometric characteristics and food consumption, pregnancy outcomes (litter size, viable pup number, pup weight) and tissue weight at sacrifice in the diabetic females.

2. What effect does the maternal intrauterine environment have on the offspring's ability to handle glucose as young developing animals?

Rationale.

Various authors have suggested a non-genetic encoding of information due to alterations in maternal uterine milieu during gestation (Gauguier et al., 1991; Grill, 1991). This environmental control has been thought to impact not only first but second generation offspring. If this is the case, it would appear that any perturbation of the diabetic environment either prior to, or during gestation might subsequently influence the offspring outcomes. In addition, one could suggest that these alterations may persist even

into later life. This study evaluates offspring growth (body weight) over the first four weeks of life. Additionally, response to a glucose tolerance test is assessed at 28 days of age. Tissue weights and plasma glucose and insulin concentrations were determined (28 days). Qualitative morphological abnormalities were also documented throughout the study.

Due to the nature of this dissertation, the following chapter will provide a review of the literature which addresses directly pertinent research examining the topic of exercise, pregnancy and diabetes (Chapter 2). Chapter 3 and 4 delineate each of the experiments conducted; specifically the background research, the methods utilized, the outcomes and a discussion of the data. Finally, Chapter 5 provides a global interpretation of the two studies and how they relate to the role of the environment in offspring outcome as well as describing future work based on the information gained in this dissertation project.

Chapter 2

Literature Review

LITERATURE REVIEW

The present series of experiments are designed to evaluate the interactive effects of the triad of exercise, pregnancy and diabetes mellitus on glucose homeostasis in both mother and offspring. It is the purpose of this review of literature therefore, to provide a general overview of both general metabolism during pregnancy and the metabolic pathophysiology associated with DM. Additionally, the pairwise effects of exercise, pregnancy and diabetes will be discussed (pregnancy and exercise, pregnancy and diabetes, and diabetes and exercise). Literature assessing the interactive effects of diabetes, exercise and pregnancy will be discussed. Finally, research on the effects of maternal DM on neonatal morphology and glucose homeostasis will be discussed.

GENERAL OVERVIEW OF THE ETIOLOGY OF DIABETES.

Pancreatic hormones function both in digestion and in regulating glucose, lipid and protein metabolism. The hormones are secreted from two distinct anatomical tissue types: (1) the acini which secrete digestive juices, and (2) the islets of Langerhans, which secrete insulin and glucagon directly into the blood. Human islets of Langerhans contain various cell types, of which three are critically important in normal physiological functioning; namely alpha, beta and delta cells. The alpha cells secrete glucagon, the beta cells secrete insulin and the delta cells secrete somatostatin. Somatostatin functions in the regulation of islet cell secretion. Insulin causes the uptake of glucose, amino acids and fatty acids in the tissues. Glucagon is described as an "insulin antagonist" and acts to mobilize

glucose, fatty acids and amino acids from tissue stores into the bloodstream. Both insulin and glucagon respond to the circulating levels of glucose in the blood. During hypoglycemic conditions, glucagon is released and functions to enhance hepatic gluconeogenesis and glucose release. Conversely, hyperglycemia triggers insulin secretion causing glucose uptake into the skeletal muscle and adipose tissues, thereby restoring the organism to relative euglycemia (Ganong, 1983; Guyton, 1991).

Alterations in the circulating levels of these hormones create pathophysiological conditions which often necessitate clinical intervention (Ganong, 1983). One such pathology, diabetes mellitus (DM), is a disruption in carbohydrate metabolism similar to extreme fasting. DM typically results from inadequate production or utilization of insulin. The specific cause of DM is yet unknown but the apparent physiological ramification, in almost all cases, is a diminished rate of insulin secretion by the pancreas or depressed utilization of insulin in the periphery (Berne and Levy, 1988; Guyton, 1991). Clinical findings suggest that in most cases DM is the result of a genetic disorder, but can be triggered by trauma, infection, malignancies of the pancreas and/or surgery. General clinical symptoms include polydipsia, polyuria and glucosuria. In addition, various metabolic abnormalities are evident and include alterations in protein metabolism (Ganong, 1983; Teperman, 1983) and disruptions in fat metabolism (Brown, VanBueren and Millward, 1983; Kenno and Severson, 1985). Typically, the insulin to glucagon ratio is very low which results in augmented glucose production as well as peripheral resistance to insulin, the ultimate result being a

very high plasma glucose level (300-2000 mg/dl) in humans compared to that of normals (<100 mg/dl) (Berne and Levy, 1988). Other associated clinical problems include but are not limited to neuropathy (Olson, Kelley and Smith, 1981; Reaven, Peterson and Reaven, 1973), cell structure changes (Chen and Ianuzzo, 1982) and alterations in the circulating levels of various hormones (Cameron, Cotter and Harrison, 1986; Taylor, Sharma, Avasthy, Duguid, Blanchard, Thomas and Dandona, 1987).

The American Diabetes Association and the National Diabetes Data Group both have published classification schemes for DM (Bonar, 1980; Davidson, 1985). This general classification scheme is as follows:

> ***Type I*** *(Insulin-dependent diabetes mellitus; IDDM)*
> Previously termed: juvenile diabetes (JD), juvenile-onset diabetes (JOD), ketosis-prone diabetes or brittle diabetes. This form is ketosis prone and is characterized by insulin deficiency due to islet cell loss or destruction. IDDM can occur at any age but is typical in young individuals. Experimental inducement of IDDM is commonly accomplished in research settings by pharmacologically destroying the beta cells. The pharmacological agents utilized include streptozotocin (STZ) or alloxan. IDDM in humans necessitates the use of exogenous insulin.
>
> ***Type II*** *(Non-insulin dependent diabetes mellitus - Nonobese or Obese; NIDDM)*
> Previously termed: adult-onset diabetes (AOD), maturity-onset diabetes (MOD), ketosis-resistant diabetes, stable diabetes or maturity-onset diabetes of youth (MODY).

This form is not ketosis prone and is common in adults but can occur at any age. Frequently the individuals are overweight or obese. The condition has been described as having a genetic link and is common in family aggregates. Generally, the disease does not require exogenous insulin except in specific situations such as stress or pregnancy. Evaluation of NIDDM experimentally in animals generally uses animals genetically bred to exhibit NIDDM such as Fatty Zucker or BB Wistar rats.

Gestational Diabetes (GDM)
This form of diabetes occurs during gestation. Diagnosis of diabetes during pregnancy occurs in approximately 1-2% of all pregnancies. Glucose intolerance is transitory and diagnosis (in humans) requires at least two abnormal glucose tolerance tests (3-hour). Individuals with GDM are predisposed to above-average risk of perinatal complications. The glucose intolerance remits postpartum. Generally the condition is controlled by diet with a portion of the women necessitating exogenous insulin administration.

The two forms pertinent to this study are IDDM and GDM. The specific symptomatology associated with IDDM includes beta cell lesions, insulin deficiency, insulin resistance, hyperglycemia and glucosuria. Pregnant women frequently experience symptoms of glucose intolerance during pregnancy (Keen, 1991). If the pathophysiology of glucose intolerance deteriorates to the level of diabetes during gestation and returns to normal postpartum then the individual is diagnosed as having gestational diabetes rather than the chronic condition of IDDM. Research suggests that both the maternal and fetal systems are negatively influenced by the diabetic condition (Horton, 1991; Keen, 1991).

Regardless of the form, DM is considered a chronic pathophysiological condition. Generally, depending upon the type and severity of the disease, the symptoms can be minimized and the quality of life can be enhanced by modern treatments. Common treatment methods include administration of exogenous insulin (IDDM), dietary manipulation (IDDM, NIDDM, GDM), and exercise (IDDM, NIDDM, GDM). Dietary alterations are utilized as an initial treatment modality, followed by injectable exogenous insulin if necessary (Horton, 1991).

ALTERATIONS IN NORMAL METABOLISM ASSOCIATED WITH DIABETES MELLITUS.

The general metabolic effects of DM are the following: (1) a depression of glucose entry into various peripheral tissues including adipose tissue and skeletal muscle, (2) an increase in the liberation of glucose into the circulation from the liver caused by elevated hepatic gluconeogenesis, (3) decreased entry of amino acids into skeletal muscle causing depletion of protein in the body tissues and (4) an elevation in lipolysis resulting in abnormal fat metabolism and deposition of lipids in the vascular walls (Ganong, 1983; Guyton, 1991). The focus of this section includes a review of perturbations in normal glucose homeostasis resulting from IDDM as well as a description of the alterations in cardiac and skeletal muscle metabolism in diabetics.

Insulin biosynthesis and secretion occurs in the beta cells of the pancreas. Insulin is synthesized as a part of a large polypeptide precursor called preproinsulin (Ganong, 1983). Preproinsulin is

cleaved as it enters the endoplasmic reticulum and converts into the folded molecule proinsulin. Proinsulin is composed of two primary chains (A and B) and a connector chain. Ultimately the connector chain (C-peptide) is cleaved and is secreted along with insulin. Insulin levels in the plasma are the net result of insulin secretion and insulin removal. To adequately evaluate insulin metabolism, both the total volume secreted and the circulating levels must be determined. Since C-peptide is released in a one-to-one relationship with insulin, this peptide can be utilized to determine the secretion rate of insulin into the circulation (Krotkiewski and Gorski, 1986; Polonsky and Rubenstein, 1984). Naturally occurring lesions or pharmacological destruction of the beta cells causes the reduction in insulin secretion evident in IDDM. The reduction in circulating levels of insulin causes a cascade of events that perturb normal glucose metabolism.

The net level of blood glucose is a function of the balance between glucose entry into and exit from the bloodstream. Factors controlling entry and exit of glucose include dietary intake, rate of uptake by skeletal muscle and adipose tissue, utilization by other organs (brain) and glucostatic activity of the liver. Typically, 30-40% of ingested glucose is stored as fat while five percent is converted to glycogen by the liver. The remainder is metabolized by muscle or other tissues (Ganong, 1983).

Either in naturally occuring or experimentally (STZ) induced diabetes a reduction in basal insulin levels and an elevation in basal glucose levels is evident (Wallberg-Henriksson, 1986). The hyperglycemia associated with DM is caused by several factors. The

rise in glucose is due in part to decreased peripheral utilization of glucose. In addition, glucose uptake by the liver is reduced although uptake by the brain and erythrocytes is normal. Another influential cause of the hyperglycemia in DM is the alteration in hepatic glucostatic function (Ganong, 1983). Insulin enhances the synthesis of glycogen while inhibiting hepatic gluconeogenesis. In DM, hepatic output of glucose remains elevated despite high blood glucose levels in part because of the hyperglucagonemia. Ultimately, the insulin deficiency in DM causes enhanced levels of extracellular glucose with concommitant intracellular deficits (Ganong, 1983; Wallberg-Henriksson, 1986).

Due to the intracellular reduction in glucose, the energy needs of the cell must be met by alternative fuel sources such as stored glycogen, fats, and proteins. A reduction in intracellular glycogen is common in both liver and skeletal muscle in diabetic humans and animals. In addition to glycogen, catabolism of amino acids is increased. Amino acids are utilized as gluconeogenic precursors in the liver. The availability of circulating amino acids is due to a reduction in insulin stimulated protein synthesis. Elevated glucagon associated with DM stimulates gluconeogenesis in the hepatic tissue (Ganong, 1983, Guyton, 1991). Finally, fat metabolism is altered in diabetes by accelerated lipid catabolism.

The catabolism of lipids results in increased formation of ketone bodies and decreased formation of fatty acids and triglycerides. In severe DM ketones build up in the bloodstream and spill over into the urine causing ketonuria (Ganong, 1983; Guyton, 1991). The degree of altered metabolic homeostasis associated with DM can be

evaluated by measuring circulating levels of various hormones such as insulin and glucagon. Additionally, the basal level of glucose as well as the response of insulin and glucose to a glucose load (glucose tolerance test) reveals important information regarding the status of the diabetic subject.

Peripheral utilization of glucose is reduced, especially in skeletal muscle. This reduction is due to a depression in insulin (in IDDM) and/or an enhanced insulin resistance at the muscle level (Goodyear et al., 1988). Therefore, skeletal muscle metabolism is altered in the diabetic state and can provide an additional gauge of the severity of the disease. The following section will review evidence supporting the alterations in metabolic function of skeletal muscle accompanying IDDM.

Muscle tissue alterations due to perturbed glucose utilization provide experimental evidence as to the hormonal and metabolic changes in diseases such as DM. Research suggests that the metabolism of both cardiac and skeletal muscle is altered in response to the diabetic state. Muscle metabolism, whether glycolytic or oxidative, has traditionally been evaluated using enzymatic markers. Alterations in the activities of key glycolytic (phosphofructokinase, PFK; hexokinase, HK; lactate dehydrogenase, LDH) and oxidative (citrate cynthase, CS; succinate dehydrogenase, SDH) enzymes indicate metabolic changes occuring within the various muscle fibre types.

Ianuzzo and Armstrong (1976) determined that PFK levels were decreased in soleus and gastrocnemius muscle from STZ-induced, chronically diabetic rats. Soleus and middle portion of the

gastrocnemius represent slow oxidative (SO) fibres and fast-oxidative, glycolytic (FOG) fibres, respectively. Similar findings in the diaphragm (SO and FOG primarily) (Ianuzzo, Patel, Chen, O'Brien and Williams, 1977) and the plantaris muscle, a mixed muscle (Chen and Ianuzzo, 1982) were reported. In a broad assessment of multiple enzymatic markers, Noble and Ianuzzo (1985) demonstrated that the diabetic condition negatively affected enzymes such as PFK, hexokinase (HK), phosphorylase (PHOS), glycerol-3-phosphate dehydrogenase (G3PDH) and lactate dehydrogenase (LDH) in skeletal muscle. These changes resulted in depressed glucose entry into cells as well as an increase in glucose release from the liver.

The fibre type specificity of PFK response was clearly demonstrated in muscles possessing high oxidative capacity which were the most significantly affected (Chen and Ianuzzo, 1982; Ianuzzo and Armstrong, 1976; Noble and Ianuzzo, 1985). Additionally, reductions in phosphatase (PHOS) and HK levels add evidence to support the depressed glycolytic potential in the various fibre types (Chen and Ianuzzo, 1982; Ianuzzo and Armstrong, 1976; Noble and Ianuzzo, 1985). PHOS activity was reduced only in the diaphragm and in the plantaris muscle while HK activity was depressed in all fibre types (Noble and Ianuzzo, 1985). These alterations in glycolytic potential in response to the diabetic condition indicate that the disruption in glucose entry triggers various processes that depress function of the glycolytic pathway.

Oxidative metabolism has been shown to be affected by the chronic diabetic state (Gollnick and Ianuzzo, 1972; Ianuzzo and Armstrong, 1976; Noble and Ianuzzo, 1985). Typical oxidative

enzymes such as citrate synthase (CS) and succinate dehydrogenase (SDH) were evaluated in all three types of skeletal muscle (FG, FOG, SO). SDH was reported to be decreased in each of the three fibre types (Ianuzzo and Armstrong, 1976; Noble and Ianuzzo, 1985). Noble and Ianuzzo (1985) reported, however, that a fibre type specificity was evident for the oxidative enzymes as well. Significant reductions in SDH activity were found in the plantaris muscle (mixed) and the red gastrocnemius (FOG) but only slight changes occurred in the soleus (SO) and the white gastrocnemius (FG). Large decreases were indicated in CS activity in the soleus (SO), plantaris (mixed) and the red gastrocnemius (FOG) muscles (Noble and Ianuzzo, 1985).

Adaptations in lipid metabolism in response to DM is common in both humans and animals. Increases in lipolysis and free fatty acid mobilization are clearly apparent. The up-regulation of beta oxidation in skeletal muscle is evident in elevations of 3-hydroxyacyl CoA dehydrogenase (HADH) activity. Ianuzzo and co-workers (1977) reported increases in HADH activity as great as 60% in diabetic rat diaphragm. Noble and Ianuzzo (1985) reported no significant change in HADH activity of chronically diabetic rats, however, other studies suggested an elevation in the activity of this enzyme in all fibre types (Chen and Ianuzzo, 1982). Overall, these results indicate a fibre type specificity in beta oxidation as well.

Alterations in protein metabolism and/or synthesis due to a chronic insulin deficiency have been investigated. Goodman and Ruderman (1982) describe a general perturbation of protein metabolism with marked protein catabolism. Due to the function of

insulin in protein anabolism, the apparent reduction in DM causes protein degradation to exceed protein synthesis. Ultimately, a net loss of protein will ensue and muscle tissue will be wasted (Ganong, 1983; Goodman and Ruderman, 1982). The degradation of protein elevates the levels of circulating urea and amino acid as well as increases the loss of nitrogen in the urine (Goodman and Ruderman, 1982). The circulating amino acids can be utilized for synthesis of glucose through gluconeogenesis, thus exacerbating the hyperglycemic condition.

CHANGES IN METABOLISM ASSOCIATED WITH PREGNANCY.

Pregnancy can be described as an *in vivo* correlate of tissue culture *in vitro* (Freinkel and Metzger, 1979; Hollingsworth, 1983). The mother provides metabolic substrates for the developing conceptus in a fashion similar to that of the tissue culture medium as it provides nutrients to the growing tissue. Early in pregnancy the maternal system is preparing fuel stores, such as fat, through enhanced food intake. During late pregnancy, the placenta and the fetus undergo marked growth. As suggested in the tissue culture paradigm, the regulation of fetal fuels by maternal controllers has potential implications for fetal, neonatal and even adult offspring development and maturation.

Modifications in maternal metabolism occur during pregnancy in an attempt to meet the energy requirements of the developing fetus. Glucose homeostasis is progressively altered throughout the duration of pregnancy with maximal effects occuring during the last portion of gestation. Additionally, changes in lipid metabolism

during the late gestational period have been clearly defined (Herrera, Gomez-Coronado and Lasuncion, 1987; Herrera, Knopp and Freinkel, 1969; Russ, Eder and Barr, 1954). Furthermore, the diabetogenicity of late pregnancy is well documented (Cousins, 1991; Freinkel, 1980; Freinkel and Metzger, 1979; Horton, 1991; Leturque, Hauguel, Ferre and Girard, 1987). Increased insulin resistance or decreased insulin sensitivity are both mechanisms which researchers speculate to be the cause of the diabetogenicity associated with pregnancy (Cousins, 1991).

During normal pregnancy, glucose mediated insulin secretion is progressively enhanced (Cousins, Rigg, Hollingsworth, Brink, Aurand and Yen, 1980; Spellacy and Goetz, 1963). Evaluation of the long term responses of insulin and glucose during normal pregnancy reveal increased sensitivity to exogenous insulin during the first trimester, followed by increased serum insulin-glucose ratios over the later portion of gestation (Cousins, 1991; Yen, 1973). Research on glucose, insulin and C-peptide levels over a 24 hour time period during late pregnancy indicates that the insulin-glucose ratio in the fasting condition is significantly increased (Cousins et al., 1980). Furthermore, the literature suggests that insulin sensitivity is also decreased in late pregnancy period. Generally, clinicians and researchers agree that enhanced insulin resistance is also prevalent during late pregnancy, although longitudinal data evaluating this phenomenon is limited.

The interrelationship between the mother and the conceptus is intregal to the alteration in maternal metabolism associated with pregnancy. The following section of this review will provide an

overview of the literature regarding the triangular metabolic arrangement between mother, placenta and fetus which attempts to provide adequate substrates to the growing conceptus.

The interplay between the maternal hormone insulin and placental hormones such as progesterone, estrogen, and human chorionic somatomammotrophin (*h*CS or human placental lactogen *h*PL) have been evaluated as potential causes of the diminished peripheral insulin sensitivity and enhanced insulin secretion observed in pregnancy. Various authors implicate the placenta in controlling fetal nutrition relative to maternal substrates (Hay, 1991; Hollingsworth, 1983). The placenta functions as a complex endocrine gland which plays a role in the delivery and regulation of maternal fuels to the growing fetus. Fetal growth is determined by an integrated process of transmission of maternal fuels to the fetus (Figure 2), therefore the placenta has been described as a fuel modulator (Hay, 1991; Hollingsworth, 1983).

Glucose passes across the placenta by a process of gradient dependent, saturable, carrier mediated transport (Hay, 1991). Hay (1991) describes a three pool glucose model during pregnancy. Glucose is contained in one of three pools, either in the maternal, placental or fetal pool. The transfer of glucose occurs between these general segments of glucose. The transport of glucose is mediated by a group of proteins called glucose transporters (Horton, 1991). These structures carry glucose from one tissue to another.

Amino acids passage requires energy for transporter to concentrate the particles within the intracellular portion of the placenta. Subsequently, the amino acids pass to the fetal system

down a concentration gradient. Finally, lipds cross the placenta by several pathways: transporters carry the free fatty acids directly across, free fatty acids are synthesized into complex lipid within the placenta and then released, or larger lipid substances are hydrolyzed into free fatty acids followed by their release. Insulin does not cross the placental barrier but remains within its original pool - maternal or fetal (Freinkel, 1980). Therefore, any alteration in either pool of insulin will result in a direct perturbation of only one physiological system.

Release of *h*CS, progesterone and estrogen parallel the growth of the fetus and the decline in insulin action in the maternal system. Each of these hormones has been shown to affect both pancreatic insulin secretion and target cell responsiveness to insulin (Felig, 1977; Kalkhoff and Kim, 1979; Kalkhoff, Kissebah and Kim, 1978; Josimovich and MacLaren, 1962; Josimovich, Kosor, Bocella, Mintz and Hutchinson, 1970; Saxena, Emerson and Selenkow, 1969).

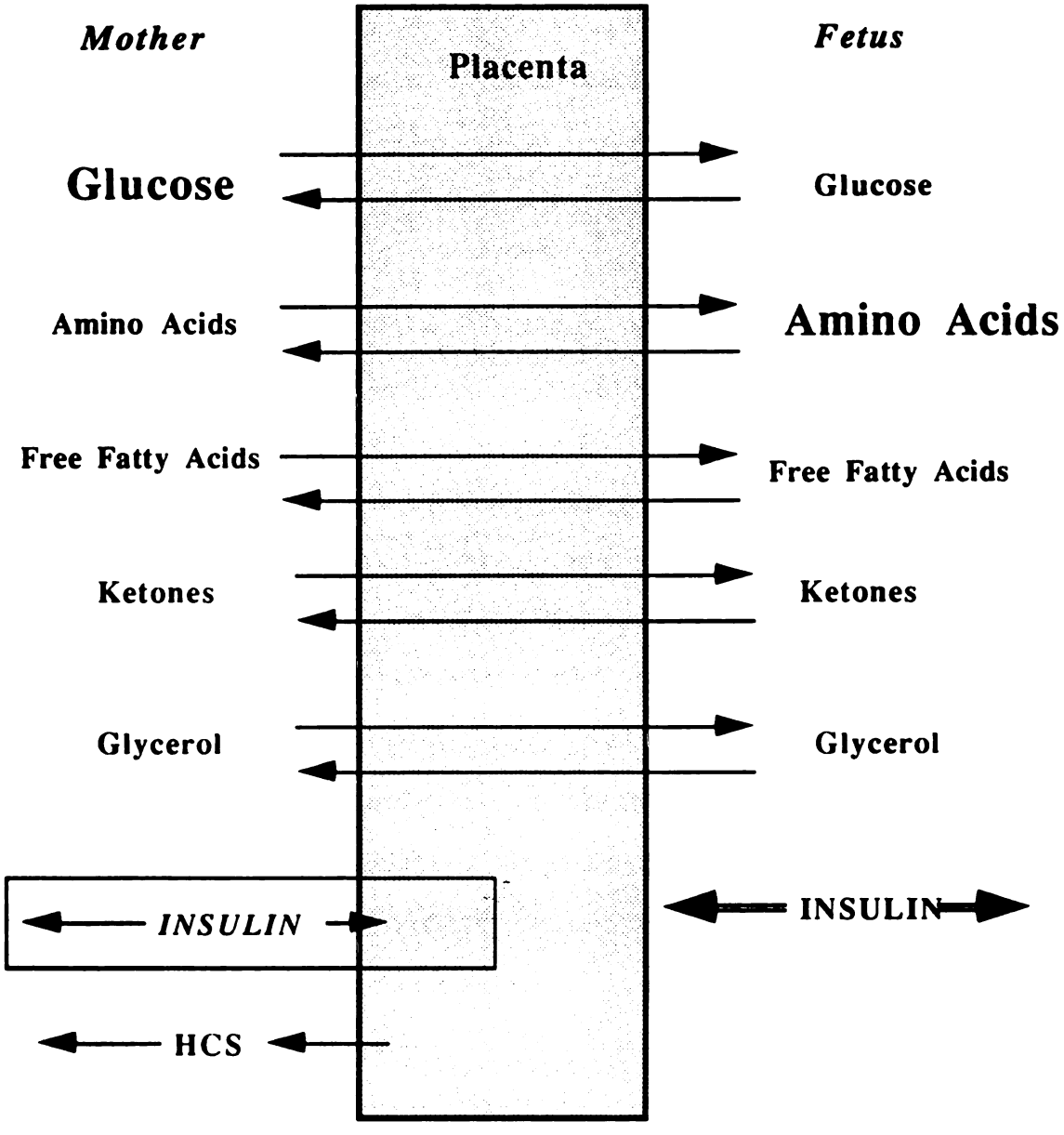
Human chorionic somatomammotropin (*h*CS) or human placental lactogen (*h*PL) is a peptide synthesized and released by the placenta. *h*CS is both immunochemically and biologically similar to pituitary growth hormone and possess both insulinotropic and lipolytic capabilities (Josmovich and MacLaren, 1962; Kaplan and Grumbach, 1981). Specifically, *h*CS causes enhanced glucose induced insulin release *in vitro*, altered glucose to insulin ratio following glucose administration (Beck and Daughaday, 1967; Josmovich et al., 1970; Kalkhoff, Richardson and Beck, 1969) and decreased insulin sensitivity in hypophysectomized rats (Kaplan and Grumbach, 1981). Although the function and control of *h*CS in human gestation is

unclear, research suggests that *h*CS may modulate the metabolic function of the maternal-placental regulation of fetal fuels especially glucose (Kaplan and Grumbach 1981). Furthermore, serum levels of *h*CS in pregnant diabetics are directly correlated with elevations in placental weight (Josimovich et al., 1970; Saxena, Emerson and Selenkow, 1969). Several other anabolic placental hormones are secreted during gestation such as (a) corticotropin, (b) lipotropin, (c) beta-endorphin and (d) vasoactive intestinal peptide (Hollingsworth, 1983). However, the function of these hormones remains unclear.

