Joint Effects of Exercise and Dietary Carbohydrate on Pregnancy Outcome and Early Neonatal Survival in Rats

Katja Leccisi-Esrey
School of Dietetics and Human Nutrition
McGill University, Montreal
July 1991

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Masters of Science

© Katja Leccisi-Esrey
1991
Acknowledgements

I would like to thank my supervisor, Kris Koski, whose encouragement was a key factor in my decision to pursue graduate studies in the first place. Throughout my MSc she provided inspiration and guidance. Thank you also to my committee, Hélène Perrault and Elliot Block whose help and keen interest in my research were greatly appreciated. Thanks to all three and to Dr. Touchburn for their constructive review of this thesis.

Thanks to Louise Coté and Michael Leccisi's help I was able to catch a few hours of sleep and take a day off when I really needed it. Thanks also to the other graduate students in Kris' lab who helped me out at crucial times. Thanks to my friends who listened to me talk endlessly about my work.

Special thanks to my husband, Steve, who not only provided his time, sound statistical advice, and very constructive criticism, but who gave me intellectual stimulation through our many discussions as well as all the emotional support I needed. Thanks for believing in me.

Finally, I would like to dedicate this thesis to my parents. Thanks for supporting me over the years.
ABSTRACT

Exercise and dietary carbohydrate restriction during pregnancy independently reduce maternal weight gain and offspring survival. It was hypothesized that the combined stress of exercise and dietary carbohydrate restriction would decrease offspring survival more than the independent effects. Within the exercise and sedentary groups pregnant rats were randomly assigned to be fed either 60%, 40%, or 20% dietary carbohydrate ad libitum. No statistical interactions were found between exercise and diet. Main effects were found for litter weight, maternal feed intake and weight gain, but not for litter size, pup birthweight, or pup survival in the first two days postpartum. Exercised rats gained less weight and ate more on a per gram body weight basis than sedentary rats. Rats fed carbohydrate restricted diets ate less and gained less weight than the rats fed 60% carbohydrate. These results demonstrate that the neonatal rat is not vulnerable to the effects of moderate maternal exercise and carbohydrate restriction during pregnancy.
Résumé

L'hypothèse proposée est qu'il existe un effet additif de ces deux facteurs tel qu'une diète pauvre en glucides associée à un entraînement physique régulier aura des conséquences plus marquées que chacun de ces facteurs par lui-même. A l'intérieur de groupes de rates gestantes entraînées ou sédentaires, les animaux ont été assignés de façon aléatoire à des diètes *ad libitum* composés de 20, 40, ou 60% de glucides. Aucune interaction significative n'a été observée entre la diète et l'exercice. Des effets significatifs indépendants ont cependant été observés pour l'ingestion maternelle de nourriture et la prise de poids mais non pour la taille de la portée, le poids de naissance ou la survie du rejeton à deux jours post-partum. Une réduction de la prise de poids et une augmentation de l'ingestion de nourriture par gramme de poids ont été observées chez le groupe entraîné par rapport au groupe témoin. Les rates soumises aux régimes pauvres en glucides ont démontrées une diminution de l'ingestion de nourriture et de la prise de poids par rapport au rates ingérant la diète de 60% glucides. Ces résultats suggèrent que l'association d'exercice modéré à une diète pauvre en glucides au cours de la grossesse n'entraîne pas de conséquences néfastes à court terme sur le nouveau-né.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>i</td>
</tr>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Résumé</td>
<td>iii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of Tables and Figures</td>
<td>vi</td>
</tr>
</tbody>
</table>

## Chapter I
Review of the Literature

### A. Overview of the Physiological Adaptations to Normal Pregnancy

1. Weight Gain and General Body Changes ........................................ 1
2. Thermoregulation ........................................................................ 4
3. Cardiovascular ........................................................................... 5
4. Respiration and Blood Gases .................................................... 7
5. Maternal Metabolism and Endocrinology ....... 7
6. Distribution of Blood Flow ...................................................... 13
7. Oxygen and Nutrient Delivery to the Fetus .................................. 14

### B. Overview of the Effects of Changes in Dietary Carbohydrate

1. In Exercise .............................................................................. 16
2. In Pregnancy ............................................................................ 17

### C. Interactions of Pregnancy and Exercise

1. Physical Working Capacity During Pregnancy ................................ 24
2. Weight Gain, Body Composition, and Food Intake .................................. 25
3. Thermoregulation ........................................................................ 29
4. Cardiovascular ........................................................................... 32
5. Respiration and Blood Gases .................................................... 33
6. Metabolism and Endocrinology, and Implications for Nutrient Delivery to the Fetus .................................................. 34
7. Blood Flow Distribution and Oxygen Delivery to the Fetus .................. 38
8. Acute Uterine and Fetal Responses ............................................. 41
9. Antepartum Course, Length of Gestation and Course of Labour ............. 43
10. In Utero Fetal Growth and Development ........................................ 47
11. Postnatal Offspring Effects ..................................................... 51
Chapter II

Joint Effects of Exercise and Dietary Carbohydrate on Pregnancy Outcome and Early Neonatal Survival in Rats.

A. Introduction......................... 64

B. Methods

1. Experimental Design.................... 65
2. Animal Care and Exercise Protocol..... 66
3. Experimental Diets...................... 69
4. Data Management and Statistical Analyses.................................. 71

C. Results

1. Feed Intake During Pregnancy............ 73
2. Weight Gain During Pregnancy........... 74
3. Fetal Growth and Survival in utero...... 75
4. Neonatal Mortality........................ 76

D. Discussion................................ 76

E. References for Chapter II................ 86

Appendix I. Summary of Statistical Procedures........ 103
List of Tables and Figures

Figure

1. Experimental Design.................................................... 90
2. Time Line for Experimental Protocol................................. 91

Table

1. Composition of Control and Carbohydrate-Restricted Diets........... 92
2. Cumulative Maternal Feed Intake in Pregnancy
   Among Sedentary and Exercised Rats Fed Varying Levels of Dietary Carbohydrate........... 93
3. Cumulative Maternal Weight Gain in Pregnancy
   Among Sedentary and Exercised Rats Fed Varying Levels of Dietary Carbohydrate.......... 94
4. Length of Gestation Among Sedentary and Exercised Rats Fed Varying Levels of Dietary Carbohydrate................................. 95
5. Number of Implantation Sites Among Sedentary
   and Exercised Rats Fed Varying Levels of Dietary Carbohydrate.................................. 96
6. Number of Fetal Resorptions Among Sedentary
   and Exercised Rats Fed Varying Levels of Dietary Carbohydrate................................. 97
7. Number