Progesterone administration has been shown to enhance the levels of insulin secretion (Kalkhoff, Jacobson and Lemper, 1970) as well as increase gluconeogenesis (Landau and Lugibihl, 1967). The role of estrogen, the complimentary hormone to progesterone, has yet to be clearly defined. It has been suggested that *h*CS, progesterone, and estrogen function to augment insulin secretion while depressing insulin action in the periphery (Freinkel, 1980).

As illustrated in Figure 2.1, transplacental transfer of nutrients plays a central role in the growth and development of the fetus. Each of the primary fuel sources (proteins, fats and carbohydrates) is passed from the maternal component to the fetal component by various means of transport. The present work focusses on glucose metabolism, yet both fat and protein substrates function in collaboration with the metabolism of carbohydrates. Therefore, each of the aforementioned fuel sources will be discussed, with glucose transfer being the central point.

Figure 2.1. Integration of Maternal, Placental, and Fetal Fuel Sources During Normal Pregnancy.



(Freinkel, 1980)

Amino acids are essential to the adequate growth and development of the conceptus. Transport of amino acids from the maternal plasma into the placental trophoblasts occurs via active transport (Phelps, Metzger and Freinkel, 1981). Passage into the fetal system is dependent upon a decreasing concentration gradient (Hill and Young, 1973). The energy dependent transport of amino acids can be reduced through inhibition of glycolysis and oxidative metabolism (Hay, 1991).

Metabolism of proteins can occur through multiple pathways such as gluconeogenesis, glycogenesis, protein synthesis, oxidation and ammoniagenesis (Edwards, Rattenbury, Varnam, Dhand, Jeacock and Shepherd, 1977). Additionally, the placenta utilizes amino acids in a variety of other capacities including (a) oxidation, (b) production of other amino acids by transamination pathways and (c) synthesis of placental hormones (chorionic gonadotropin, luteinizing hormone and human placental lactogen) (Hay, 1991). Studies indicate that pathways exist for placental-fetal amino acid cycling of specific amino acids. This is a cooperative process important in fetal metabolism (Hay, 1991; Marconi, Sparks, Battaglia and Meschia, 1989). Presently, the consequences of placental-fetal amino acid and ammonia cycling are unknown. The potential negative effects on normal fetal growth and development due to pathological conditions have yet to be determined.

Maternal, placental and fetal lipid metabolism is species dependent. Maternal (human and rat) body fat accumulates during the first half of pregnancy followed by maintenance of this "new" body composition for the remainder of the gestational period (Gorski,

1983; Lopez-Luna, Monoz and Herrera, 1986). Elevations in maternal levels of circulating free fatty acids (FFA), plasma triglycerides (TG) and very low density lipoproteins (VLDL) are common throughout pregnancy with highest concentrations occurring during the third trimester (2-4 times normal in FFA and 3 times normal in TG - human) (Arguiles and Herrera, 1981; Gorski, 1983; Knopp et al., 1973).

Flux of lipids across the placenta occur by three primary processes: (a) direct transfer of fatty acids, (b) synthesis of lipids from fatty acids within the placenta coupled with their release, and (c) hydrolysis of maternal triglycerides, lipoproteins and phospholipids followed by release of fatty acids into fetal circulation (Coleman, 1986). Transfer of lipids across the placenta is hindered during the third trimester, therefore, the elevation in plasma lipids suggests maternal utilization of this substrate (Herrera, Gomez-Coronado and Lasuncion, 1987).

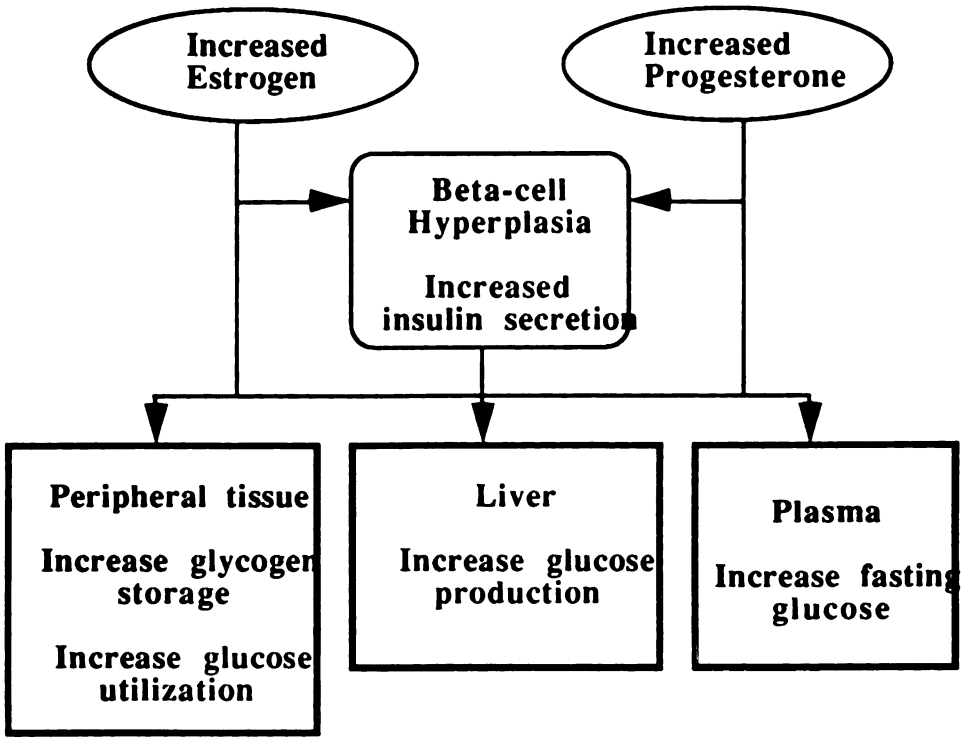
Unlike the maternal timecourse, fetal fat deposition increases primarily during the second half of gestation (Sparks, Girand and Battaglia, 1980). Fetal fat content varies in direct relation to placental lipid transport (Hay, 1991). Once within the fetus, lipid metabolism includes esterification of triglycerides with subsequent deposition into brown and white adipose tissue, utilization in membrane synthesis and oxidation for energy production (Coleman, 1986; Hay, 1991). Additionally, lipids modulate transfer of other metabolites to the fetus (Herrera, Gomez-Coronado and Lasuncion, 1987) whereby the entire nutrient milieau is affected by passage of lipids across the placenta..

Major alterations in maternal, placental and fetal fat metabolism are described in the following section. Triglyceride formation is elevated in the liver, facilitated by enhanced adipose tissue lipolysis which ultimately increases the levels of triglycerides and VLDL in the plasma. Concomitant with this process, maternal hyperphagia causes augmented dietary intake of fats thereby increasing the chylomicron triglycerides; the synergistic effects resulting in hypertriglyceridemia (Herrera, Gomez-Coronado and Lasuncion, 1987). Hypertriglyceridemia prevails throughout pregnancy due to the reduction of adipose tissue lipoprotein lipase activity coupled with the enhancement of triglyceride mobilization and dietary intake of fats. Fats serve to augment carbohydrates as both a maternal and fetal fuel source especially during periods of maternal food restriction. During these periods, reduced glucose stimulates ketogenesis. The ketogenic products along with glycerol from the adipose tissue are utilized to provide adequate maternal and fetal fuels (Herrera, Gomez-Coronado and Lasuncion, 1987; Metzger and Freinkel, 1987).

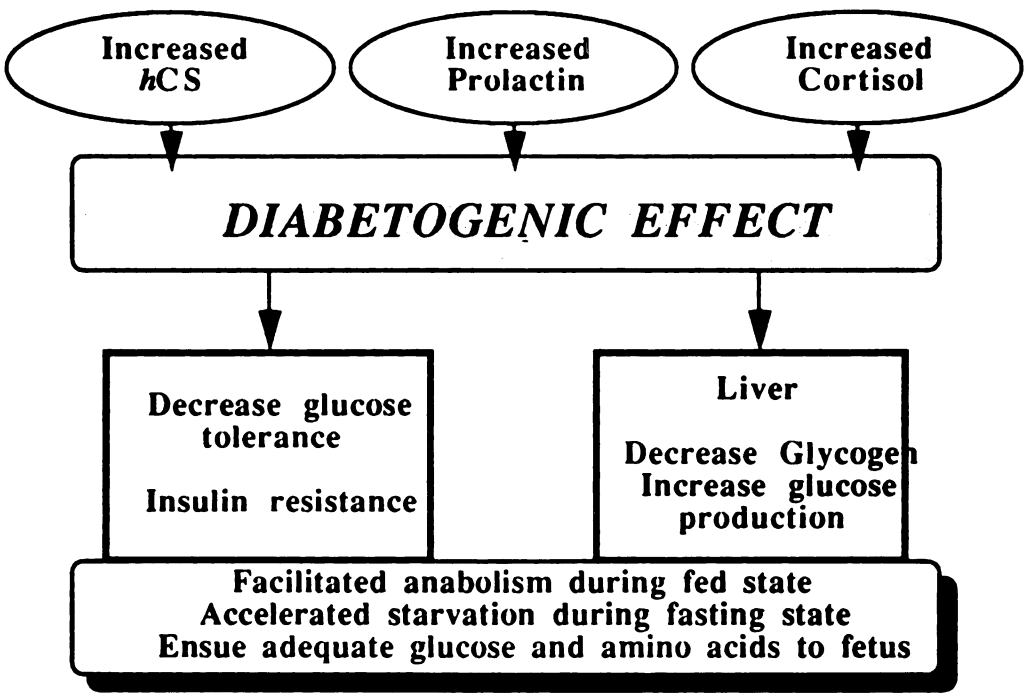
Maternal carbohydrate metabolism is controlled throughout pregnancy by alterations in various hormones. Figure 2.2 illustrates the multiple factors affecting glucose metabolism in humans during both early (<20 weeks) and late (20-40 weeks) gestation. Initially, increased maternal serum estrogen and progesterone levels act on the pancreas increasing insulin secretion with resultant beta cell hyperplasia (Figure 2.2a). The resultant hyperinsulinemia causes increases in tissue glycogen storage, decreased hepatic glucose production, increased peripheral glucose

Figure 2.2. Carbohydrate Metabolism During Normal Pregnancy from 0-20 Weeks (A) and from 20-40 Weeks (B) Gestation. (Hollingsworth, 1983).

A. Early metabolism (< 20 weeks)



B. Late metabolism (20 -40 weeks)



utilization and decreased fasting plasma glucose levels (Costrini and Kalkhoff, 1971). Continuing pregnancy alters glucose metabolism due to the enhanced fetal demand for adequate fuels. The increased demand during late pregnancy is primarily due to elevations in *h*CS, prolactin and cortisol (Freinkel, 1980). These hormones precipitate a maternal diabetogenic response evidenced by depressed glucose tolerance, insulin resistance, decreased hepatic glycogen stores and increased hepatic glucose production (Hollingsworth, 1983).

In summary, alterations in metabolism during pregnancy are designed to provide an adequate supply of substrates to the developing and maturing fetus. Transplacental flux of the various substrates is controlled by placental and maternal hormones, with the placenta playing a critical role in controlling the fetal metabolic mileau.

EXERCISE ASSOCIATED ALTERATIONS DURING PREGNANCY.

Metabolic adaptations during pregnancy are triggered and controlled by endocrine factors. The organ systems most frequently evaluated by researchers for alterations/adaptations include the cardiovascular, pulmonary and metabolic systems. Various authors report that exercise during pregnancy presents a dichotomous physiological situation (Lotgering, Gilbert and Longo, 1985).

The interaction of the two physiological stresses (pregnancy and exercise) may result in a convergence of maternal and fetal metabolic needs (Lotgering, Gilbert and Longo, 1985). Changes in

metabolism caused by exercise and pregnacy are the focus of this section.

Fetal utilization of maternal glucose is the primary energy substrate for growth and maturation (Jones and Rolph, 1985). Treadway and Young (1989) evaluated glucose uptake by the fetus following an acute bout of maternal exercise. Glucose and a [1-^{3}H]2-deoxyglucose tracer were injected post exercise with subsequent analysis of maternal and fetal tissues for the radioactive label. The authors reported increased glucose uptake in the skeletal muscle of untrained pregnant rats following acute exercise. Additionally, the authors found decreased fetal glucose uptake under the same conditions when compared with control animals. These results suggest potential negative effects of exercise on fetal fuel substrate availability (Treadway and Young, 1989). Conversely, Bell and associates (1983) and Chandler and colleagues (1985) reported fetal hyperglycemia during acute exercise in pregnant ewes. Initially they suggested that increased umbilical uptake of maternal glucose was the mechanism controlling fetal glucose elevations (Bell et al., 1983). In subsequent work the authors were unable to fully explain the mechanism by blood flow alterations occurred. However, they described various postulates such as decreased fetal glucose utilization or hepatic glycogenolysis as a potential reason for their results (Chandler et al., 1985).

Further evaluation of carbohydrate metabolism during exercise typically focuses on the utilization of glycogen as a metabolic substrate. The effects of exercise on both maternal and fetal glycogen stores is controversial. Acute exercise on day 20 of

gestation reportedly caused decreased maternal and fetal liver glycogen in untrained rats (Gorski, 1983). In a replication of Gorski's work, there existed a stimulus for maternal liver glycogen reduction with no alteration in fetal rat liver glycogen stores (Carlson et al., 1986). Mottola and co-workers (1988) evaluated chronic exercise training in rats both prior to, and during gestation, on maternal and fetal glucose and glycogen levels and reported significant reductions in fetal liver glycogen. Further work by Mottola and Christopher (1991) assessed liver and skeletal muscle glycogen content under five conditions (control, pregnant non-running control, pregnant control, pregnant running and non-pregnant running) in the rat. The authors suggested that the decreased liver glycogen in pregnant non-running rats was due to enhanced liver glucose synthesis to meet maternal energy demands. In addition, the pregnant exercising animals showed neither augmented nor blunted resting liver glycogen levels as is typical in non-pregnant runners and normal pregnancy, respectively (Mottola and Christopher, 1991). These results were similar to those resported in previous work (Baldwin, Fitts, Booth, Winder and Holloszy, 1975; Jones and Rolph, 1985) and suggested a normalization of maternal liver glycogen when exercise and pregnancy are synergistically combined (Mottola and Christopher, 1991).

Alterations which are observed in skeletal muscle glycogen content are indicative of muscle, or fibre type specificity, in response to exercise in pregnant and non-pregnant rats (Mottola and Christopher, 1991). Soleus muscle glycogen storage was unchanged in any treatment combination of exercise and pregnancy. However,

in the same study, Mottola and Christopher (1991) reported increased glycogen content in both the red and white gastrocnemius of exercised non-pregnant animals. Furthermore, chronically exercising pregnant mothers had elevated glycogen in the red gastrocnemius with moderate enhancements in the white portion of the muscle coupled with increased size of the muscle (Mottola and Christopher, 1991). Finally, this work provided atypical results in non-exercising pregnant animals whereby the glycogen content in both red and white gastrocnemius increased without a concommitant increase in muscle size. These, and other authors suggest that this phenomenon is caused by a reduced insulin insensitivity in postural muscles, resulting in enhanced glucose uptake and glycogen storage in those muscles (Mottola and Christopher, 1991; Treadway and Young, 1989).

Lactate, the byproduct of anaerobic glycolysis, tends to increase during maternal exercise in both maternal and fetal blood (Bell et al., 1983; Chandler et al., 1985). Fetal lactate concentration is moderately elevated as a function of increased rates of tissue glycolysis stimulated by increased levels of blood glucose, and relative fetal hypoxia (Bell et al., 1983; Chandler et al., 1985). These alterations in anaerobic glycolysis are typical during acute bouts of exercise during pregnancy.

Although maternal exercise during pregnancy provides benefits to the mother, it is unclear as to the influence of exercise on fetal growth and development. Research is conflicting regarding fetal outcome and maturation. Human studies present data suggesting beneficial effects (Hall and Kaufman, 1987), negative effects (Clapp

and Dickstein, 1984; Dale, Mullinax and Bryan, 1982), or no effects (Collings, Curet and Mullin, 1983; Kulpa, White and Visscher, 1987; Lutter, Lee and Cushman, 1984), on fetal outcome.

Research using animal models presents conflicting results similar to what has been indicated with human data. Adverse effects of four to eight weeks of endurance training on fetal outcome include decreased fetal weight and increased resorption sites or greater fetal mortality (Wilson and Gisolfi, 1980; Terada, 1974). Other research reports no significant effects of four weeks of maternal training prior to, and during gestation on fetal outcome in the rat (Bagnall, Mottola and McFadden, 1983; Mottola, Bagnall and McFadden; 1983; Mottola, Bagnell, Belcastro, Foster and Secord, 1986; Mottola and Christopher, 1991; Rodgers, Mottola, Corbett and Taylor, 1991) in the rat.

Literature regarding the effects of maternal exercise on fetal/neonatal muscle metabolism is limited. Various authors have evaluated the response of fetal cardiac muscle to maternal exercise and have shown indirect training effects resulting in both contractile and structural alterations (Bonner, Buffington, Newman, Farrar and Acosta, 1978; Parizkova, 1975; Parizkova and Petrasek, 1978). Parizkova (1975) found enhanced structural parameters including increased capillary number, increased muscle fibre size and increased capillary to fibre ratio in the hearts of 50-100 day old offspring from trained mothers (treadmill walking at 40% maximal oxygen consumption). Bonner and co-workers (1978) evaluated myocardial tissue from five day old neonates *in vitro*. The offspring were from either untrained or swim trained mothers (30-40 minutes per day at 50% of maximal aerobic power). The functional

adjustment in Bonner's work (1978) suggested a reduction in intrinsic contractile activity of cultured fetal cardiac cells from exercised mothers. In a recent paper regarding alterations in skeletal muscle, Rodgers and co-workers (1991) reported that chronic maternal training does not appear to affect skeletal muscle metabolism in neonates. Animals in this study trained for four weeks prior to, and during pregnancy at 26.8 m/min, 1 hour/day, 5 days per week which was twice the speed reported by Parizkova (1975). It is important to point out however, that the animals in the aforementioned work were twenty-eight days old at the time of tissue harvesting. This factor may have affected the results due to the possible detraining effects in the offspring during the postpartum period.

INTERACTIVE CHANGES IN METABOLISM IN A DIABETIC PREGNANCY.

A. Maternal and Fetal Alterations.

Freinkel (1980) describes pregnancy as creating a complication for the diabetic individual and refers to this complication as a "fascinating connundra in cell biology". Early pregnancy is typically a period of maternal weight gain while the later portion of gestation is a period of fetal growth and development. Classically, the later period presents a physiological situation that is highly diabetogenic in nature (Freinkel, 1980). During this period, diabetic patients experience increased exogenous insulin requirements, a portion of

non-diabetic individuals are diagnosed with gestational diabetes, and insulin resistance increases in all pregnancies (Freinkel, 1980; Hollingsworth, 1983).

As stated previously, fetal substrate demands can modify or be modified by maternal fuel availability during pregnancy. Due to the concentration-dependent nature of transplacental passage of fuel substrates, diffusion to the fetal system would be enhanced during heightened maternal supply postprandially (Freinkel, 1980). When the mother is in a fed state, she can be described as hyperglycemic. This condition allows for enhanced glucose transfer to the fetus. Additionally, the rise in plasma triglycerides augments the glucose transfer by providing alternative fuel for maternal metabolism (Freinkel, 1980; Hollingsworth, 1983). Freinkel (1964) described maternal metabolism during fed conditions as "facilitated anabolism".

During the period of relative fasting, the maternal system can siphon fuels from the fetus in an attempt to maintain maternal homeostasis. Therefore, researchers have suggested that augmented maternal fat utilization would spare critical fetal nutrients during these periods, a process described as "accelerated starvation" (Freinkel, 1980). Maternal hormones elevated during pregnancy have been related to the initiation of the "accelerated starvation" process (Buse, Roberts and Buse, 1962; Freinkel, 1980; Herrera, Knopp and Freinkel, 1969; Hollingsworth, 1983). The implications both clinical and practical relative to "accelerated starvation", have been investigated by various groups. Short duration fasting such as avoiding breakfast during pregnancy can result in major metabolic alterations at any point during late pregnancy (Freinkel, 1980).

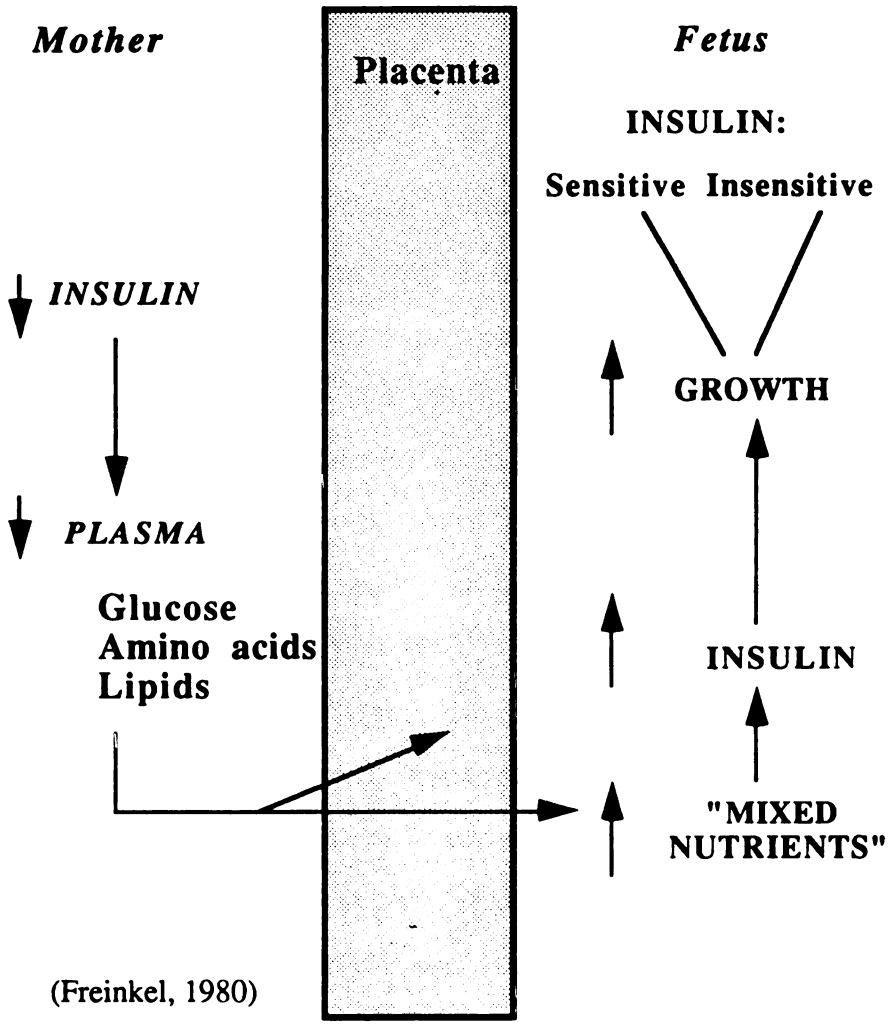
Throughout gestation, oscillations of "accelerated starvation" and "facilitated anabolism" occur regularly (Freinkel and Metzger, 1975). The physiological perturbations associated with these oscillations in a diabetic pregnancy present serious clinical problems. Administration of exogenous insulin in such a manner as to normalize the cyclic fuel levels is critical in perinatal care of mother and fetus, and postnatal outcome of the offspring (Freinkel, 1980).

Hormonal and metabolic alterations are distinctly different in Type I and Type II diabetic pregnant subjects. Metabolism of IDDM females is more metabolically perturbable than NIDDM women (Hollingsworth, 1983). The alterations seen during diabetic pregnancies can be linked with modified fetal growth.

Figure 2.3 illustrates the Pedersen hypothesis developed in the early sixties (Pedersen, 1967). Pedersen hypothesized that pregnancy provided a condition of maternal hyperglycemia which resulted in fetal hyperinsulinemia and fetal macrosomia. Further evaluation of this hypothesis by Freinkel and Metzger (1979) suggested that inadequate insulin release in response to maternal feeding may result in failure to utilize metabolic substrates (Figure 2.3). Ultimately the mother has elevated glucose, lipid and amino acid levels (Freinkel, 1980). These changes affect the placenta causing increased fetal nutrition with concomitant fetal growth, ultimately

Figu

Figure 2.3. Redefined Hyperglycemia-Hyperinsulinemia Hypothesis.



(Freinkel, 1980)

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resulting in the classic macrosomic state often observed in newborns of diabetic mothers.

During early gestation, oscillatory hypo- and hyperglycemia result in abrupt changes in the availability of glucose to the developing fetus. Additionally, increased levels of circulating ketone bodies and branched-chain amino acids are evident in the maternal circulation (Eriksson, Borg, Forsberg and Styrud, 1991; Pedersen, 1977). Altered fuel substrates could impact organogensis which occurs within the first six weeks postconception (Eriksson et al., 1991; Metzger, 1991; Mills, 1982; Mills, Baker and Goldman, 1979; Tanigawa, Kawaguchi, Tanaka and Kato, 1991). Alterations during mid and late gestational periods could be damaging to other tissues including brain cells, muscle cells, adipocytes and pancreatic beta cells (Freinkel, 1980; Metzger, 1991). In addition, recent evidence suggests that metabolic alterations during this period could influence behavior patterns (IQ, alertness, concentration) in infants and children (Johansson, Meyerson and Ericksson, 1991; Silverman, Rizzo, Green, Cho, Winter, Ogata, Richards and Metzger, 1991). Altered fuel for fetal utilization has clearly been linked with developmental alterations in both animals and humans.

Pedersen (1977) described the influence of diabetes on fetal outcome as macrosomia or neonatal obesity. Two years later, Freinkel and Metzger (1979) modified Pedersen's theory by incorporating the multiple effects of other nutrients aside from glucose. In his Banting Lecture in 1980, Freinkel described a condition typical of diabetic pregnancy which he called fuel-mediated teratogenesis (Freinkel, 1980). Freinkel aggressively

pursued this concept of altered fetal development beyond traditional teratology (anomalies or permanent anatomical alterations due to events during development) to incorporate anatomical, physiological and behavioral changes (Figure 2.4). Offspring of IDDM subjects are at risk throughout pregnancy while the offspring of GDM mothers are subject to heightened risk primarily during late pregnancy (Freinkel, 1980; Hollingsworth, 1983). Ultimately, the interaction of pregnancy and diabetes is not without complication and presents varied physiological alterations yet to be completely understood.

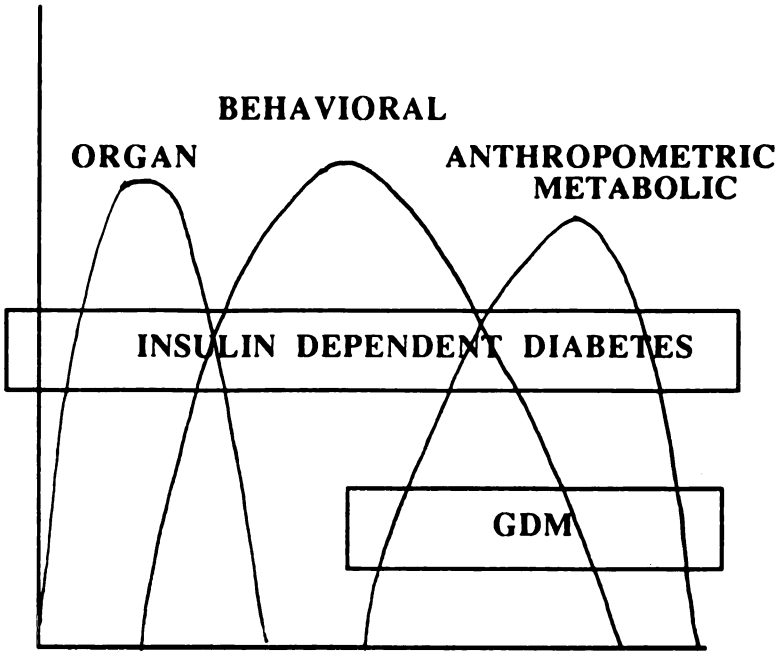
B. Neonatal Alterations.

Incidence of congenital malformations in the offspring of diabetic (IDDM) mothers is approximately three to four times greater than in non-diabetic individuals. Rosenn and colleagues (1990) evaluated 171 insulin-dependent diabetic humans relative to the interaction between glycemic control and congenital malformations in their offspring. Glycosylated hemoglobin (HbA_{1c}) levels in the blood were utilized as an indicator of the degree of hyperglycemia. The researchers reported that poor glycemic control during the later portion of pregnancy increased the risk of neonatal abnormalities. Further work in humans by Kitzmiller and associates (1991) supported the findings of Rosenn. Utilizing the HbA_{1c} method, the physicians reported enhanced risk for congenital abnormalities in infants from mothers with poor glycemic control during gestation.

Brownscheidle and co-workers (1983) evaluated the interaction between the pregnant diabetic state and various exogenous insulin infusions on fetal development and neonatal

Figure 2.4. Temporal Relationship of Development/Maturation to Fuel-Mediated Teratogenesis.

POTENTIAL TERATOLOGY



outcome in spontaneously diabetic BB Wistar rats. These authors reported that control of glucose homeostasis in the BB rats was related to increased litter and fetal size, decreased mortality, reduced congential malformations, enhanced postnatal growth and normal neurological maturation. Moreover, rat newborns rendered experimentally macrosomic by insulin infusion, remained larger than their control counterparts through the first eight weeks postpartum (Cha, Gelardi and Oh, 1987). Following the eighth week the animals from both groups were similar in size.

Beyond the morphological condition of the offspring which is affected by DM, researchers have debated the role of both the genetic component and the uterine environmental component on glucose homeostasis in the offspring of diabetic mothers. Kervran and associates (1978) experimentally induced DM in virgin rats with an intravenous injection of 30-50 mg/kg body weight STZ. Following inducement of DM, the animals were bred and allowed to carry the fetus until day 19.5-21.5 of gestation at which time the fetuses were evaluated for morphological and metabolic status. Fetuses from severely diabetic dams were slightly smaller than control offspring. In addition, the fetuses from the severely diabetic mothers demonstrated impaired insulin synthesis and secretion (Kervran, Guillaume and Jost, 1978). These results were supported by further animal and human studies. STZ diabetic pregnant rats had decreased litter sizes, elevated blood glucose levels, increased glucose to insulin ratios, small fetal body sizes and reduced pancreatic insulin levels versus control animals (Eriksson, Andersson, Efendic, Elde and Hellerstrom, 1980).

Data from human studies indicated an attenuated glucose uptake in response to a glucose load in infants from IDDM mothers (King, Tserng and Kalhan, 1982). Artal and colleagues (1988) assessed other hormonal parameters involved in glucose homeostasis and reported depressed glucagon and catecholamine levels in offspring from diabetic mothers. The depression in these hormones, which assist in regulating glucose during periods of hypoglycemia, may contribute to the neonatal hypoglycemia typical of infants born of diabetic mothers.

Research attempting to elucidate the mechanism controlling altered glucose homeostasis in offspring from diabetic mothers has been increasing over the past decade. Placental control of fetal fuel substrates was investigated using STZ diabetic rats in a study that reported that the diabetic state did not alter transplacental passage of fuels to the fetus (Herrera, Palacin, Martin and Lasuncion, 1985). Furthermore, the flux of glucose is primarily concentration gradient dependent which is responsible for the elevated transfer of glucose to the fetus during maternal hyperglycemia. Due to the limited ability of the fetus to handle glucose, this elevation in glucose flux may augment the potential diabetogenic effects on the fetus. One potential limitation of the aforementioned work was the inducement of DM on the eighth day of gestation. The teratogenic effects of STZ administration at this point during pregnancy add a potential for confounding the results.

Further evaluation of the mechamisms controlling the diabetogenic influences of maternal DM on neonatal outcome has concentrated on the intrauterine metabolic mileau. First generation

offspring from rats infused with glucose during pregnancy were found to have depressed insulin release in response to a glucose load as well as mild hyperglycemia (Bihoreau, Ktorza, Kinebanyan and Picon, 1986). Insulin release in response to glucose and arginine was reduced in newborns from mildly STZ diabetic dams while the neonates from severely STZ diabetic mothers demonstrated elevated insulin secretion under similar conditions. Glucose tolerance *in vivo* was normal in the offspring from the severely diabetic dams but insulin resistance was evident as indicated by an elevation in the insulin to glucose ratio (Aerts, Sodoyez-Goffaux, Sodoyez, Malaisse and vanAssche, 1988).

Recently, Gaugier and colleagues (1990) evaluated a simulated moderate hyperglycemic state during pregnancy (gestation day 15 through term) in non-diabetic dams. They reported that the newborns from these hyperglycemic mothers showed the main features of newborns from diabetic mothers (hyperglycemia, hyperinsulinemia and macrosomia). These metabolic alterations persisted into adulthood. Subsequent work by Grill and co-workers (1991) and vanAssche and associates (1991) evaluated maternal fuel metabolism and its role in diabetogensis without genetic influences. Both groups used STZ (Grill 40 mg/kg body wt; vanAssche 30 mg/kg body wt) prior to conception to induce the diabetic condition. Results indicated that severe diabetes during gestation causes decreased insulin sensitivity in the offspring (Grill, Johansson, Jalkanen and Ericksson, 1991). Similarily, vanAssche's group stated that offspring from diabetic dams demonstrated hyperglycemia and hyperinsulinemia as well as increased amino acid metabolism. As

adults, these animals had decreased glucose tolerance in response to a glucose load with enhanced protein metabolism (vanAssche, Aerts and Holemans, 1991).

In summary, these data suggest that a perturbed uterine environment can alter fetal outcome without the presence of a genetic link thus emphasizing that the effects of diabetes on both maternal and fetal physiological function.

EXERCISE INDUCED ALTERATIONS IN DIABETICS.

Endurance training in euglycemic subjects causes decreased insulin and increased glucagon secretion. These responses are postulated to be controlled by the decreased catecholamine response during exercise (McKardle, Katch and Katch, 1989). In addition, training enhances insulin sensivitiy in the skeletal muscle tissue which has been shown to be a standard characteristic of endurance exercise training (Wallberg-Hendriksson, 1986). Figure 2.5 illustrates the metabolic fuels and hormonal alterations in response to exercise in normal subjects. It is clear that glucose release by the liver and uptake by the peripheral tissues (muscle) is increased during exercise stress under normal conditions.

Various researchers have suggested that endurance exercise can have beneficial effects on the diabetic condition. IDDM present a difficult clinical situation whereby the individual must utilize exercise as a theraputic modalitity controlling IDDM while avoiding major acute complications such as exercise induced hypoglycemia (Zinman, 1986).

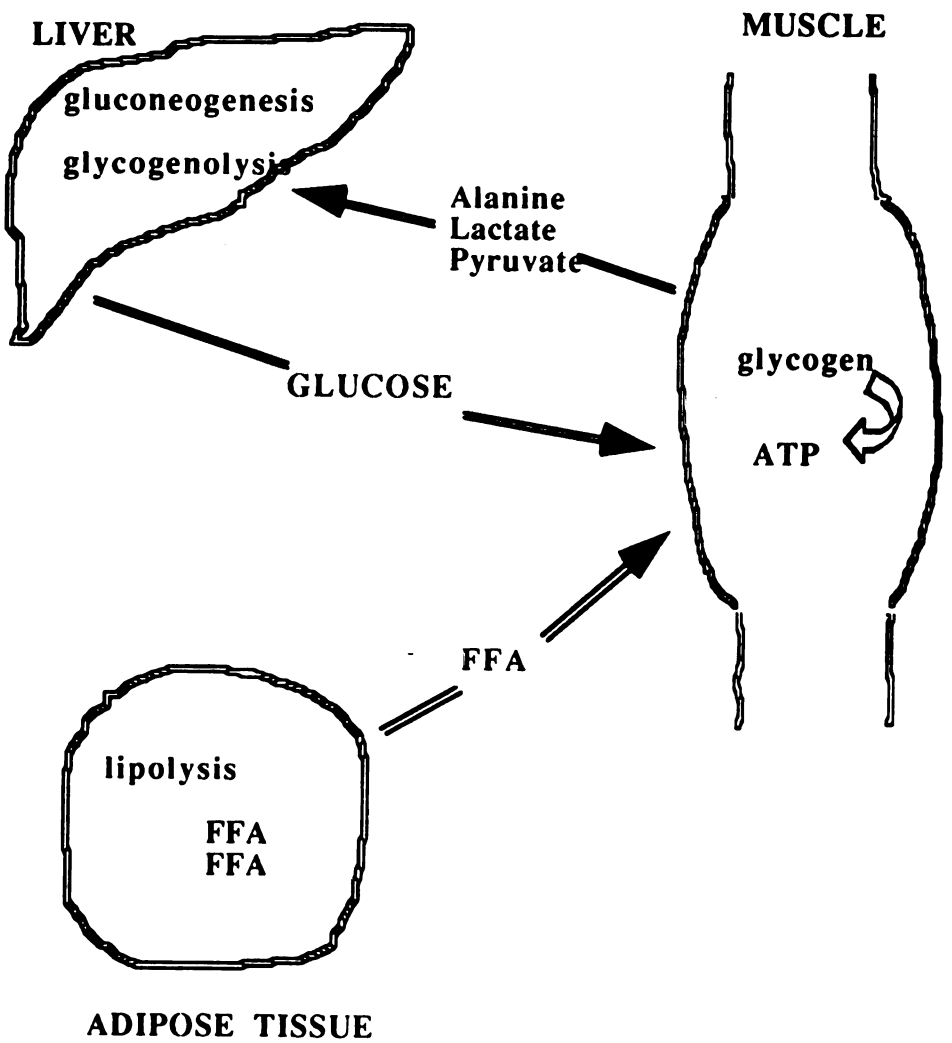
Exercise performed in individuals utilizing traditional exogenous insulin injections causes differing responses dependent upon the insulin level prior to exercise. When excess insulin is present, hepatic glucose production, plasma glucose concentration and free fatty acid production are reduced, while muscle glucose utilization is enhanced (Zinman, 1986). Under conditions of insulin deficiency muscle glucose utilization remains unchanged with concommitant elevations in hepatic glucose production, plasma glucose concentration and blood ketone production (Zinman, Zuniga-Guajardo and Kelly, 1984; Zinman, 1986). If the subcutaneous exogenous insulin depot is in an exercising appendage, rapid depletion of the depot will quickly occur during exercise and results in the overall state of hyperglycemia (Zinman, Murray, Vranic, Albisser, Beibel, McClean and Marliss, 1977). Recent use of osmotic insulin pumps aids in maintaining glycemic control during exercise. The effects of chronic training on glucose homeostasis are believed to produce beneficial results yet research directly confirming this phenomenon is limited (Zinman, 1986).

Skeletal muscle metabolism under chronic insulin deficiency has been described previously in this review. To summarize, oxidative function and metabolic capacity of skeletal muscle is reduced under DM conditions (Armstrong and Ianuzzo, 1976; Chen and Ianuzzo, 1982; Gollnick and Ianuzzo, 1972). Under normal conditions, chronic training causes increased insulin sensitivity and increased aerobic capacity (Baldwin and Winder, 1977; Berger, Kemmer, Becker, Hagberg, Schwenen, Gjinavci and Berchtold, 1979; Richter, Garetto, Goodman and Ruderman, 1982). Utilization of

Figure 2.5. Hormonal and Metabolic Alterations During Exercise in Euglycemic Individuals. (Ganong, 1983)

HORMONAL ALTERATIONS

INSULIN	⇩
CATECHOLAMINE	⇧
GLUCAGON	⇧
CORTISOL	⇧



exercise as a theraputic modality during chronic insulin deficiency has been evaluated relative to skeletal muscle metabolism and was found to effect fibre type specific responses (Gollnick and Ianuzzo, 1972; Ianuzzo et al., 1984; Noble and Ianuzzo, 1985).

Ianuzzo and co-workers (1984) studied three experimental groups (normal, STZ diabetic, and STZ diabetic insulin-treated) of both trained and sedentary male rats. Enzymatic activites of various metabolic markers (HK, PHOS, PFK, and HADH) were determined in the diaphragm. The results suggested that training caused increased glycolytic and aerobic capacities. Noble and Ianuzzo (1985) continued this work in skeletal muscle. The study utilized six primary conditions: (a) normal, (b) normal trained, (c) diabetic, (d) diabetic-trained, (e) diabetic insulin treated, and (f) diabetic insulin treated trained. As in the previous work, enzymatic markers were evaluated as indicative of skeletal muscle metabolism. These authors postulated that exercise training of Type I diabetic rats mimicked the effects of insulin treatment and caused a near normalization of enzyme markers in skeletal muscle (total phosphorylase, citrate synthase, succinate dehydrogenase, HADH, phosphofructokinase, and hexokinase). In addition to the enzyme markers, Noble and Ianuzzo (1985) determined blood glucose and free fatty acid levels in the blood. Although the skeletal muscle changes were evident, blood glucose and free fatty acids were both elevated in the diabetic trained group similar to the untrained levels. Further evaluation of parameters associated with glucose homeostasis and skeletal muscle metabolism must be performed to fully elucidate the mechanisms assoicated with the alterations reported in the literature.

EXERCISE MEDIATED ALTERATIONS IN A DIABETIC PREGNANCY.

The synergistic effects of exercise, diabetes mellitus and pregnancy on physiological function present a complex puzzle of diverging and converging processes. Physical training is reported to reduce plasma insulin concentration while enhancing peripheral sensitivity to insulin in skeletal muscle and adipose tissue in both normal and NIDDM patients (Horton, 1991). Researchers have speculated as to the potential of exercise as a therapeutic modality for the diabetic patient. Research on the effects of exercise during diabetic pregnancies in both human and animal models is relatively limited to date. Generally, research has focused on the gestational diabetic population, although various studies have evaluated the effectiveness of exercise intervention in naturally occurring and experimental IDDM.

Hollingsworth and Moore (1987) evaluated glycemic control in the pregnant IDDM population. The authors reasoned that although vigorous activity has been avoided in IDDM patients during gestation, normal women are typically allowed to participate in moderate physical activity without negative consequence. They evaluated postprandial walking in 42 Type I diabetic women and 28 non-diabetic controls (Hollingsworth and Moore, 1987). Results indicated that exercise afforded enhanced glycemic control in the diabetic patients coupled with lower fasting triglyceride levels. In addition, the authors report that no adverse effects in glucose handling were

evident in IDDM pregnant females who participated in a walking program (Hollingsworth and Moore, 1987).

The results in humans have been supported in rats exposed to experimentally induced (STZ) IDDM. Urie-Hare and colleagues (1989) trained both diabetic and non-diabetic rats on a treadmill for three weeks prior to, and throughout gestation. Results indicate that the untrained diabetic dams had fetuses which were lighter, smaller and malformed. Exercise, however, blunted the typical diabetic response thereby resulting in increased fetal weight and decreased fetal morphological malformations. This work presents initial insights into the effects of chronic training on fetal outcome in experimentally induced diabetes.

The primary goal during GDM is to achieve and maintain euglycemia (Jovanovic-Peterson and Peterson, 1991). Typically, dietary manipulations are sufficient yet specific cases necessitate insulin as adjunct therapy. Recently, obstetric care began to evaluate the therapeutic potential of exercise in this population of pregnant women. Utilization of exercise that caused cardiovascular conditioning also resulted in lower levels of glucose compared with dietary manipulation alone. The alterations in glucose homeostasis were evident after four weeks of training and resulted in depressed hepatic glucose output and enhanced glucose clearance. Furthermore, the subjects were able to avoid augmented insulin through exogenous sources which the authors suggested to be a critical finding. Findings from this research were supported by the work of Bung and co-workers (1991) in the gestational diabetic.

These authors suggest individualization of exercise as a critical element in the efficacy of the exercise treatment.

In summary, physiological alterations during any one of the conditions pregnancy, exercise, or diabetes mellitus encroach on the limitations of the organism's capabilities to re-establish a functional homeostatic balance. Any combination of these stresses provides impetus for multiple converging and diverging physiological adaptations resulting in both negative and/or positive outcomes for both the mother and the offspring. Freinkel's (1980) description of diabetic pregnancy both as a "tissue culture experiment" and a "fascinating connundra in cell biology" is only enhanced by the addition of exercise training into the cell medium. The available literature as well as the apparent lack of research in various areas provides impetus for the present work.

Chapter 3

Effects of Exercise Training Prior to, and During Gestation on Maternal Glucose Handling and Offspring Outcome.

Introduction

Diabetes mellitus (DM) is a serious pathological condition which when combined with the stress of pregnancy creates a metabolic environment which is life threatening to both the mother and the fetus (Freinkel, 1980; Metzger, 1991). The natural diabetogenic state of pregnancy further complicates control of the diabetic condition in the mother (Hollingsworth, 1987) creating a maternal environment which exposes the fetus to abnormal fuel levels and precipitates a wide variety of fetal abnormalities associated with "fuel-mediated teratogenesis" (Freinkel, 1980; Metzger, 1991). Research has indicated that in addition to a greater risk for developing diabetes, the offspring of diabetic women typically show "diabetic-like" symptoms such as glucose intolerance and enlarged body size (Herrera, 1985).

Although some of the observed fetal alterations might be directly attributable to a genetic factor, recent data has suggested that the maternal environment, specifically the disease associated altered fuel metabolism, may in itself be a primary contributor to the "diabetic-like" alterations which occur in the fetus (Gauguier et al., 1990; Silverman et al., 1991; VanAssche, Aerts and Hoelmans, 1991). It has long been recognized that the placental interrelationship between the mother and fetus is a primary determining factor of fetal metabolic substrates and fetal outcome (Hay, 1991; VanAssche et al., 1991).

A recent study by Gauguier et al. (1991) simulated a moderate hyperglycemic state during the latter part of pregnancy and found that the offspring of the hyperglycemic dams showed changes similar

to those which are typically exhibited by newborns from diabetic mothers. Furthermore, the altered glucose metabolism which was observed persisted in to adulthood. These data highlight the contribution that modifications in placental transfer have on the overall well-being of the fetus. It stands to reason therefore that any intervention either prior to or during pregnancy which would improve the metabolic status of the mother, and/or improve the nature of placental transfer, would positively benefit both mother and fetus. It is the premise of this research that exercise might be one such intervention.

Although research examining the triangular relationship between exercise, diabetes and pregnancy is limited, pairwise evaluation of these three parameters has been reported in the literature. Examination of the interactive effects of exercise and DM has indicated that endurance exercise can elicit positive changes in fuel metabolism, skeletal and cardiac muscle function, and overall patient well-being (Noble and Ianuzzo, 1985; Landon, Gabbe and Sachs, 1990). Depending on the severity of DM the prescribed exercise may be used in conjunction with standard insulin treatment or as an adjunct to dietary manipulations.

Exercise during gestation in non-diabetic individuals has been shown to have either positive, negative, or no significant effects on both maternal and fetal physiological function (Clapp and Dickstein, 1982; Collings, Curet and Mullin, 1983; Wolfe, Ohtake, Mottola and McGrath, 1989). Bung et al., (1991) suggest that the use of exercise as a therapeutic modality in patients with gestational diabetes mellitus (GDM) has greater potential than insulin treatment due to

the reduced peripheral insulin sensitivity which evolves from the hormonal alterations during pregnancy. In a study with Type I diabetic patients Hollingsworth (1983) showed a positive effect of exercise during gestation on maternal lipid profile with no significant detrimental effect on neonatal outcome. To date, the effects of prior endurance exercise training on the ability of the mother to handle the metabolic alterations associated with a diabetic pregnancy have yet to be examined. Furthermore, no research exists which examines the combined effect of cessation of regular exercise and onset of pregnancy on maternal and fetal outcome in a diabetic model.

Recent work by Mottola and Christopher (1991) indicates that fetuses from non-diabetic mothers who stopped exercise upon conception were smaller and had lower levels of liver glycogen than fetuses from mothers who continued to exercise during gestation. In a condition such as diabetes where carbohydrate metabolism is already significantly impaired failure to continue to exercise may in fact predispose the mother and fetus to greater risk than that of euglycemic women. Since exercise programs are often described as an adjunct treatment for diabetes mellitus, yet are typically terminated with pregnancy, determining whether or not it is better to continue to exercise through gestation or terminate regular exercise upon conception is particularly important. Therefore, the purpose of this study was to examine the role of endurance exercise training prior to and during gestation on maternal metabolic response to pregnancy and fetal outcome.

Methods

Animal care and monitoring.

Sixty female Harlan Sprague-Dawley (city) rats (150-175 grams) were housed in seperate nalgene cages in a pathogen-free University Laboratory Animal Research facility at Michigan State University. Food (Purina® rat chow 5008) and water were supplied *ad libitum* and the animals were kept on a twelve hour light-dark cycle (0100:1300). Room temperature (70-74' F.) and humidity (50-54%) were controlled throughout the study to ensure the health of the animals. Upon arrival at the facility, the animals were allowed six days to accommodate to their new environment. Each animal was weighed daily during this time, to familiarize them with their caretakers. Subsequently, all animals were weighed weekly during the pre-conception portion of the study and daily during gestation. Food intake was also measured on a weekly basis for each animal.

Experimental Procedures.

Diabetes Inducement.

A two hour fasting arterial blood sample (1 ml) was drawn from the tail artery (Bober, 1988) of each animal following completion of the accomodation phase. Samples were immediately centrifuged and the plasma stored at -20'C for later analysis of blood glucose concentration representative of the prediabetic status of the animals. Two days following their respective draw, animals were injected with a 50 mg/kg•body wt dosage of streptozotocin (Sigma® Chemical, no. S-0130) into the tail vein. The STZ was dissolved in 40 mM citrate buffer (pH of 4.5) and care was taken to ensure that the

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STZ injection occurred within five minutes of dissolution in citrate to allow for the STZ to be in its active state (Chen and Ianuzzo, 1982). All animals were anesthesized by methoxyflurane inhalation prior to arterial blood sampling and STZ injection.

Diabetes Evaluation.

Following STZ injection, animals were maintained for a fourteen day period without further intervention to enable the diabetogenic effects of STZ to be expressed. An arterial blood sample (1 ml) was then drawn and analyzed for plasma glucose and insulin levels (Time 0) to determine each animal's level of diabetes. Forty-eight hours following Time 0 measurements, an intraperitoneal glucose tolerance test (IPGTT; GTT1) was administered to those animals who had attained a minimal blood glucose level of 20 mmol/l. Each animal (n=31) received a 2g/kg•body weight dose of 30% glucose intraperitoneally in a fasted state (2 hours). Blood samples (0.1 ml) were collected from the tail vein (Bober, 1988) of restrained animals. Samples were drawn ten minutes prior to injection of glucose and ten, thirty, sixty and one-hundred twenty minutes following injection. Samples were stored and later analyzed for plasma glucose concentration at each timepoint.

Diabetic animals were subsequently, matched in groups of three based on body weight (day eight) and blood glucose response to GTT1 (initial and peak value). One animal from each trio was randomly assigned to either the sedentary prior, sedentary during gestation group (SS; n=10), or the exercise prior, sedentary during

gestation group (ES; n=10), or the exercise prior, exercise during gestation (EE; n=10) treatment group.

Exercise Program.

EE and ES animals initiated their exercise program by running on a motor driven treadmill for a fourteen day accommodation period. The animals began running at 10 m/min, 0% grade, 15 min/day, 5 days/week and increased to a level of 20 m/min, 0% incline, 60 min/day, 5 days/week by day 14.

The animals then continued to train at this final intensity for eight weeks prior to the inducement of pregnancy. The non-exercising group remained sedentary during this time period but was exposed to the same environmental conditions as the runners during the training sessions. All exercise sessions were conducted during each animal's dark cycle.

Arterial blood and urine samples were obtained from all animals at weeks two, four and six of the training program. Upon completion of this pre-pregnancy training program (after week 8), a second IPGTT (GTT2) was performed. Animals in the EE group continued to exercise during gestation while ES and SS animals were sedentary.

Impregnation Procedures.

Twenty-four to fourty-eight hours following GTT2, each of the animals was injected with 50 ug leutenizing hormone releasing hormone (LHRH; Sigma® Chemical no. L-4563). The LHRH was carried in a 0.1% bovine serum albumin solution with the total

injected volume of the active hormone being 0.2 ml per animal (Mottola and Christopher, 1991). The LHRH allows for esterus during the twelve hour dark cycle following a 110 hour post injection period. During the twelve hour dark cycle the females were placed into a cage with a breeder male (Harlan Sprague-Dawley). The male and female remained together for the duration of the twelve hour cycle (Mottola and Christopher, 1991). Following the mating period, each of the females was evaluated for a vaginal sperm plug and a positive vaginal sperm smear. Positive evaluations were described as day zero of gestation (GD0). If the animal was not determined to be pregnant, the non-pregnant (NP; n=7) animal was reclassified as NPEE, NPES, and NPSS and allowed to continue in their experimental condition until the termination of the study. No animal was allowed to be injected or mated more than one time during the course of the study.

A final IPGTT (GTT3) was administered on day nineteen of gestation (GD19). This time point was chosen to ensure that the animals were in a third trimester condition as well as to minimize the risk of negatively influencing birthing procedures. The mothers were then allowed to birth their pups naturally. Within eight hours postpartum, the neonates were weighed and evaluated for gross morphological abnormalities. Following birth, animals from all groups (PEE, PES, PSS, NPEE, NPES, NPSS) were anesthetized via methoxyflurane inhalation prior to surgical removal of tissues (uterus, ovary, heart, liver and skeletal muscle). Upon completion of the surgical tissue harvesting, the animals were exsanguinated using a pneumothorax technique.

Blood and Urine Evaluation.

Glucose levels were determined using a Kodak Ektachem DT60® analyzer. Briefly, this procedure involves determination of glucose based on the glucose oxidase catalyzed reaction where gluconic acid and hydrogen peroxide are produced. Subsequently, the hydrogen peroxide is coupled with 4-amminoantipyrine and 1,7-dihydroxynaphtalene. The reaction which was catalyzed by peroxidase resulting in the formation of a red dye. The reflectance density of the dye is then measured spectrophotometrically. Higher glucose values corresponded to higher colour density of the dye. Insulin levels were analyzed using radioimmunoassay according to the methods of Morgan and Lazarow (1963) (Linco® Rat Insulin kit).

Urine samples were also taken to provide an estimate of each animal's baseline level of acetonuria and glucosuria (Chemstrip® uGK, Boehringer Manheim no. 00513).

Statistical Analysis.

Analysis of variance with repeated measures was used to evaluate the body weight, food intake, glucose and insulin values determined over the course of the study. Additionally, a two-way ANOVA was utilized to determine the effects of treatment and time for the GTT data. Moreover, the area under the glucose curve was determined and analyzed for each treatment and time. Tissue and body weights at the time of sacrifice were analyzed using a factorial ANOVA. Finally, litter size, resorption number, and viable neonate number were compared using a factorial analysis of variance. Post

hoc Scheffe' analysies were utilized when appropriate. The level of significance was chosen at $p < .05$ in all instances.

Results

Preconception.

Body Weight and Food Intake.

Figure 3.1 describes mean body weight during the preconception period for all six animal groups. Body weight increased steadily in all groups until week eight, and then achieved a plateau. There was no significant difference in body weight between any of the groups which ultimately became pregnant (PEE; PES; PSS) at any of the time points examined . In contrast, NPEE animals weighed significantly more than NPES animals at week twelve. Weekly food consumption in all groups increased up through week four, and then was maintained at this new level through the remainder of the preconception phase (Figure 3.2). The animals appeared to adjust to their treatment conditions and controlled food intake in response to these parameters.

Basal Glucose and Insulin Levels.

Administration of a 50 mg/kg•body weight dose of STZ caused a significant increase in arterial blood glucose concentration within two weeks of injection. Subsequent two hour fasting plasma glucose levels did not differ significantly between groups at any of the time points examined during the eight weeks prior to mating, or at sacrifice (Figure 3.3).

Figure 3.1 Body weight (grams) prior to diabetes injection (Pre), at injection (STZ) and throughout the exercise training period until mating in virgin female Sprague Dawley rats. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). Asterisks (*) indicate a significant ($p<0.05$) difference in body weight between NPEE and NPES animals at week 12. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.

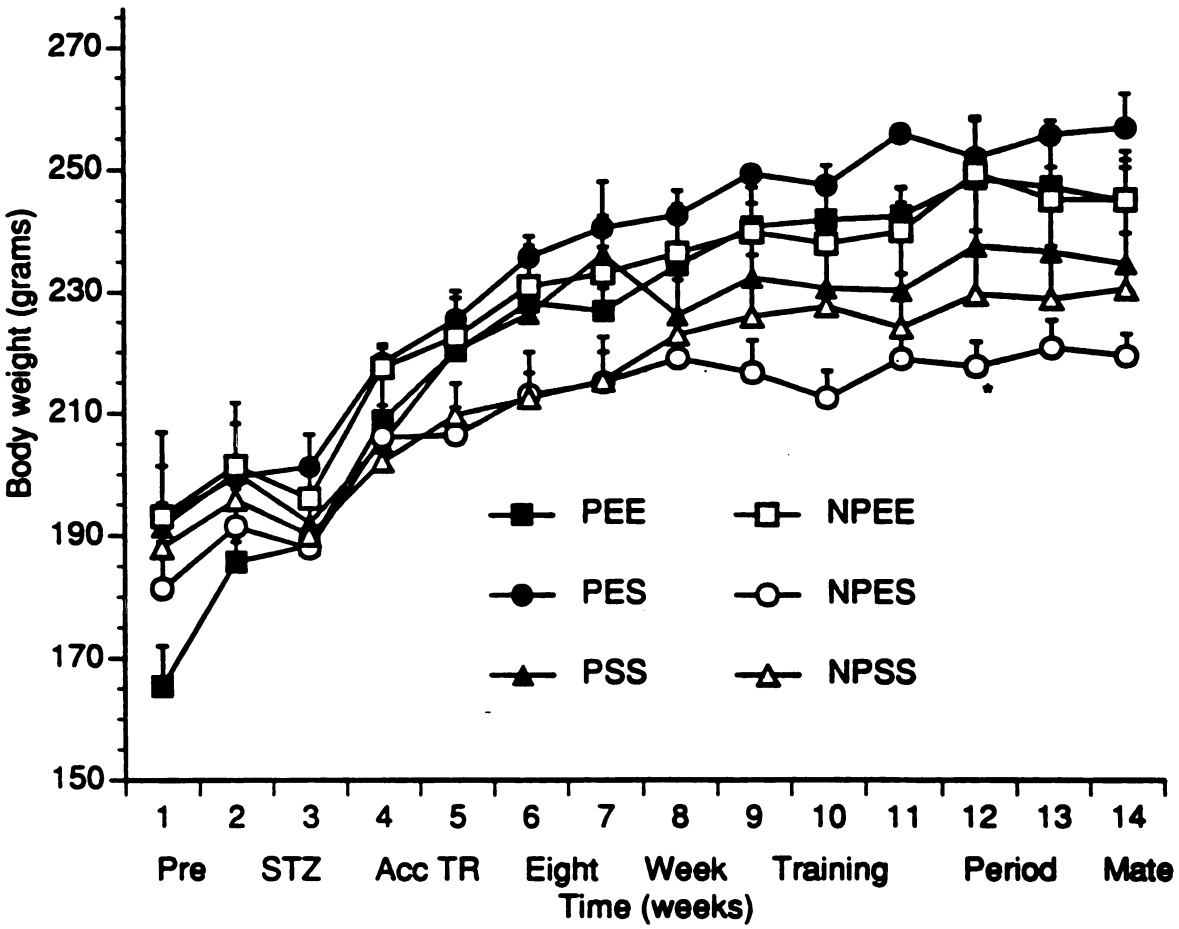

270
250
230
210
190
170
150
Body weight (grams)
PEE
NPEE
PES
NPES
PSS
NPSS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Pre
STZ
Acc TR
Eight
Week
Training
Period
Mate
Time (weeks)

Figure 3.2 Food consumption (grams) prior to diabetes injection (Pre), at injection (STZ) and throughout the exercise training period until mating in virgin female Sprague Dawley rats. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). There was no significant difference in food consumption between the six animal groups over this time period. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.

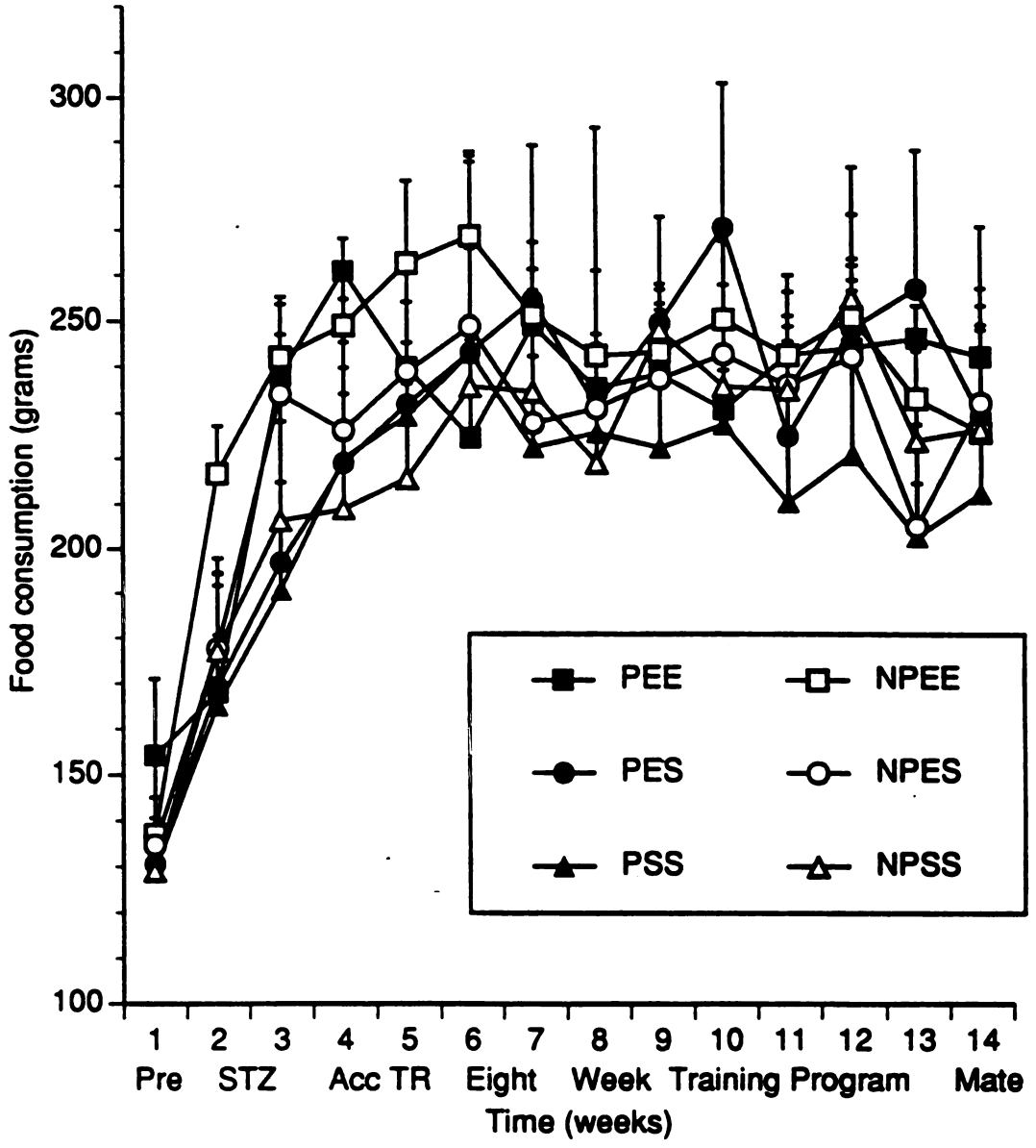
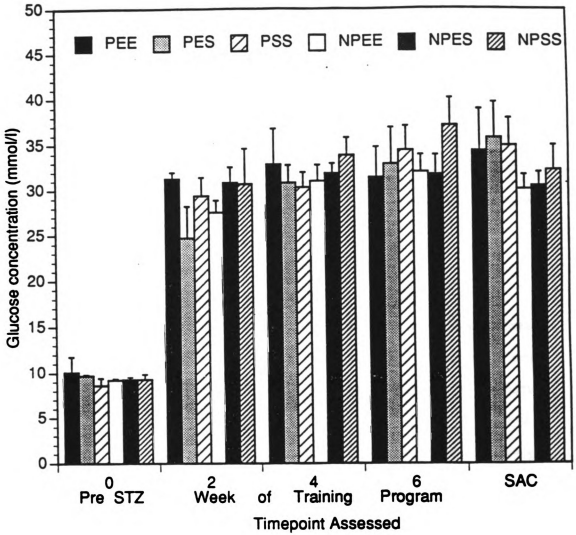

Food consumption (grams)
300
250
200
150
100
PEE
NPEE
PES
NPES
PSS
NPSS
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Pre STZ Acc TR Eight Week Training Program Mate
Time (weeks)

Figure 3.3 Twelve hour fasting plasma glucose concentration (mmol/l) prior to injection of STZ (time 0), at 2, 4 and 6 weeks of exercise training, and at sacrifice (SAC) in virgin female Sprague Dawley rats. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). There was no significant difference in plasma glucose concentration between groups at any of the timepoints assessed Plasma glucose concentration at time 0 was significantly less than at all other timepoints for all groups. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.


PEE
PES
PSS
NPEE
NPES
NPSS
Glucose concentration (mmol/l)
50
45
40
35
30
25
20
15
10
5
0
0
Pre STZ
2
Week
of
4
Training
6
Program
SAC
Timepoint Assessed

Response to an Intraperitoneal Glucose Tolerance Test.

During the preconception period two IPGTTs were administered; the first (GTT1) two weeks following administration of STZ (Figure 3.4a) and the second (GTT2) at the end of the eight week training phase (Figure 3.4b). Animals in all groups exhibited an elevated response to the intraperitoneal glucose load typical of their diabetic state during both GTT1 and GTT2. There was no significant between group difference in plasma glucose levels achieved at any of the time points during either of the IPGTTs, nor was there a difference in the overall pattern of their response as indicated by the similarities in the area under the plasma glucose curve (Figure 3.5).

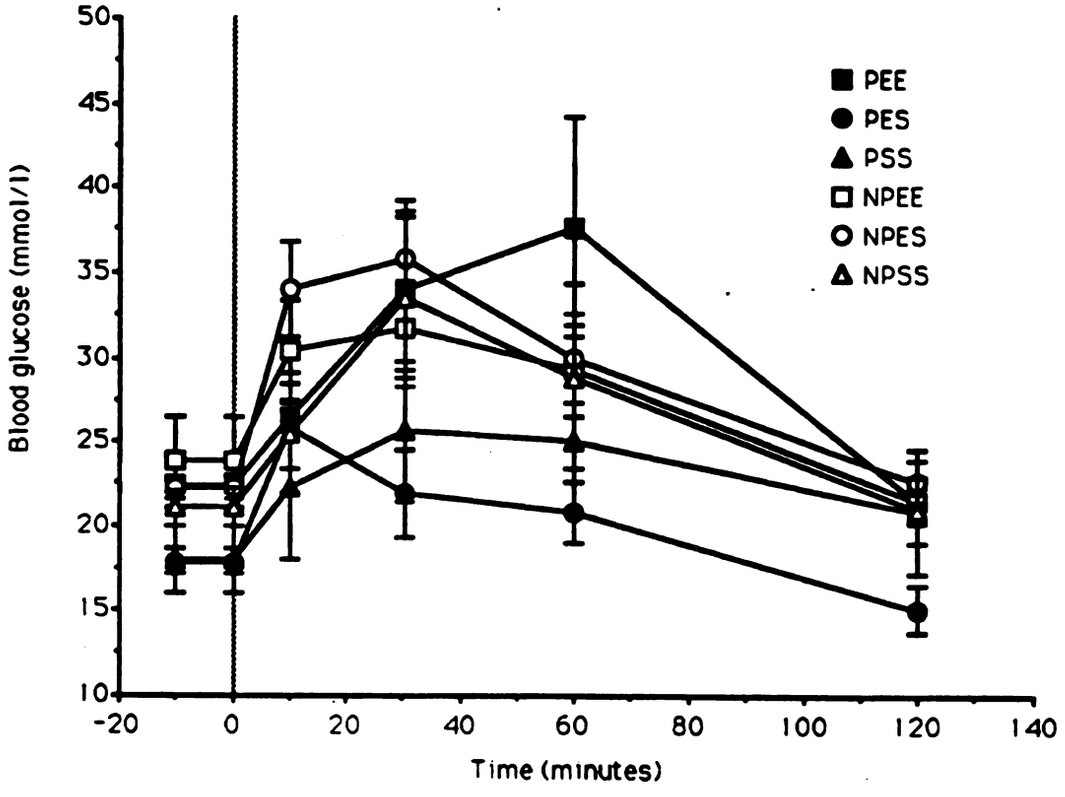
Gestation.

Body Weight and Food Intake

Figures 3.6 and 3.7 illustrate body weight and food intake respectively, during gestation. Body weight was measured daily throughout pregnancy. All six groups had similar values at gestation day zero (GD0) with group averages ranging from 216 $\pm$ 5.86 to 258 $\pm$ 8.99 grams. As expected, there was a steady increase in body weight over time in those animals that became pregnant. There was no significant difference in body weight gain during gestation between PEE, PES, and PSS groups. PES animals were however consistently heavier throughout gestation than either of the other two pregnant groups. This finding was in contrast to what was observed between those groups of animals which did not become pregnant where the NPEE group was consistently the heaviest. It was also interesting to note that several (n= 6) animals in the "non-

Figure 3.4 Twelve hour fasting plasma glucose concentration (mmol/l), plasma glucose concentration prior to injection (time 0) of a 2.0g/kg body weight glucose load, and at 10, 30, 90 and 120 minutes following injection in virgin female Sprague Dawley rats. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM) and are from glucose tolerance tests which were administered two weeks following STZ injection (3.4a) and at the end of the eight week training program (3.4b). There was no significant difference in plasma glucose concentration between groups at any of the timepoints assessed. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.

a


Blood glucose (mmol/l)
50
45
40
35
30
25
20
15
10
-20
0
20
40
60
80
100
120
140
Time (minutes)
PEE
PES
PSS
NPEE
NPES
NPSS

b

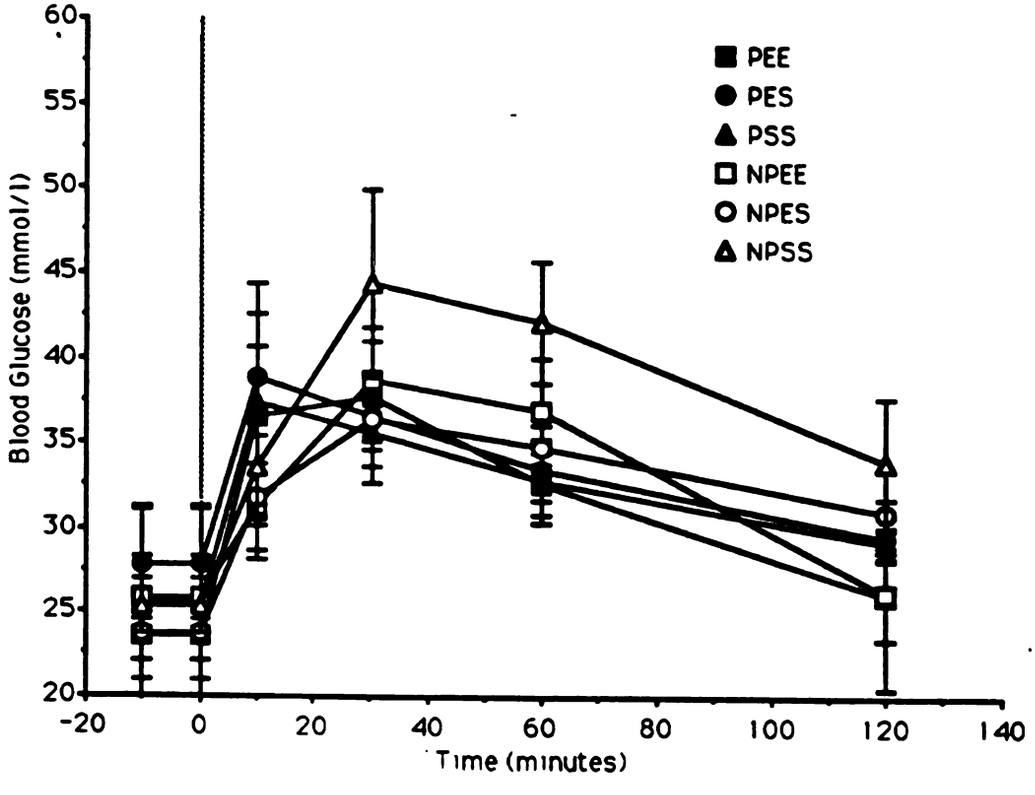

Blood Glucose (mmol/l)
60
55
50
45
40
35
30
25
20
-20
0
20
40
60
80
100
120
140
Time (minutes)
PEE
PES
PSS
NPEE
NPES
NPSS

Figure 3.5 Area under the plasma glucose curve following injection of a 2.0g/kg body weight glucose load in virgin female Sprague Dawley rats. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean (± SEM) and are from glucose tolerance tests which were administered two weeks following STZ injection (3.5a) and at the end of the eight week training program (3.5b). There was no significant difference in area under the plasma glucose curve between groups at either of the timepoints assessed. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.

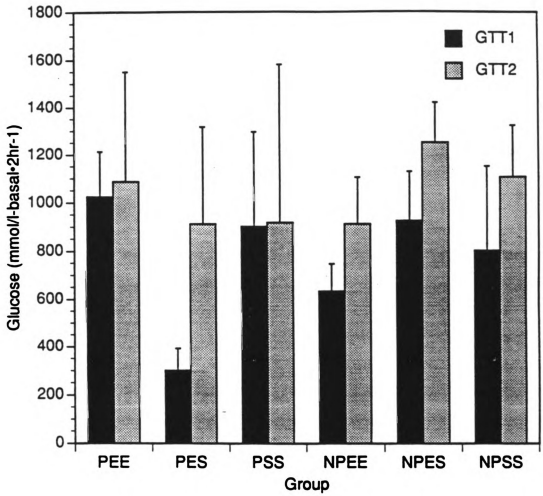

1800
1600
1400
1200
1000
800
600
400
200
0
Glucose (mmol/l-basal*2hr-1)
GTT1
GTT2
PEE
PES
PSS
NPEE
NPES
NPSS
Group

Figure 3.6 Body weight (grams) in diabetic female rats for 22 days following mating. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.

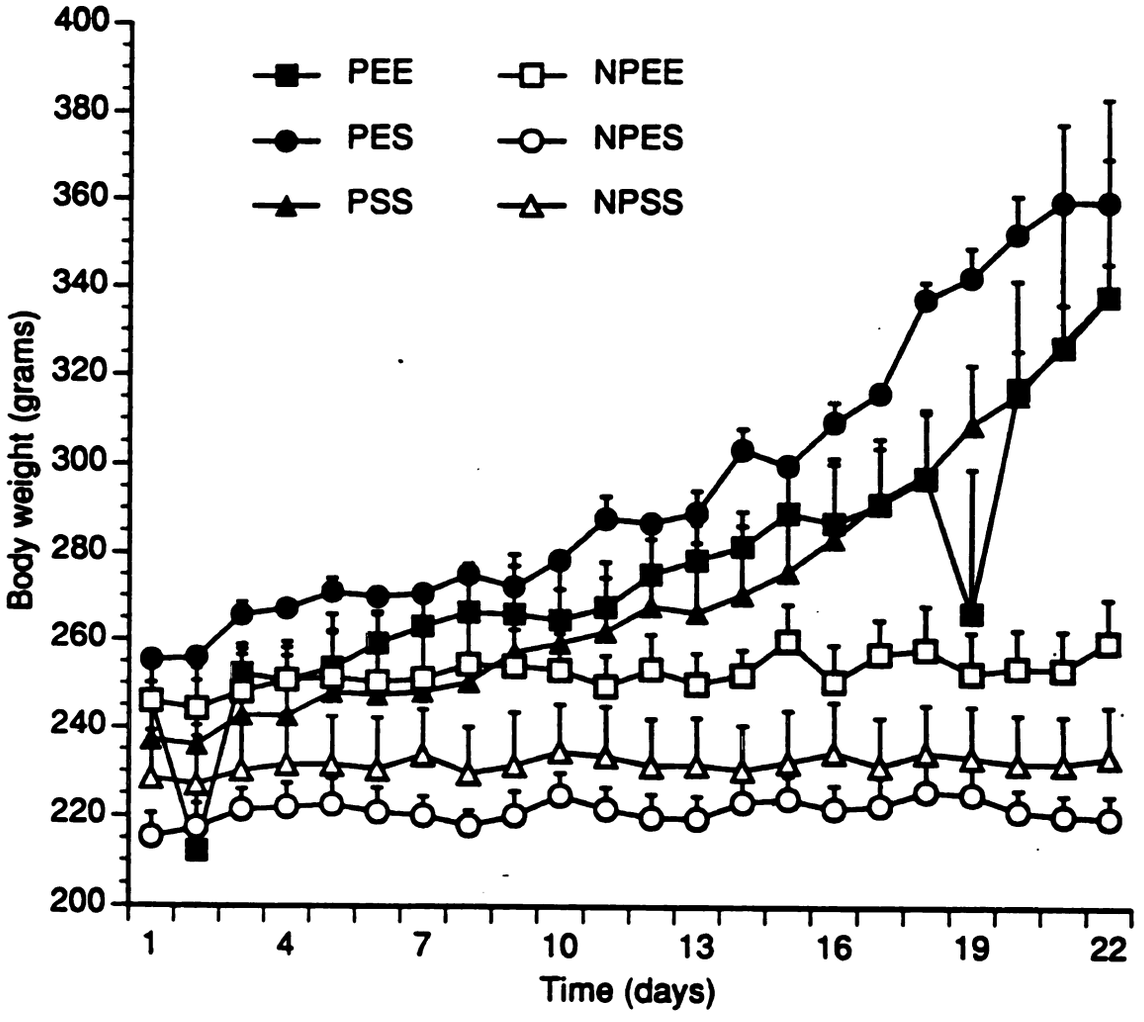
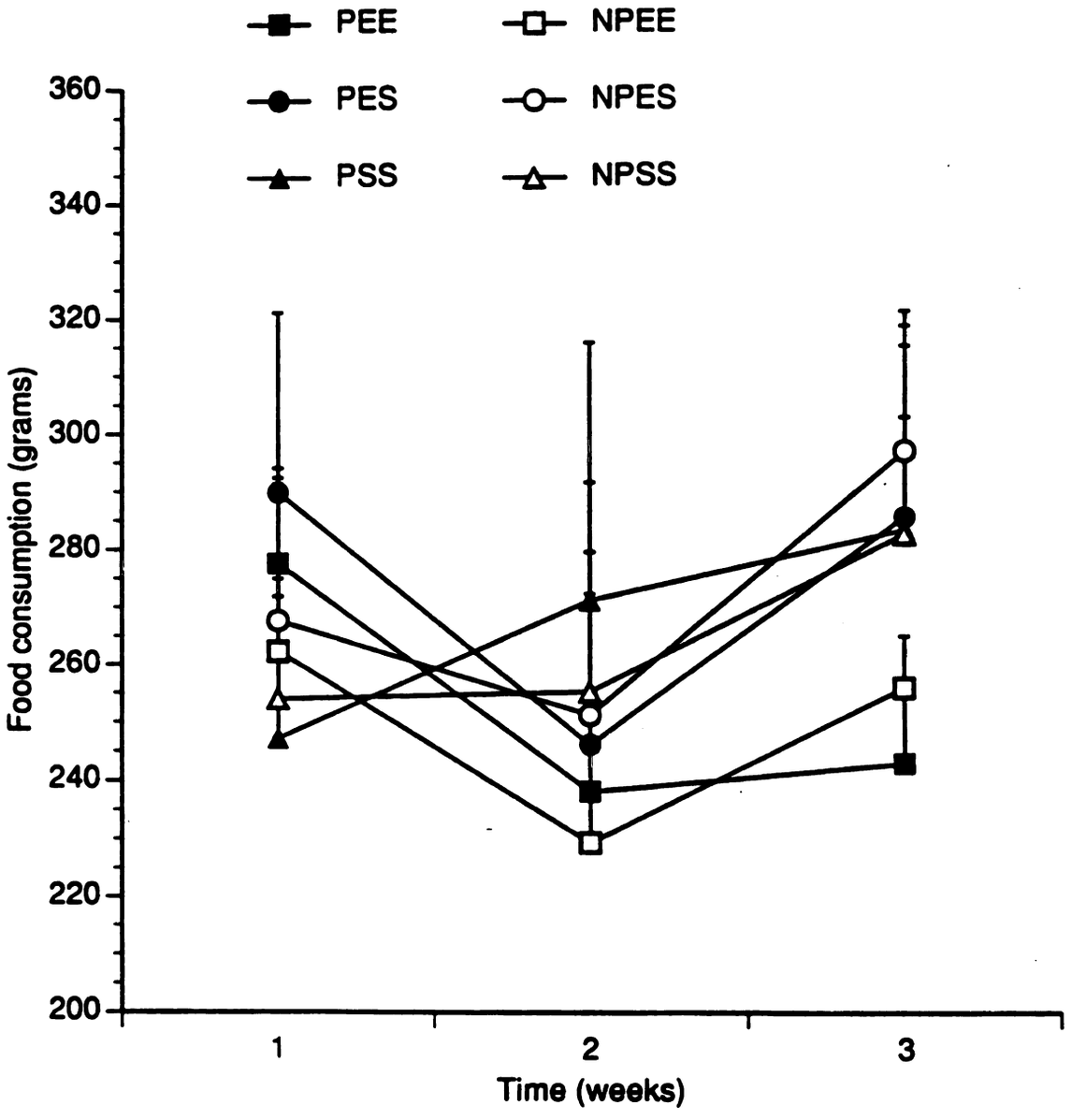

PEE
NPEE
PES
NPES
PSS
NPSS
Body weight (grams)
400
380
360
340
320
300
280
260
240
220
200
1
4
7
10
13
16
19
22
Time (days)

Figure 3.7 Food consumption (grams) in diabetic female rats for 22 days following mating. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean (± SEM). Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.


PEE
NPEE
PES
NPES
PSS
NPSS
360
340
320
300
280
260
240
220
200
Food consumption (grams)
1
2
3
Time (weeks)

pregnant" groups showed an increase in body weight over the first 14 days typical of that observed in those animals which ultimately became pregnant. In the instance of the "non-pregnant" animals however body weight returned to GD0 levels by day 21. The NPES group had the largest number of false pregnancies (n=3) with NPEE group showing the least (n=1).

Food intake during the gestational period was not significanlty different between groups, although some differences in trends were noted. PSS tended to increase food intake over the entire gestational period while both PEE and PES showed a decline in food intake, with only PES intake levels returning to that of PSS levels at the third week of gestation. Data from the NPSS group mimicked that of the PSS group over the complete gestational timecourse. NPES and NPEE food intake declined at week two but increased again by week three. Moreover, NPES increased its intake by week three to a higher volume than NPSS.

Response to an Intraperitoneal Glucose Tolerance Test.

Results from the GD19 glucose tolerance test are provided in Figure 3.8. As expected, animals in the pregnant groups had a blunted response to the intreperitoneal glucose load in comparison to their post training, pre-conception GTT. Data were not however, significantly different between groups at GD19. Calculation of area under the glucose curve indicated that in those animals which became pregnant the greatest area was attained in the PES group, and the smallest in the PSS group (Figure 3.9). Conversely, in the animals which failed to attain/maintain pregnancy the NPSS area

Figure 3.8 Twelve hour fasting plasma glucose concentration (mmol/l), plasma glucose concentration prior to injection (time 0) of a 2.0g/kg body weight glucose load, and at 10, 30, 90 and 120 minutes following injection in virgin female Sprague Dawley rats 19 days following mating (D19). Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). There was no significant difference in plasma glucose concentration between groups at any of the timepoints assessed. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.

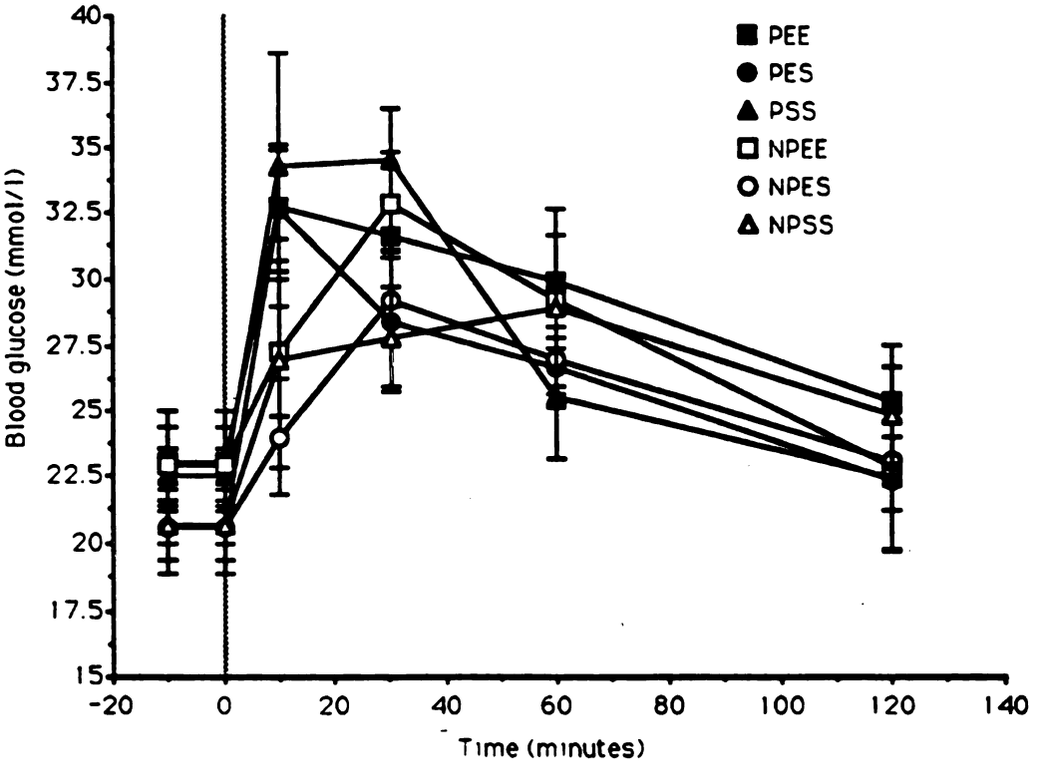
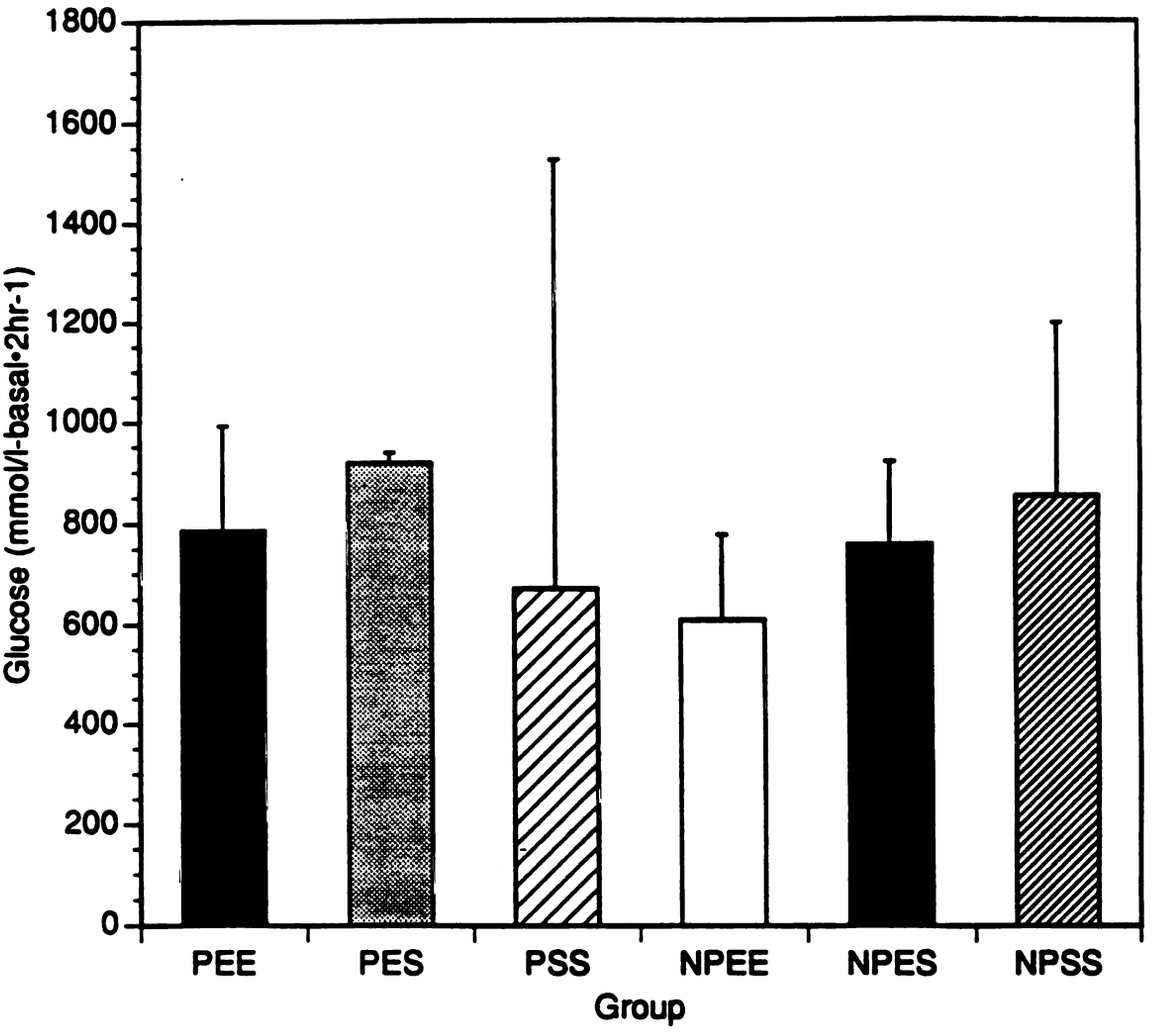

PEE
PES
PSS
NPEE
NPES
NPSS
Blood glucose (mmol/l)
40
37.5
35
32.5
30
27.5
25
22.5
20
17.5
15
-20
0
20
40
60
80
100
120
140
Time (minutes)

Figure 3.9 Area under the plasma glucose curve following injection of a 2.0g/kg body weight glucose load in virgin female Sprague Dawley rats at 19 days following mating (D19). Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). There was no significant difference in area under the plasma glucose curve between groups. Total number of animals evaluated in each group were; PEE=3; PES=3; PSS=3; NPEE=7; NPES=7; NPSS=7.


1800
1600
1400
1200
1000
800
600
400
200
0
Glucose (mmol/l-basal•2hr-1)
PEE
PES
PSS
NPEE
NPES
NPSS
Group

was the largest and the NPEE area the smallest. In no instance were these data significantly different between groups.

Offspring Outcome.

Offspring outcome data for the three pregnant groups is presented in Table 3.1. Number of litters and litter size did not differ significantly between the three groups. There were no viable offspring from the PES dams while the number of offspring from PEE and PSS mothers was similar (PEE = 17; PSS = 14). An equivalent number of males and females were born across the three groups. Average female viable pup weight was significantly greater in litters born of PEE dams (5.04 $\pm$ 0.12 grams) than those born of PSS dams (4.4 $\pm$ 0.0gm).

Sacrifice Data.

Table 3.2 represents the tissue weight and ratio data for all animals at sacrifice. Only the left ovary weight from the PES group was significantly different from the PSS group. No other comparisons were statistically different.

It is interesting to note, however, that generally the exercising animals tended to have a larger heart, larger muscles and a higher heart to body weight ratio than the sedentary animals. Additionally, the liver weight of the EE groups tended to be larger than in either of the other two groups.

Insulin values and insulin to glucose ratios at sacrifice are provided in Figure 3.10 a and b. Neither the insulin nor the ratios showed any between group differences.

Table 3.1. Offspring Outcomes.

Parameter	PEE	PES	PSS
Litters - total (n)	3	3	3
Pups - total (n)	29	27	25
Litter size (average)	9.67 ± 1.45	9.00 ± 1.53	8.33 ± 0.33
Viable pups - total (n)	17	0	14
average/litter	5.67 ± 3.18	0	4.67 ± 2.40
Male offspring - total (n)	14	12	13
Female offspring - total (n)	15	15	12
Pup body weight			
Males - viable (average)	5.49 ± 0.44	4.9 ±0.30	6.8 ±0.10
- non-viable (average)	7.4 ±0	6.2 ±0.27	0
Females - viable (average)	5.04* ±0.12	4.4 ±0	5.67 ±0.18
- non-viable (average)	4.7 ±0	6.06 ±0.22	6.95 ±0.35

* vs PSS

Data represent mean ± sem. ($p \leq .05$)

Table 3.2. Diabetic animal tissue weights at sacrifice.

	Pregnant			Nonpregnant		
Tissue Weight (grams)	**SS**	**ES**	**EE**	**SS**	**ES**	**EE**
Liver	16.07 ±2.27	15.74 ±1.48	17.74 ±1.15	12.35 ±.88	12.7 ±.23	13.33 ±.49
Uterus	2.36 ±.17	2.77 ±1.55	2.04 ±.49	.45 ±.11	.33 ±.09	.52 ±.06
Ovary -right	.07 ±.02	.06 ±.01	.07 ±.01	.06 ±.01	.05 ±.01	.08 ±.01
Ovary - left	.07 ±.01	.05±* 3.7E-3	.07± 1.4E-3	.06 ±.01	.05 ±.01	.08± 4.6E-3
Heart	.94 ±.09	.96 ±.09	1.06 ±05	.88 ±.05	.91 ±.06	1.0 ±.03
Soleus - right	.09± 4.9E-3	.09 ±.01	.11 ±.01	.08 ±.01	.08 ±.01	.09 ±.01
Plantaris - right	.17 ±.02	.18 ±.01	.17 ±.01	.18 ±.02	.16 ±.02	.18 ±.02
Gastrocnemius - total	1.07 ±.04	1.09 ±.1	1.1 ±.06	.97 ±.12	.86 ±.06	1.1 ±.05
White Gastrocnemius	.52 ±.04	.54 ±.02	.64 ±.03	.47 ±.06	.47 ±.03	.53 ±.04
Red Gastrocnemius	.54 ±.08	.54 ±.09	.47 ±.03	.51 ±07	.36 ±.05	.55 ±.05

Data represent mean ± sem.
* vs. SS ($p<.05$)

Figure

Dawley
depende
ultimate
exercise
pregnan
unable t
mating
(NPSS);
signific
groups.
pregnan

Figure 3.10. Insulin concentration (ng/ml) in female Sprague Dawley rata at sacrifice. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). There was no significant difference in insulin concentration at sacrifice between groups. Total number of animals evaluated in each group were: all pregnant groups n=3, all non-pregnant groups n=7.

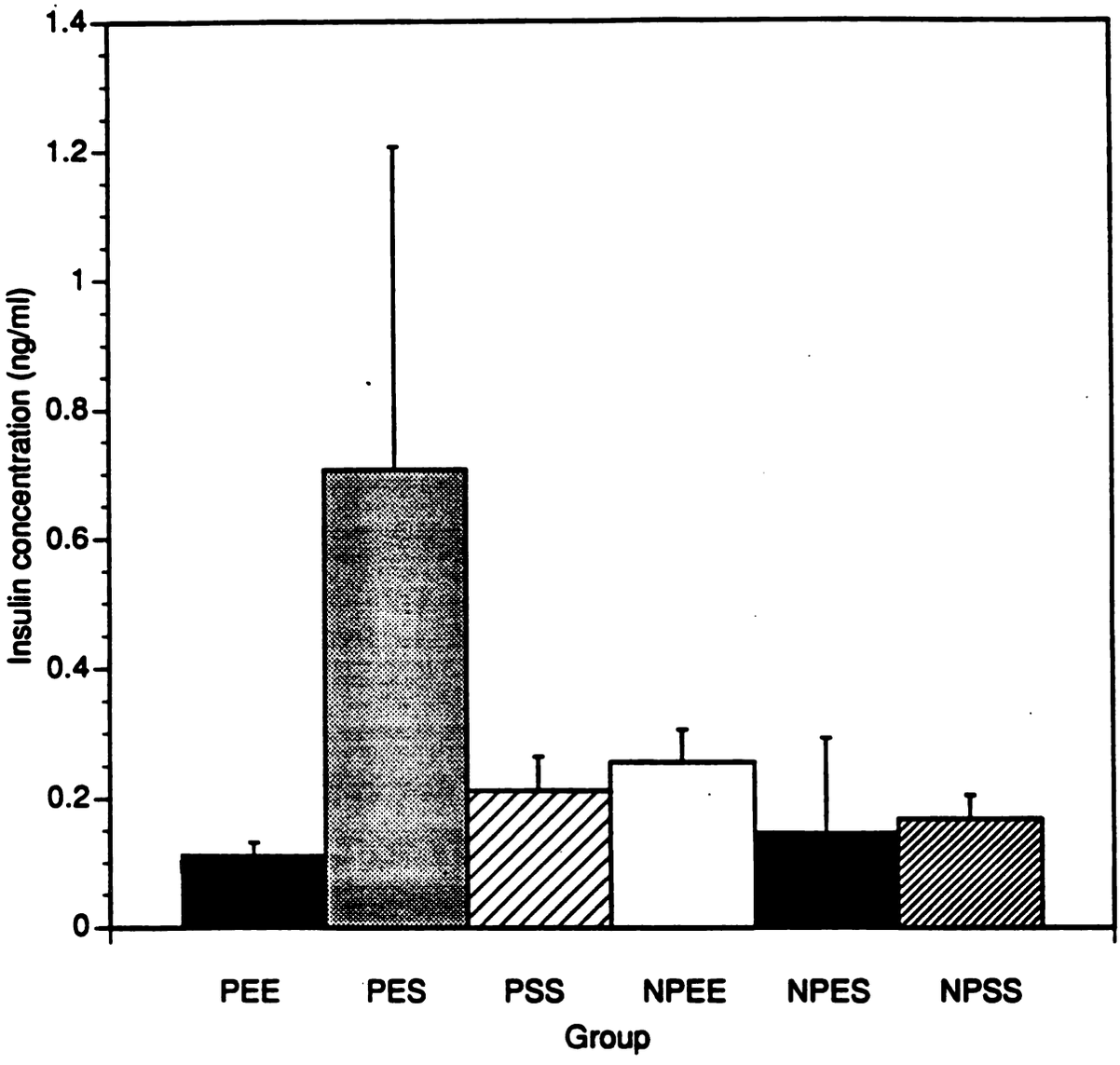
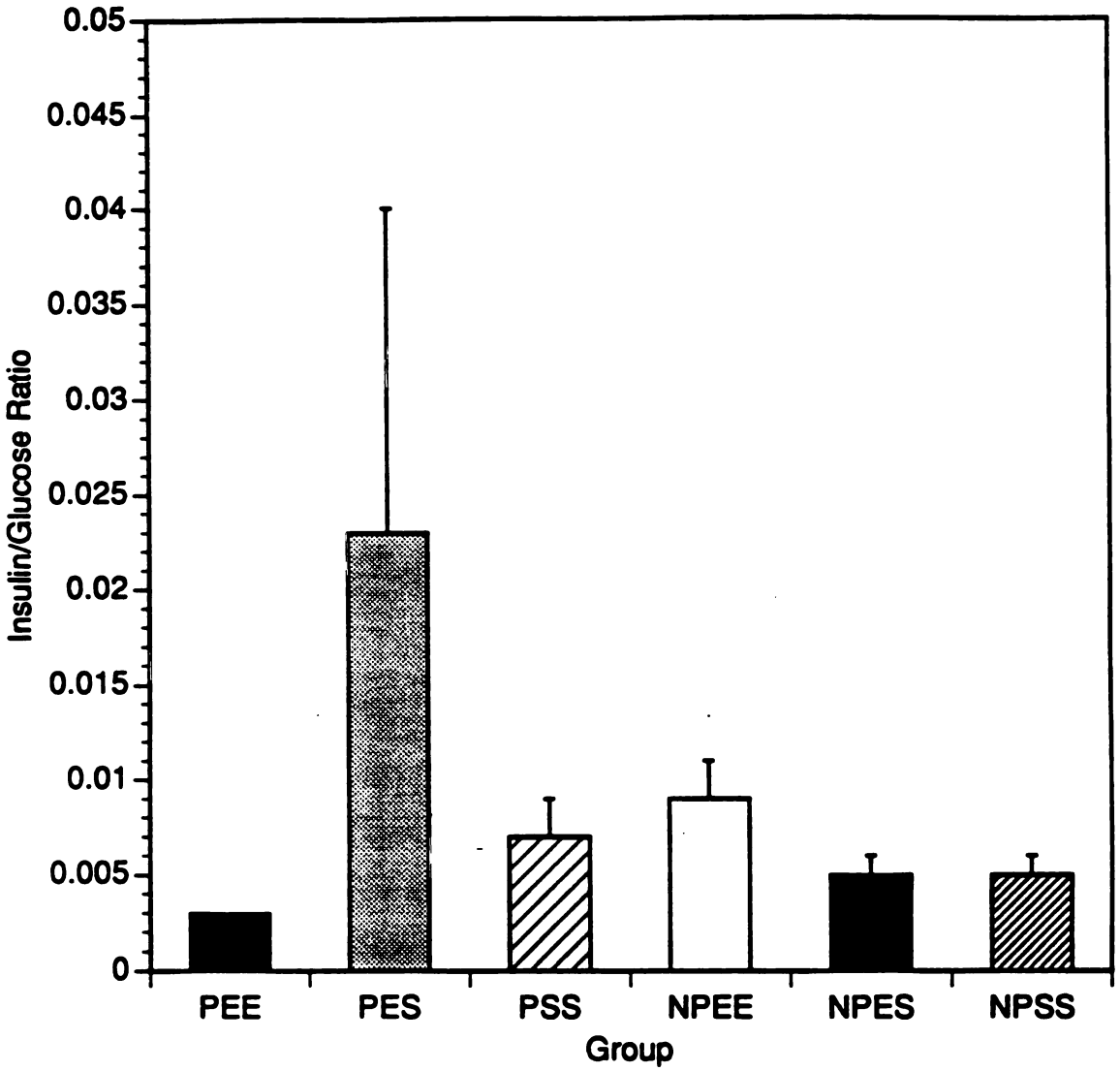

1.4
1.2
1
0.8
0.6
0.4
0.2
0
Insulin concentration (ng/ml)
PEE
PES
PSS
NPEE
NPES
NPSS
Group

Figure 3.11. Insulin to glucose ratio (I/G) in female Sprague Dawley rats at sacrifice. Animals were in one of six groups dependent upon their activity status and whether or not they were ultimately able to conceive; animals which became pregnant (P) and exercised prior to and during gestation (PEE); exercised prior to pregnancy only (PES); did not exercise (PSS); and animals which were unable to conceive (NP) and exercised prior to and for 21 days after mating (NPEE); exercised prior to mating only (NPES); did not exercise (NPSS); Data are expressed as mean ($\pm$ SEM). There was no significant difference in I/G ratio at sacrifice between groups. Total number of animals evaluated in each group were: all pregnant groups n=3, all non-pregnant groups n=7.


0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Insulin/Glucose Ratio
PEE
PES
PSS
NPEE
NPES
NPSS
Group

Discussion

The present study was designed to evaluate the effects of chronic endurance training on glucose homeostasis in female rats with uncontrolled Type I diabetes. Moreover, the impact of this training prior to and during gestation on offspring outcomes was assessed. To evaluate the influence of exercise three training regimes were utilized -- (1) exercise prior to, and during gestation, (2) exercise prior to gestation with termination of exercise upon conception, and (3) sedentary status prior to, and during pregnancy.

In order to induce the Type I diabetic state virgin Sprague Dawley rats were injected with a 50 mg/kg body weight dose of STZ. STZ is a methylnitrosourea derivative of 2-deoxy-glucose which destroys pancreatic beta cells, subsequently inhibiting insulin production and causing an elevation in plasma glucose concentration (Like and Rossini, 1976). Only thirty-one of the sixty animals which were injected became diabetic. This rate of success is typical when using female animals and supports earlier data by Goodyear et al., (1991) and Calles et al., (1986) where STZ administration in female animals had approximately a fifty percent success rate. Paik and colleagues (1982) reported similar results and suggest a potential link between estrogen-androgen levels and the diabetogenic potential of animals. Further merit to this potential relationship is provided by work by Bjorntrop (1992) which shows a direct correlation between high levels of testosterone and Type II diabetes in females, with the direct opposite relationship being true in males. Although this difference in susceptibility to diabetes is not directly pertinent to the outcomes of this study, the abiity to enhance the STZ

success rate in female animals would make work in this area certainly less costly. Careful analysis of estrogen and androgen levels during the estrous cycle in female rats might identify a specific phase in the cycle where a more conducive diabetogenic hormonal mileau exists. It is interesting to note that the typical cycle is four days in duration and during two of these days estrogen levels are significantly higher than on the remaining two days. Considering the probability of randomly selecting either a high or low estrogen day when injecting a large number of female animals a fifty percent success rate seems quite appropriate. Although this hypothesis has yet to be tested, it is qualitatively supported byt he data.

Only thirty percent of the diabetic animals became pregnant. Exercise training did not either hinder or facilitate the ability of diabetic rats to become pregnant. Although most authors do not report conception rate data, Ryan and co-workers (1993) indicate a similar level of fertility in Wistar-Furth rats receiving a 55 mg/kg body weight dose of STZ shortly before mating. The use of a priming technique such as was used in this study (LHRH injection) did not enhance the conception rate of the diabetic animals as has been shown to occur in non-diabetic animals (Mottola, unpublished report). While the priming method may be more effective in controlling onset and timing of ovulation its lack of effect on enhancing conception rate, in conjunction with the altered natural reproductive behaviour patterns of the diabetic animals suggests that it might be of greater benefit to allow the animals to mate naturally in an attempt to increase the number of pregnancies attained.

As a consequence of the low pregnancy rate it became possible to assess the data from an additional perspective, that being whether or not there were any observable differences prior to mating in those animals that became pregnant that might have enhanced their ability to conceive.

During the period prior to mating there was no significant difference in body weight or food consumption between the six groups. This finding differs from previous work where exercise trained diabetic animals had lower body weights (Goodyear et al, 1991; Goodyear et al., 1988) and lower food intakes (Goodyear et al., 1991) than their control counterparts. A fundamental difference between the work by Goodyear et al. (1991) and the present study was the volume of exercise stress placed on the animals. Goodyear and co-workers (1988) ran the animals at a pace 10m/min. faster than the present study as well as twice the daily duration (2x 60 minute sessions/day). The enhanced stress place on these animals could have depressed their appetite resulting in a decrease in food intake and a consequent decrease in body weight. Moreover, the mere fact that animals were physically removed from food for at least twice the total time per week could have impinged on their ability to consume the volume of food necessary relative to their energy expenditure and relative to their control counterparts.

Although there was no significant difference in food consumption or body weight between those animals which ultimately became pregnant and those animals which were unable to conceive there was an observable decline in food intake in all three non-pregnant groups at week seven of training. A simultaneous decrease

in body weight in the NPSS and NPES group was also observed. In light of this trend one might speculate that body weight at the time of conception played a significant role in the ability of the diabetic animals to conceive. The decline in food consumption and body weight suggests that some animals were under more stress than others due to their diabetic condition. The lack of significant difference in plasma glucose and insulin concentration, and in ability to respond to a glucose challenge would suggest that the degree of severity of the diabetic state is an unlikely cause of this "additional" stress although, this concept cannot be totally ruled out since glycosylated hemoglobin (HbA1c) levels were not assessed. Recent evidence suggests that HbA1c levels may be a more critical measure of diabetes control than either glucose levels or IPGTT values. Regardless of the specific identity of this additional stress its consequences were such that the animal was unable to maintain homeostatic energy balance and were ultimately unable to conceive. This "natural selection" for positive conception is common for most of the animal kingdom.

Moderate endurance exercise training prior to pregnancy had no effect on two-hour fasting plasma glucose concentration, insulin levels, or response to a glucose challenge. This lack of exercise effect has been reported previously in equally severely diabetic female animals (Vallerand, Lupien and Bukowiecki, 1986; Goodyear et al., 1988) and in more critically diabetic males (Noble and Ianuzzo, 1975). It appears that a minimal level of insulin is necessary for enhancements in glucose utilization to occur in response to training (Goodyear et al., 1988). Those studies which do report a positive

effect of exercise training typically utilize animals with a lesser degree of hyperglycemia (Dall'aglio, Chang, Hollenback, Mondon, Sims and Reaven, 1983, Goodyear et al., 1991). One important finding of the present study was the failure of plasma glucose levels to continue to rise throughout the training program. Goodyear et al., (1988) report an increase in levels of plasma glucose over the first three weeks of exercise training in diabetic animals that were placed on an eight week progressive training program. This finding is similar to work by Burch et al. (1992) who treadmill trained Fatty Zucker rats (NIDDM). In Goodyear's work (1988), exercise training began approximately five days following STZ injection, limiting the amount of time given to these animals to adjust to the new metabolic state prior to commencing exercise training, which in itself is a metabolic pertubation (Goodyear, 1988). Allowing the animals to adjust to their new "diabetic homeostasis" prior to initiation of exercise, as was done in this study appears to have provided a more appropriate picture of the impact of exercise training on a Type I diabetic model.

An important feature of this study was that it enabled a thorough examination of the impact of exercise training on response to pregnancy, and on pregnancy outcome under two distinctly different conditions; exercise training prior to and during gestation, and exercise training prior to gestation followed by no exercise during gestation. To date, no other study exists which compares these two situations in a diabetic model.

Pregnancy outcome, as indicated by litter size and viable offspring was not negatively affected by exercise training prior to

and during gestation. Average litter size was slightly larger than that which has been previously reported under diabetic conditions (Ryan et al., 1993; Brownscheidle et al., 1983; Eriksson et al., 1980) and the percentage of viable offspring in the PEE was similar to studies without exercise perturbations (Brownscheidle et al., 1983; Eriksson et al., 1980). Failure to continue to exercise during gestation did however have a significant impact on the maternal response to pregnancy and on pregnancy outcome. In the PES group two of the three dams were unable to affectively deliver their pups. Both dams delivered animals which were grossly malformed. In addition, one mother died during labor after delivering several severely abnormal offspring. Although these reports are qualitative, it appears that the sudden perturbation to glucose homeostasis due to the cessation of exercise training, coupled with the normal diabetogenic effects of pregnancy acted synergistically to negatively influence both the mother and the fetus. Support for this hypothesis can be further drawn from the data acquired on the dams during gestation. As was to be expected, mothers in all groups increased body weight during gestation. However, food consumption in both the PES and PEE group declined during the first week, but rebounded in the PES group from week two to three to a level equivalent to PSS animals. Not only did this rapid enhancement in food intake cause PES animals to attain the greatest body weight at term but it also clearly demonstrated a significant change in metabolism that was not solely due to the effects of pregnancy. Research has shown that diabetic dams with higher body weights tend to have offspring with greater morphological and metabolic abnormalities (Grill et al., 1991;

Metzger, 1991). In addition, previous studies have shown a "blunting" of the glucose tolerance curve during gestation in diabetic animals. In this study, although the glucose challenge data were not significantly different between groups, both the response in plasma glucose and area under the curve data suggested a trend whereby the PEE and PSS groups appeared to improve their diabetic status while the condition worsened in the PES group. In light of Pedersen's hypothesis (1977) which emphasizes the role of maternal hyperglycemia on fetal development, the observed macrosomia of the non-viable pups would therefore be expected and probably played a signficant role in the overall pregnancy outcome in these animals. It is also likely that the potential of "fuel-mediated teratogenesis", whereby the fetus is bathed in a nutrient rich medium exposing them to the highly teratogenic effects of hyperglycemia, hypertriglyceridemia and ketosis (Freinkel, 1980: Metzger, 1991), was greatly enhanced in PES offspring. Martin and Herrara (1991) report a marked hypertriglyceridemia during late gestation in diabetic dams. Since exercise during diabetic pregnancy has been shown to improve lipid profile in humans (Hollingsworth, 1983) it is possible that PEE animals were better able to handle the changes in lipid levels than either PSS or PES animals. Although one would expect that the benefits of exercise training on lipid metabolism would not be completely eliminated over the short gestation period it is possible that removal of the exercise stimulus may have altered lipolytic activity in a fashion that made handling the pregnancy induced hypertriglyceridemia more difficult instead of easier. The enhanced weight gain and food consumption which was

observed in PES animals certainly substantiates an inability to adjust to a sedentary level of caloric expenditure. Further research examining changes in lipid profile under these conditions is essential.

In conclusion, moderate exercise training in severely diabetic female rats did not improve oral glucose tolerance or hyperglycemia. It is important to note, however, that the statistical power is low due to the small sample size in each of the pregnant subgroups. The ability of diabetic female rats to become pregnant was neither hindered or facilitated by exercise training. Furthermore, continuation of exercise training during gestation did not appear to negatively affect pregnancy, as indicated by litter size and percentage of viable offspring. In contrast, cessation of exercise in conjunction with initiation of pregnancy appears to perturb the level of glucose homeostasis established under diabetic exercise conditions in such a way as to negatively affect the response of the dams to pregnancy and pregnancy outcome.

Chapter 4

Concommittent Effects of Exercise and Type I Diabetes on Offspring Anthropometric Profile and Metabolic Development.

Introduction

It is well known that the offspring of diabetic mothers are negatively affected by the pathophysiology which accompanies the maternal diabetic condition. In most instances these negative effects are a result of a cascade of events, initiated by maternal hyperglycemia and resulting in fetal hyperglycemia and hyperinsulinism (Pedersen, 1977). These conditions, concomitant with the enhanced maternal circulating fuel supply, result in a fetal environment which precipitates macrosomia and various other teratological abnormalities (Pedersen, 1977; Freinkel, 1980; Metzger, 1991).

Neonatal macrosomia has been reported to increase the risk for both mother and offspring at delivery (Hollingsworth and Moore, 1987). Furthermore, increased size at birth has been associated with obesity in later life (Vohr, Lipsitt and Oh, 1980; Pettitt, Baird, Aleck, Bennett, and Knowler, 1983; Cha, Gelardi and Oh, 1987). The dysmorphogenesis associated with elevated nutrient levels has been primarily attributed to enhanced levels of glucose and beta-hydroxybutyrate (Sadler, 1991; Metzger, 1991). Recently, Eriksson (1993) also documented the teratogenic influence of oxygen free radicals. Abnormal levels of glucose and beta-hydroxybutyrate were found to cause severe malformations and depressed growth rate in developing embryos *in vitro*. Administration of antioxidant substances such as superoxide dismutase provided protection from the teratogenic effects of both glucose and beta-hydroxybutyrate (Eriksson, 1993). It is believed that these effects are mediated by an oxygen free radical mechanism (Eriksson, 1993; Cagliero et al., 1993).

Additionally, these authors found a differential reduction in teratogenesis when a mitochondrial pyruvate transport inhibitor was added to the agar growth medium, thus suggesting that the fetal mitochondria produce free radicals in response to exposure to abnormal maternal fuel supply, ultimately resulting in dysmorphogenesis (Eriksson, 1993).

Additional information supporting the important role of the maternal environment on offspring outcome is provided by Gauguier and co-workers (1990) who studied the role of maternal hyperglycemia during late gestation on inheritance of altered glucose metabolism in the offspring. In this study, normal dams were infused with high levels of glucose during the last week of gestation. Offspring born of these mothers were macrosomic and had glucose handling problems typical of those seen in offspring from Type I diabetic dams. Moreover, these differences persist into adult life. These and other similar findings provide impetus for work attempting to manipulate the maternal environment during a diabetic pregnancy.

The use of exercise as a means by which to manipulate the maternal environment has been studied in a limited number of projects. Hollingsworth and Moore (1987) examined the effect of exercise on glycemic control in pregnant females with IDDM. Results indicated that mild to moderate intensity postprandial exercise (walking) improved glycemic control and lowered fasting triglyceride levels. In addition, there were no significant negative effects of the training program on the mothers (Hollingsworth and Moore, 1987).

Urie-Hare and colleagues (1989) trained both diabetic and non-

diabetic rats on a treadmill for three weeks prior to, and throughout gestation. Results indicated that the untrained diabetic dams had fetuses which were lighter, smaller and more malformed than offspring of trained dams (Urie-Hare et al., 1989). Furthermore, exercise appeared to blunt the characteristic diabetic response normally seen in offspring of diabetic dams. Although this work presents initial insights into the effects of chronic training on fetal outcome in experimentally induced diabetes, it is clear that three weeks of training is a rather limited exercise stimulus. In light of the adjustment periods associated with both STZ initiation of diabetes (Goodyear et al., 1988) and accommodation to training (McKardle, Katch and Katch, 1989), a different maternal environment may have resulted if the animals had been allowed to fully accommodate to a new level of homeostasis in response to both diabetes and training. Furthermore, since the offspring were sacrificed at birth, the long term effects of maternal exercise on offspring growth and glucose homeostasis have yet to be examined.

Therefore, the purposes of the present study were to: 1) extend the training duration utilized in the work by Urie-Hare et al. (1989) to determine the effects of a longer exercise stimulus prior to gestation on both growth and glucose tolerance in offspring, 2) compare the effects exercise prior to, and during pregnancy on offspring outcome, and 3) illustrate the relationship between neonatal growth patterns and glucose handling.

Methods

General Procedures.

Maternal.

Virgin female Sprague-Dawley rats (150-175 grams; Harlan, Madison, Wisconsin) used in the study were housed in seperate nalgene cages at the University Laboratory Animal Research facilities at Michigan State University. Food (Purina® chow 5008) and water were supplied *ad libitum.* and the animals were kept on a twelve hour light-dark cycle (0100:1300). Room temperature (70-72' F.) and humidity (52-55%) were controlled throughout the study to ensure the health of the animals. Following six days of accomodation, animals were injected with a 50 mg/kg•body wt dosage of streptozotocin (STZ; Sigma® Chemical, no. S-0130) dissolved in 40 mM citrate buffer (pH 4.5) through the tail vein. The STZ injection occurred within five minutes of dissolution in citrate to allow for the STZ to be in its active state (Chen and Ianuzzo, 1982).

After a fourteen day period, to allow for complete diabetogenic action of the drug, a 1 ml arterial blood sample was drawn from the tail artery (Bober, 1988). Blood was centrifuged (1500•g) and the supernatant removed and stored at -20' C. until later analysis for plasma glucose concentration. Glucose levels were determined using a Kodak Ektachem DT60® analyzer according to a standard glucose oxidase reaction. Animals with two hour fasting blood glucose values above 20 mmol/l were considered diabetic. Those animals that did not respond to the drug and showed normal fasting plasma glucose as well as a normal response to a glucose challenge were defined as control animals. Because these animals had been injected

with the drug, they were utilized as a control. Control (n=30) and diabetic (n=30) animals were placed into matched pairs based on body weight and fasting plasma glucose levels. One animal from each pair was then randomly assigned to either an exercise (control-exercise = CE; diabetic-exercise = DE) or sedentary (control-sedentary = CS; diabetic-sedentary = DS) treatment group.

Animals in the exercise groups were accomodated to running on a motor driven treadmill for fourteen days, during which time both speed and duration were increased until the training intensity of 20 m/min, 0% incline, 60 min/day, 5 days/week was reached. The animals continued to train at this intensity for an additional eight weeks. Sedentary animals remained in their cages throughout this period although these animals were handled by their caretaker in similar fashion as those animals in the exercise groups.

Following completion of the initial eight week training program, all animals were mated with euglycemic breeder males (Harlan Sprague-Dawley, Madison, WI; 300-350 grams). DE and CE animals continued to exercise during their gestational period at the same intensity as they ran prior to pregnancy. Animals in all four groups were allowed to birth their pups naturally. Within twelve hours postpartum, pups from diabetic dams were given to euglycemic foster mothers to minimize any influence of milk contribution on growth and insulin sensitivity of the offspring. Pups from control animals remained with their birth mothers for the remainder of the study. All animals were allowed to nurse freely and were weaned at twenty-one days of age.

Neonatal.

Offspring were sexed, weighed and assessed for gross morphological abnormalities at the time of birth. In addition, the pups were weighed weekly for the remaining four weeks.

At 28 days of age offspring were given a fasting (12 hour) intraperitoneal glucose tolerance test (3g/kg•body weight^{-1}; 50% glucose). Blood samples (0.1 ml) were collected prior to injection of glucose load and ten, thirty, sixty and one-hundred twenty minutes following injection from the tail vein (Bober, 1988). Samples were centrifuged (1500•g; 20 minutes) and stored at -20' C until later analysis of plasma glucose levels at each timepoint.

Twenty four hours after administration of the glucose tolerance test, each animal was anesthetized with methoxyflurane and surgical removal of the triceps surae muscles, heart and liver was performed. Non-fasting blood was obtained from the heart prior to exsanguinated by pneumothorax and later analyzed for plasma glucose and insulin concentrations. Each animal's naso-anal length was determined for later calculation of the Lee index (Lee, 1929).

Statistical Analysis.

Analysis of variance with repeated measures was used to determine between group differences in offspring body weight. A two-way ANOVA was utilized to determine the effects of treatment and time on glucose tolerance. Area under the glucose curve, sacrifice glucose concentration and insulin concentration were compared between groups using a one-way ANOVA. Heart, liver, skeletal muscle weight and Lee index were analyzed using a factorial

ANOVA. Post hoc Scheffe' analysis was utilized when appropriate. The level of significance was set at $p < .05$ for all analyses. Data are reported as means + SEM unless otherwise noted in the text.

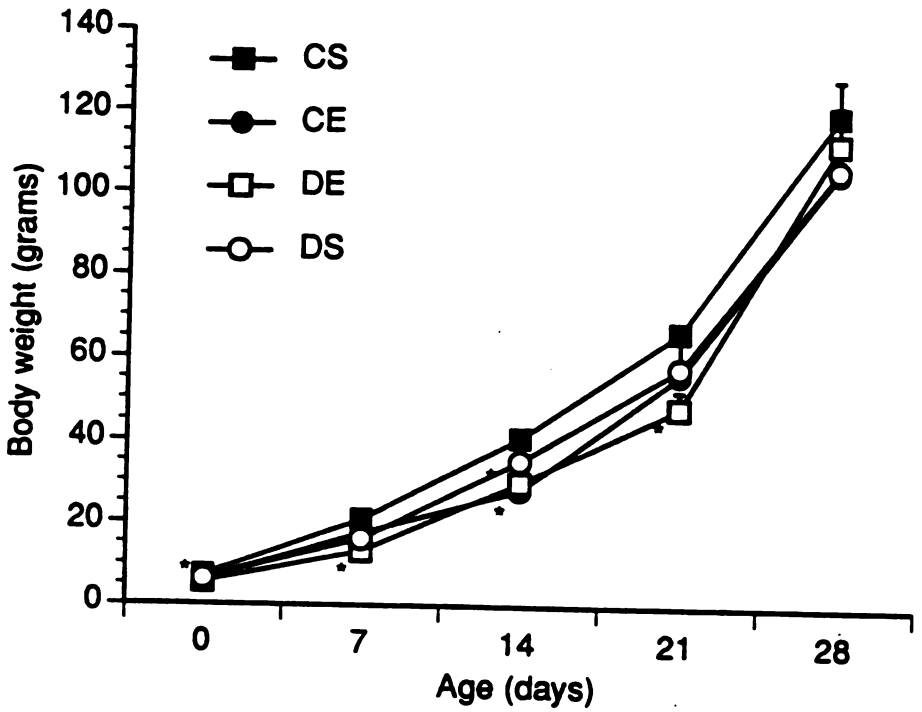
Results

The effect of maternal activity status on offspring body weight is illustrated in Figure 4.1. Due to the difference in response pattern between male and female offspring these data have been subdivided across gender. At days 0, 7, 14 and 21, CS male offspring were significantly heavier than male offspring of DE dams (Figure 4.1a) (Day 0: CS=7.09 $\pm$ 0.14 gm; DE=5.43 $\pm$ 0.29 gm; Day 7: CS=20.8 $\pm$ 0.79 gm; DE=13.2 $\pm$1.41 gm; Day 14: CS=40.81 $\pm$ 0.64 gm; DE=29.88 $\pm$2.92 gm; Day 21: CS=66.46 $\pm$1.86 gm; DE=48.1 $\pm$ 4.09 gm). Male offspring from CS dams were also significantly heavier than their counterpart control males (CE offspring) at day 14 (CS=40.81$\pm$1.64 gm; CE=27.95$\pm$2.86 gm). Maternal activity condition had no significant effect on male offspring body weight at day 28.

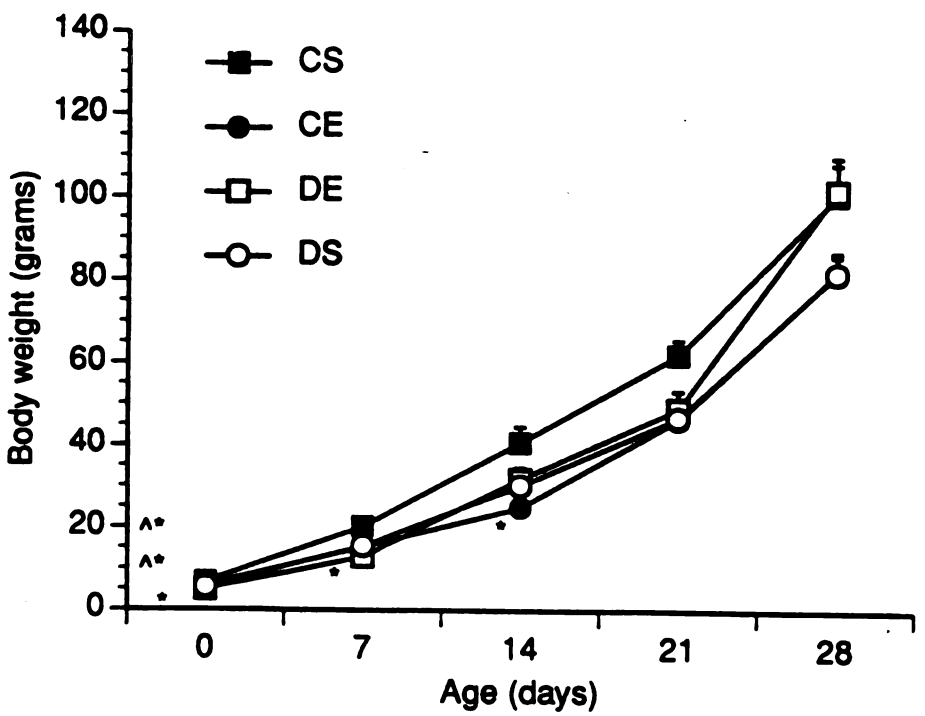
Female offspring of CS dams were significantly heavier than CE, DE, and DS female offspring at Day 0 (Figure 4.1b) (CS=6.63$\pm$24 gm; CE=5.88$\pm$13 gm DE=4.77$\pm$07 gm; DS=5.43$\pm$.09 gm). Furthermore, CE female offspring were significantly heavier than DE female offspring at birth. Female offspring of DE dams also weighed less than their sedentary counterparts (DS offspring) at this time. CS offspring were significantly heavier than DE offspring (CS=20.2 $\pm$1.91 gm; DE=12.9$\pm$ 1.58 gm) at day 7 and CE offspring at day 14 (CS=40.73$\pm$3.83 gm; CE=24.95$\pm$1.86 gm). Maternal activity condition had no significant effect on female offspring body weight at either 21 or 28 days.

Figure 4.1 Body weight (grams) at birth (0 days), 7, 14, 21 and 28 days in male **(4.1a)** and female **(4.1b)** offspring of mothers who were; diabetic and exercised prior to and during gestation (DE); diabetic and sedentary (DS); non-diabetic and exercised prior to and during gestation (CE); non-diabetic and sedentary (CS). Data are expressed as mean (± SEM). Asterisks (*) indicate a significant ($p<0.05$) difference in body weight between male offspring of CS mothers and male offspring of DE dams (Day 0,7,14 and 21) and CE dams (Day 14) and between female offspring of CS dams and CE, DE, and DS dams at Day 0; and vs. DE offspring at Day 7 and CE offspring at day 14. Female offspring of DE dams were also significantly lighter than female offspring of DS and CE dams at Day 0 (as represented by ^). Total number of offspring evaluated in each group were; DE: 6 males; 3 females; DS: 2 males; 3 females; CE: 4 males; 4 females; CS: 7 males; 3 females.

a.


140
120
100
80
60
40
20
0
Body weight (grams)
CS
CE
DE
DS
0
7
14
21
28
Age (days)

b.


140
120
100
80
60
40
20
0
Body weight (grams)
CS
CE
DE
DS
0
7
14
21
28
Age (days)

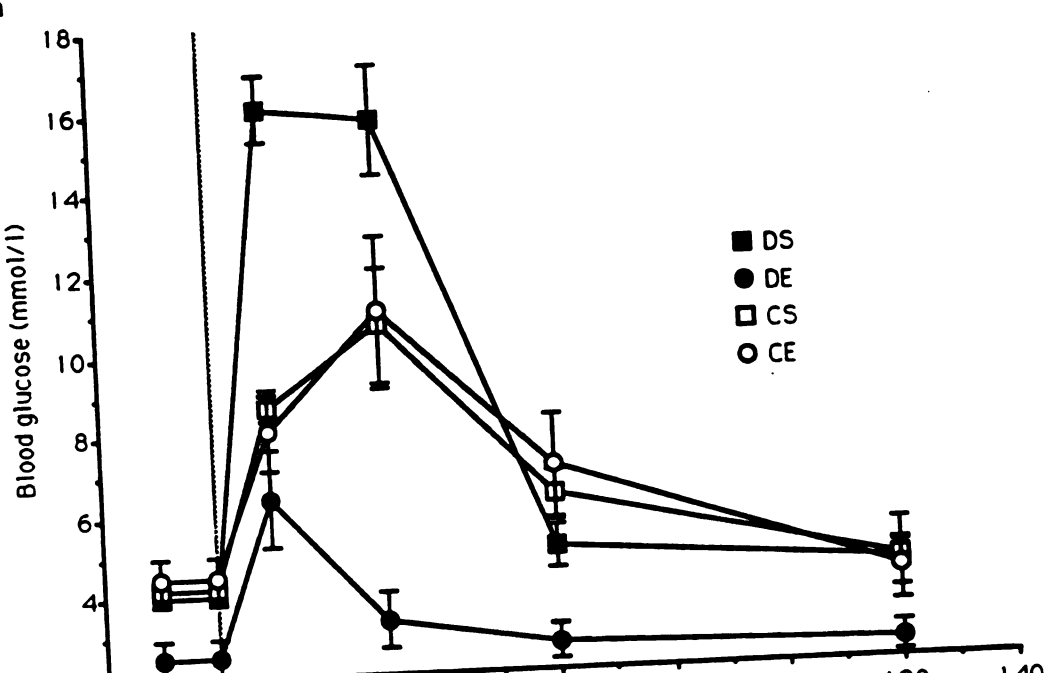
An intraperitoneal glucose tolerance test was administered to all offspring at 28 days of age. Plasma glucose response to the glucose load is illustrated in Figure 4.2. In general, offspring from diabetic exercising mothers presented a blunted response while pups from diabetic sedentary dams showed augmented glucose levels. The impact of maternal activity status on this parameter was particularly apparent in the male offspring (Figure 4.2a).

Twelve hour fasting plasma glucose concentration was significantly lower in male offspring of DE dams than in offspring of CS and CE mothers (DE=2.53+ 0.47 mmol/l; CS=4.24+ 0.26 mmol/l; CE=4.48+ 0.54 mmol/l). The difference between CS and DE offspring persisted throughout the remainder of the GTT, except at minute ten. Similarly, CE offspring values were signficantly greater than those attained by DE male offspring at minutes 30 and 60. In addition, at 10 minutes male offspring from CS, CE and DE dams had lower plasma glucose levels than offspring of DS mothers (CS=8.69 $\pm$ 0.33 mmol/l; CE=8.10 $\pm$ 0.99 mmol/l; DE=6.40$\pm$1.22 mmol/l; DS=16.0 $\pm$.8 mmol/l). DS offspring values were higher at 30 minutes relative to the DE group (DS=15.65 mmol/l; DE=3.33+.68 mmol/l) and lower than both CS and CE values at thirty minutes (CS=10.61+1.41 mmol/l; CE=10.93+1.88 mmol/l).

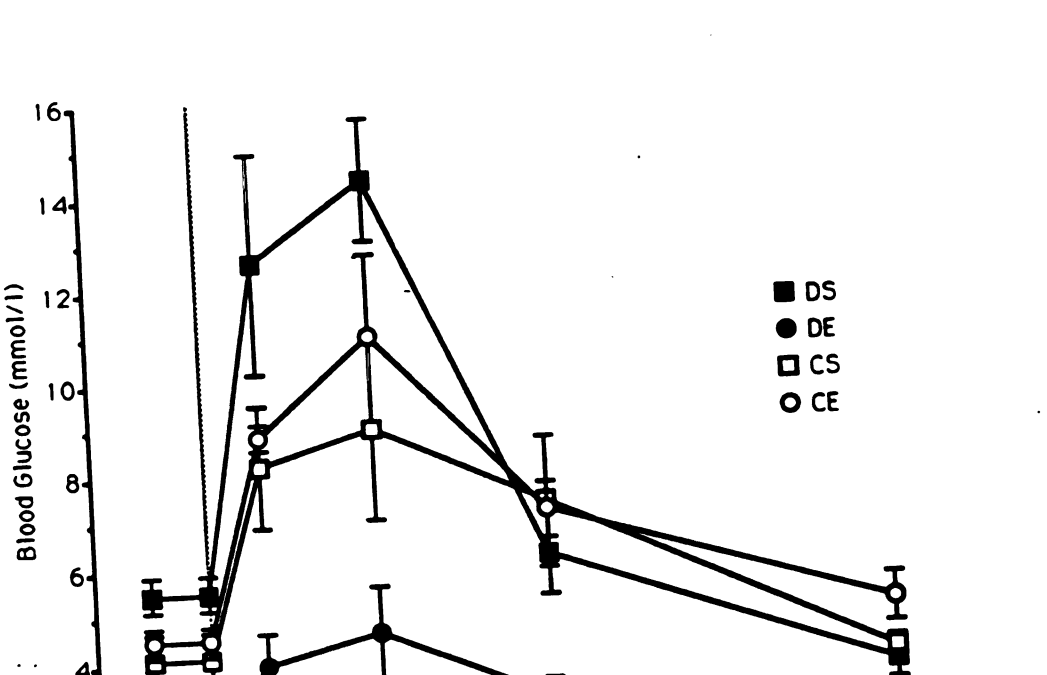
Based on response to the IPGTT, maternal activity status did not appear to impact as significantly on the female pups as was observed in their male littermates (Fig. 4.2b). At birth, female offspring from DE dams did however have lower basal levels of glucose than female offspring in the DS and CE groups (DE=2.43 $\pm$ 0.22 mmol/l; DS=5.5 $\pm$ 0.37 mmol/l; CE=4.5 $\pm$ 0.31 mmol/l). This difference

Figure 4.2 Twelve hour fasting plasma glucose concentration (mmol/l) and plasma glucose concentration prior to injection (time 0) of a 3.0g/kg body weight glucose load and at 10, 30, 90 and 120 minutes following injection in male (**4.2a**) and female (**4.2b**) offspring of mothers who were; diabetic and exercised prior to and during gestation (DE); diabetic and sedentary (DS); non-diabetic and exercised prior to and during gestation (CE); non-diabetic and sedentary (CS). Data are expressed as mean (± SEM). Asterisks (*) indicate a significant ($p<0.05$) difference in plasma glucose concentration at the time indicated between male offspring of DE dams and male offspring of group indicated. ^ indicates a significant difference at 10 minutes between male offspring of DS dams and male offspring of CS and CE dams. In Figure 4.2b asterisks (*) indicate a significant ($p<0.05$) difference in plasma glucose concentration at the time indicated between female offspring of DE dams and female offspring of the group indicated. Total number of offspring evaluated in each group were; DE: 6 males; 3 females; DS: 2 males; 3 females; CE: 4 males; 4 females; CS: 7 males; 3 females.

a


Blood glucose (mmol/l)
18
16
14
12
10
8
6
4
2
-20
0
20
40
60
80
100
120
140
Time (minutes)
DS
DE
CS
CE

b


Blood Glucose (mmol/l)
16
14
12
10
8
6
4
2
-20
0
20
40
60
80
100
120
140
Time (minutes)
DS
DE
CS
CE

between DS and DE female offspring persisted through minute 30. At minute 120, the DE offspring had significantly lower levels of plasma glucose than CE offspring (DE=2.6 ± 0.1 mmol/l; CE=4.93 ±. 0.52 mmol/l).

Additional evaluation of response to the glucose load was done by calculating the area under the glucose curve. The data are presented in Figure 4.3 for all groups according to offspring sex. Maternal activity status had no effect on area under the glucose curve in female offspring. Conversely, male offspring of DE dams had a smaller area under the curve in comparison to the other three offspring groups (DE=101.8±25.2; DS=588.3; CE=340.6 ±63.9; CS=347.7+56.9).

Glucose concentration in the plasma at sacrifice was highest for the DE animals and was significantly greater than all other groups evaluated (Figure 4.4a). The DE group had a signifcantly greater insulin value at the time of sacrifice compared to the CE group (DE=4.3 ± 1.05 ng/ml; CE=0.6 ±0.21 ng/ml)(Figure 4.5b). The insulin to glucose ratio (Figure 4.5) was significantly greater ratio for the DE pups than any other group.

Sacrifice tissue weight data for male and female offspring are displayed in Tables 4.1 and 4.2, respectively. No significant between group differences existed for any parameter assessed in 28 day old male pups. DE females had a larger red gastrocnemius than both DS and CE groups. This difference was the only statistically significant result obtained from the sacrifice data for either males or

Figure 4.3 Area under the plasma glucose curve (mmol/l-basal·2hr^{-1} for male and female offspring of mothers who were; diabetic and exercised prior to and during gestation (DE); diabetic and sedentary (DS); non-diabetic and exercised prior to and during gestation (CE); non-diabetic and sedentary (CS). Data are expressed as mean ($\pm$ SEM). Asterisks (*) indicate a significant ($p<0.05$) difference in area between male offspring of DE mothers and male offspring of DS, CE and CS dams. Maternal activity status had no effect on area under the glucose curve in female offspring. Total number of offspring evaluated in each group were; DE: 6 males; 3 females; DS: 2 males; 3 females; CE: 4 males; 4 females; CS: 7 males; 3 females.

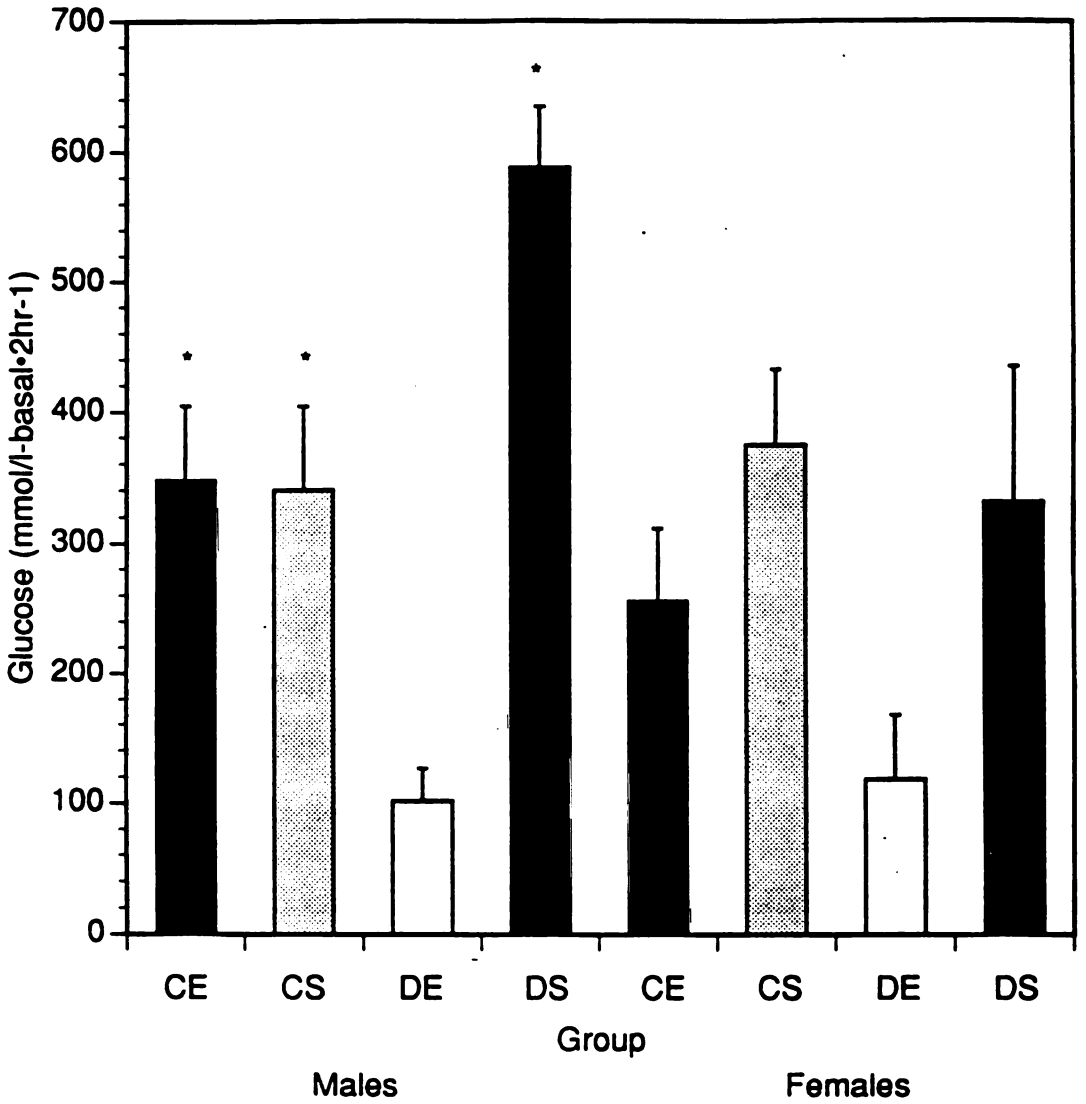
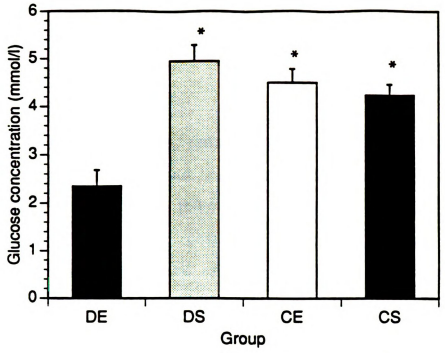

700
600
500
400
300
200
100
0
Glucose (mmol/l-basal•2hr-1)
CE
CS
DE
DS
CE
CS
DE
DS
Group
Males
Females

Figure 4.4 Glucose concentration (mmol/l) **(4.4a)** and insulin concentration (ng/ml) **(4.4b)** at sacrifice in offspring of mothers who were; diabetic and exercised prior to and during gestation (DE); diabetic and sedentary (DS); non-diabetic and exercised prior to and during gestation (CE); non-diabetic and sedentary (CS). Data are expressed as mean ($\pm$ SEM). Asterisks (*) indicate a significant ($p<0.05$) difference in plasma glucose (4.4a) and plasma insulin (4.4b) concentration between offspring of DE mothers and offspring of DS, CS and CE dams. Total number of offspring evaluated in each group were; DE: 6 males; 3 females; DS: 2 males; 3 females; CE: 4 males; 4 females; CS: 7 males; 3 females.

a.


Glucose concentration (mmol/l)
0
1
2
3
4
5
6
*
*
*
DE
DS
CE
CS
Group

b.

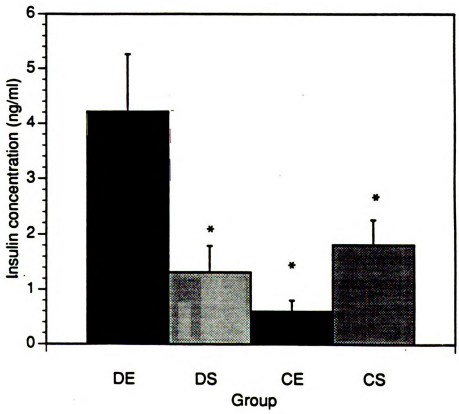

Insulin concentration (ng/ml)
0
1
2
3
4
5
6
*
*
*
DE
DS
CE
CS
Group

Figure 4.5 Insulin to glucose ratio (I/G ratio) in offspring of mothers who were; diabetic and exercised prior to and during gestation (DE); diabetic and sedentary (DS); non-diabetic and exercised prior to and during gestation (CE); non-diabetic and sedentary (CS). Data are expressed as mean (± SEM). Asterisks (*) indicate a significant ($p<0.05$) difference in I/G ratio between offspring of DE mothers and offspring of CE, CS and DS dams. Total number of offspring evaluated in each group were; DE: 6 males; 3 females; DS: 2 males; 3 females; CE: 4 males; 4 females; CS: 7 males; 3 females.

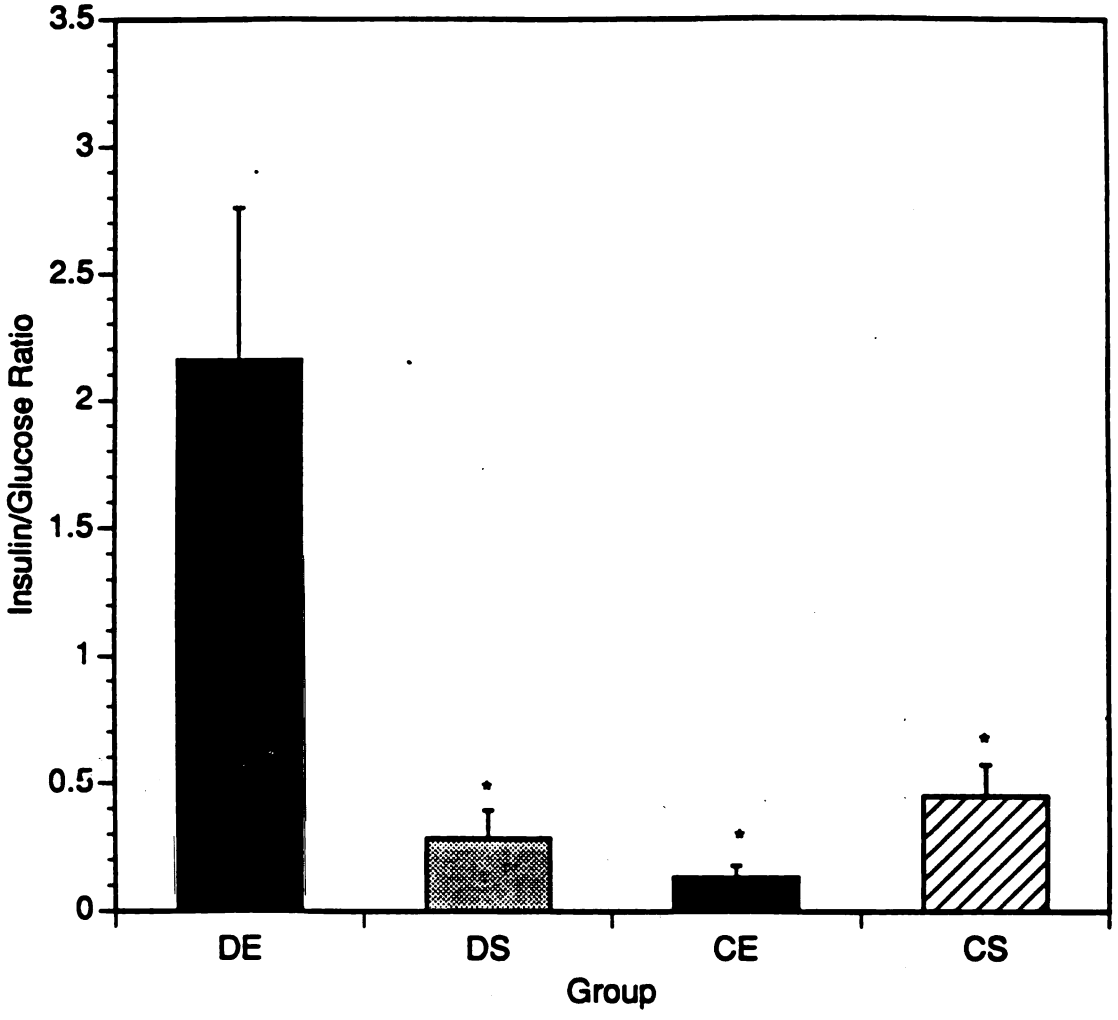

3.5
3
2.5
2
1.5
1
0.5
0
Insulin/Glucose Ratio
DE
DS
CE
CS
Group

Table 4.1. Male offspring tissue weights at 28 days.

Tissue Weight (grams)	DE (n=6)	DS (n=2)	CE (n=4)	CS (n=7)
Liver	4.73 ±.19	5.16 ±.67	4.25 ±.13	5.1 ±.29
Heart	.48 ±.02	.39 ±.06	.45 ±.01	.48 ±.04
Soleus	.04 ±2.98E-3	.04 ±1.95E-3	.05 ±2.33E-3	.05 ±4.5E-3
Plantaris	.08 ±4.39E-3	.06 ±4.45E-3	.07 ±4.42E-3	.08 ±.01
Gastrocnemius (total)	.44 ±.03	.38 ±.04	.42 ±.02	.49 ±.05
White Gastrocnemius	.25 ±.02	.23 ±.03	.26 ±.02	.27 ±.03
Red Gastrocnemius	.19 ±.02	.15 ±.01	.16 ±.01	.21 ±.03
Lee Index	.323 ±.005	.326 ±.013	.318 ±.005	.306 ±.009

Data are expressed as mean (± SEM)
$p < 0.05$

Table 4.2. Female offspring tissue weights at 28 days.

Tissue Weight (grams)	DE (n=3)	DS (n=4)	FE (n=4)	FS (n=3)
Liver	3.98 ±.43	3.66 ±.23	3.20 ±.15	4.10 ±.12
Heart	.47 .05	.32 .01	.38 .02	.43 ±.06
Soleus	.04 1.62E-3	.03 1.32E-3	.04 1.64E-3	.25 .22
Plantaris	.08 .01	.05 .01	.06 .01	.06 .01
Gastrocnemius (total)	.43 .03	.29 .03	.36 03	.30 .12
White Gastrocnemius	.21 .02	.19 .02	.24 .02	.32 .08
Red Gastrocnemius	.21 .01	.10 .01 *	.12 .01 *	.16 .03

Data represent mean ± sem.
* vs. DE ($p<.05$)

females. It is interesting to note however that the Lee index for males in the DE, DS, and CE groups as well as females in the DE, CE, and CS groups was higher than the .0.300 value reported as normal in earlier work (Lee, 1929; Bernardis and Patterson, 1968).

Discussion

Offspring outcome as impacted by exercise training and Type I diabetes, was evaluated in this study. Pups from dams exposed to one of four conditions (diabetic exercise, diabetic sedentary, control exercise, or control sedentary) were studied relative to growth pattern and glucose homeostasis at four weeks of age.

Offspring from diabetic mothers had a lower body weight at birth relative to that which has been observed in pups from control animals (Kervran, Guillaume and Jost, 1978; Eriksson et al., 1980). Furthermore, offspring from DE animals were significantly lighter than pups from CS animals at all time points assessed, up to three weeks of age. The degree of microsomia evident in the diabetic offspring was similar to that which has been reported previously (Kervran, Guillaume and Jost, 1978; Aerts and VanAssche, 1991; Grill et al., 1991). As has been proposed by the "Pedersen hypothesis" this low birth weight is most a direct result of the hyperglycemic state of the mother. Placental transfer of high levels of glucose initially results in fetal hyperglycemia and reflexive hyperinsulinemia (Pedersen, 1977). As a consequence of this insulin hypersecretion the developing beta cells in the offspring become exhausted, and ultimately experience a reduction in their ability to secrete insulin. It is believed that this exhaustion of the beta cells is

related to insensitivity to glucose rather than a shift in the glucose-insulin dose-response relationship (Kervran, Guillaume and Jost, 1978; Grill, et al., 1991). *In utero,* once the fetus becomes insensitive to glucose and is no longer able to secrete insulin, hypoinsulinism results and renders the offspring incapable of utilizing the enriched nutrient mix available. The ultimate result is a reduction in body weight or microsomia (Aerts and VanAssche, 1991). Data from offspring of DE mothers in this study would support this hypothesis.

It was interesting to note that by 28 days of age DE offspring, whose growth pattern had been significantly retarded over the first 21 days, had experienced an accelerated growth phase and were now of similar body weight as those offspring from the CS dams. In light of this trend, one might speculate that if the beta cells were not permanently damaged at the time of exhaustion their insulin secretory ability might have improved over the maturation of the animal. The offspring could, thereby, potentially reverse their insulin levels to more normal values over early life. Increases in insulin would then promote growth under conditions of adequate fuel resources. Growth patterns in the DE group appear to follow this trend. The animals were the smallest until week four when these animals 'catch up' to all other groups evaluated. Additionally, the DE pups were hypoglycemic and hyperinsulinemic at four weeks of age. One could suggest that their beta cells began to produce/secrete insulin in high quantities in response to their demands. Additional anecdotal evidence to support this theory was an increased frequency of nursing in DE pups over the first three weeks of life in

comparison to all other offspring groups, including the DS pups. The use of foster mothers to raise the diabetic pups allowed for control of nutrition during maturation of the pups. It is clear that growth rate is linked with adequate nutrition (Asplund, 1972). By using foster mothers it was possible to eliminate any confounding factors associated with availability of adequate and appropriate fuels for the pups. Additionally, use of foster mothers provided an equalizing baseline relative to nutritional content of milk between all four groups evaluated.

It appears that the reflexive hyposecretion of insulin in response to extremely high glucose levels *in utero* is followed by a normalization of insulin response in early life. Although the animals were hyperinsulinemic at four weeks of age, it is plausible that their systems were attempting to create a new homeostatic environment. If the animal's insulin sensitivity ultimately returns toward normal, it would suggest that the exercise training prior to, and during gestation might have provided limited protection for the offspring. However, if the animal's insulin profile remained elevated, one could suggest that the animal might show a response typical of NIDDM subjects in adult life. If this were the scenario exercise would still have provided a 'protective role' for the neonate since otherwise the animal would possess characteristics typical of an IDDM state.

It is important to note, that the DE animals also possessed the highest insulin to glucose ratio, a parameter associated with insulin resistance in the periphery. If these animals were able to train throughout maturation, they appear to have the greatest potential for training adaptation due to this condition.

Offspring from diabetic sedentary mothers had a hyperglycemic response to a glucose challenge and slightly depressed insulin levels at four weeks of age. The pups appeared to be developing into adults similar to their mothers with hyperglycemia and hypoinsulinemia typical of Type I diabetes. This persistence of abnormal glucose homeostasis in adulthood, without the influence of genetics has been previously reported by Gauguier and co-workers (1990). These authors suggest that perturbations in the fetal environment in utero (hyperglycemia) contribute to the development of diabetes mellitus in adulthood (Gauguier et al., 1990). Results from the present work provide additional support for this hypothesis.

The impact of elevated maternal glucose levels on teratogenic effects in offspring was assessed qualitatively in the present work. Results suggest that gross morphological abnormalities such as incomplete development of limbs were evident in offspring of the DS group. Two of the six viable offspring were born with some level of limb defect. Several other non-viable pups in both diabetic groups showed gross morphological abnormalities (incomplete development of the tail). These results appear to confirm earlier work regarding the teratogenic effects of both hyperglycemia and ketosis (elevated beta-hydroxybuterate) in the maternal environment (Sadler, 1991; Eriksson, 1991; Goldman and Goto, 1991). Due to the lack of gross abnormalities in the DE group, one could again suggest that exercise may have provided a more 'controlled' environment for the fetus which ultimately decreased their risk of dysmorphogenesis. These

data must however, be interpreted cautiously due to the low sample size in this study.

Grill and colleagues (1991) studied insulin and glucose responses of offspring in severely diabetic animals. The authors report that their data suggests no sex specificity in response to development in a diabetic mother. The present work appears to conflict with Grill et al., (1991) since male offspring tended to have lower insulin values at sacrifice. Additionally, the males showed a more abrupt response to a glucose load. The mechanism underlying these differences could be related to the androgen-estrogen levels present during development *in utero*. It has been suggested that higher levels of estrogen relative to androgen provide protection against diabetogenesis (Paik et al., 1982). Developing fetuses impact one another *in utero* such that the gonadal steroid level of one fetus impacts on neighboring fetuses (VomSaal and Bronson, 1978). The original litter composition for the DS group was more males than females with the opposite ratio for DE pups. Therefore, it is plausible that both males and females in the DS group could have experienced higher basal levels of androgens relative to estrogens *in utero*. The elevation in male gonadal steroids could have increased the likelihood for actualization of the teratogenic effects of glucose and oxygen free radicals in these pups.

Body weight response in pups from control exercising animals appears to mimic that of the diabetic sedentary group, although the glucose tolerance test results appear generally normal. It is interesting to note, however, that the insulin concentration at sacrifice was lowest in this group. The animal's inability to increase

body weight might be due to it's inability to utilize insulin stimulated transport of nutrients. This phenomenon has not been reported previously and should be evaluated over a longer timecourse to provide additional insight into the effect of maternal exercise on glucose homeostasis in adult offspring.

In summary, severe diabetes during pregnancy affects bodily growth, maturation and glucose homeostasis in the offspring. Chronic endurance training by the mother prior to and during gestation appears to reduce the negative teratological outcomes typically associated with a diabetic pregnancy, as well as causing a reversal in the normally observed hypoinsulinism and hyperglycemia at 28 days of age in diabetic offspring.

Chapter 5
Interpretations and Conclusions

GLOBAL INTERPRETATIONS AND CONCLUSIONS

A series of experiments were undertaken to examine the glucose handling response of diabetic rats to exercise training proceeding and during gestation. The impact of this exercise on offspring outcome and subsequent neonatal development was also evaluated. The training time course utilized in this study provided an exercise stimulus whose duration was not heretofore utilized in animals with uncontrolled Type I diabetes. The preconception training followed by stopping exercise at conception was also never evaluated in this type of diabetic animal. Furthermore, this study was the first to examine the effects of maternal diabetes and exercise on offspring growth rate and glucose homeostasis beyond the day of birth. It was hypothesized that those animals who trained prior to and during gestation would have an enhanced capacity to become pregnant and would have more pups that were viable over time.

Critics would suggest that animals rendered severely diabetic could not withstand the exercise stressor imposed. Furthermore, it would be unlikely that these animals could be made pregnant due to their uncontrolled level of diabetes. Finally, if the animals became pregnant, their offspring would be grossly malformed and could not survive. These criticisms are not without merit, however results from the present series of experiments present new information which could potentially refute some of these claims.

Qualitative data indicates that the animals were otherwise healthy and behaved normally throughout much of the study. The diabetic females responded to the inducement and development of the diabetic state in the following manner. Initially, the animals lost

weight and began to exhibit the early signs associated with diabetes (hyperphagia, polyuria, polydispia and glucosuria). Shortly following this initial phase, the animals adjusted to their "new condition". At approximately five to seven weeks of the study, the majority of the animals began to exhibit latter signs of severe uncontrolled diabetes-blindness and ketosis. Although their body weight declined for the short period prior to and during the initial development of blindness, animals quickly began to thrive following this second adjustment period. Once the animal had acquired the more severe clinical symptoms associated with diabetes, they continued to thrive and appeared to achieve a homeostatic baseline whereby they again behaved normally. The response of these female rats tended to contrast that observed in male rats, who tend to decline in health over time without ever acquiring a new homeostatic baseline.

Based on these results, one could hypothesize that female animals are created with the capacity to withstand a larger degree of chronic physiological stress without exhibiting an inability to thrive. Moreover, the ultimate physiological function of male and female animals indicates that the role of these two genders is very different. Simply stated, males are designed to effectively procreate while females must be able to carry and bear offspring. Therefore, each one's physiological and anatomical function is focused to maximize these primary functions.

Applying this concept specifically to physiological responsiveness it is best exemplified by the capability of females to tolerate the plethora of alterations associated with gestation. Specifically, the alteration in glucose metabolism which occurs to

such an extent in some pregnancies that the result is often a diabetogenic state. Despite this metabolic aberration females typically withstand these extreme fluctuations in glucose levels without negative consequence to either the maternal or fetal systems. Similar perturbation in males however typically result in a significant alteration in function and in ability to thrive, as was suggested by the male offspring data obtained in this study. Together, these facts are an example of the greater physiological capacity of females versus males to adapt to perturbations.

This "plasticity theory for female responsiveness" may in fact be a characteristic of females whereby they possess the ability to withstand a larger array of metabolic perturbations. Females appear to adjust to the perturbation by creating a "new level of homeostasis" whereby they function effectively under the imposed stressor. The present study supports this hypothesis in light of the fact that those animals who became pregnant appeared to have stabilized in response to the diabetic influence. The animals were blind and ketotic yet they continued to thrive physiologically and appeared 'normal' behaviorally. Animals whose weight had not stabilized or who had yet to develop the secondary characteristics of the diabetic condition (blindness) failed to become pregnant.

It also appears that any perturbation imposed on the female once they have adjusted to their new condition will negatively impact gestational outcomes. This concept is supported by the fact that animals in the PES group, whose once adjusted system was perturbed by the cessation of exercise, failed to birth any offspring that remained viable beyond eight to twelve hours. Additionally,

this group possessed the largest number of females who began to 'resorb' their pups prior to birthing. This data suggests that although females are capable of adjusting to stressors, they must be provided adequate time to create a new level of functioning within the constraints of the factors impacting on them.

If females are allowed to respond to their condition, without further perturbation, they are capable of bearing and carrying offspring to term. Furthermore, under these conditions the offspring will thrive, although their metabolic functioning will be dictated by both genetic and environmental influences.

The role of environment on offspring outcome has been clearly delineated in the present work. The pups will possess metabolic characteristics that are altered in response to their environment in utero. Utilization of STZ as a model for the nongenetic development of maternal diabetes provided an excellent technique for assessing the role of environment versus genetic influence on offspring outcome. It appears that the enhanced nutrient plethora associated with maternal diabetes provided a medium whereby neonatal outcomes and adult metabolic characteristic were impacted. Furthermore, the influence of exercise training prior to, and during gestation appears to have caused alterations in offspring outcome as well as adult responsiveness unlike that of offspring from sedentary dams. The mechanism underlying the exercise related phenomenon remains unclear. However, it appears that application of the results attained in the present work support Freinkel's modified version of Pedersen's hypothesis in regard to the diabetic condition.

RECOMMENDATIONS

Results from the present work clearly support the findings of others suggesting that the maternal environment during gestation has a significant impact on offspring outcome. Additionally, the negative influence of the diabetic condition produces teratological results in the offspring indicative of the process of "fuel-mediated teratology" (Freinkel, 1980; Metzger and Freinkel, 1981).

It appears that females possess the capacity to respond to chronic physiological stressors in a "plastic" fashion whereby they alter their function to remain within "safe" limits. This does not seem to occur in males of the same species. Further investigation into the notion of a "plasticity theory for female responsiveness" would provide additional rationale as to the viability of this theory. If the theory is supported, other research investigating the limits of perturbation acceptable for "normal" function both prior to and during pregnancy are critical. Assessment of the time necessary for adjustment to the diabetic condition prior to initiation of an exercise program and/or initiation of pregnancy would provide for a clearer evaluation of the impact of these conditions on the overall function of diabetic females.

Investigations assessing exercise as a therapeutic agent in diabetic females should vary the intensity or duration of the exercise stressor in an attempt to maximize its beneficial effects on the diabetic condition. Furthermore, additional information is necessary regarding the exercise prescription for the safest and most effective training program for both non-diabetic and diabetic pregnant individuals.

In light of previous work from Eriksson's laboratory, it is important to determine the impact of maternal exercise during gestation on fetal capacity to develop oxygen free radicals. If these molecules are the primary teratogen, it is clear that any upregulation in production of free radicals in the fetus could cause significant problems in the fetus. Additionally, evaluation of the impact of free radical production during fetal life and its subsequent effects on adult metabolism is critical.

In summary, results from this scientific research indicate the following:

(1) factors impacting the maternal system at the onset or during gestation tend to negatively impact offspring outcome

Animals who terminated exercise at the onset of pregnancy were capable of conception yet the offspring were not viable at birth. The PES mothers tended to have the largest weight gains during gestation which may have impacted the offspring in a negative fashion. Additionally, these animals had slightly larger pups than the other groups. One could speculate that the reduction in exercise stress at the onset of pregnancy created a situation whereby the mother's homeostatic balance was perturbed. The imbalance influenced the pups capacity to live once born.

(2) exercise prior to, and during gestation in a Type I diabetic animal does not appear to negatively influence the viability of offspring

The PEE group had the same number of animals conceive, relatively equal numbers of pups born and approximately the same number of viable pups after twelve hours of life as the PSS counterparts. It appears that the continuation of exercise throughout pregnancy in trained animals does not negatively affect offspring in uncontrolled diabetic animals.

(3) chronic exercise training in diabetic females prior to, and during pregnancy seems to provide some protection for offspring relative to growth and glucose handling compared to pups from diabetic sedentary mothers

Pups from the PSS mothers (DS) exhibited symptoms similar to their mothers (hyperglycemia and hypoinsulinemia) as well as a hyperglycemic response to a GTT. Conversely, the pups from PEE dams (DE) responded to a GTT in a hypoglycemic manner. These animals also were hypoglycemic and hyperinsulinemic at sacrifice. One could speculate that the DE pups were responding to the adjustments made *in utero* whereby the exercise condition had some affect on their glucose handling capabilities. The present data do not provide ample information regarding the mechanism driving this

condition. However, the controller could be either central in nature such as the at the neonatal pancreas or peripheral where the skeletal muscle alters transport of glucose or sensitivity to insulin. Further research is needed to delineate the precise mechanism involved in this phenomenon.

(4) females appear to possess the capacity to adjust to metabolic perturbations especially those typical of gestation

The diabetic females increased weight gain and exhibited behavioural characteristics similar to those seen in animals of similar age. A review of data from other studies suggested that this response is different from that seen in male animals who tend to declined in weight over time in response to STZ diabetes. From these results, it appears that some factor enables the female species to adjust more appropriately to the diabetic condition once established.

(5) there seems to be a potential gender specific response pattern to maternal conditions in offspring from diabetic dams

Male offspring tended to have a more magnified response to a glucose challenge than female offspring within the same group. Furthermore, the males were the only animals with dysmorphogenic malformations at birth. When assessing the data from both portions of this project, it

seems plausible that the female phenotype possesses a protective trait not evident in males. Care must be taken in interpreting these data due to small sample size when the data is divided into gender groups.

The results from the present work provide support for earlier findings as well as generating numerous potential research questions for additional study. Research investigating the mechanisms involved in the potential protective effects of exercise training are critical. Additionally, replication of the present work enabling a larger sample size to be obtained would add support to the current findings. This work provides new insights into the outcomes of utilizing chronic exercise as a therapy in uncontrolled diabetic animals. Although the current study had a small sample size, results suggest that individuals with Type I diabetes should be informed as to the potential negative affects of terminating exercise concomitant with the initiation of pregnancy. This study was the first to indicate that exercise termination at conception is linked with negative offspring outcome. Furthermore, these data support a potential protective role for exercise in the mother as it relates to increasing the benefits to the neonate.

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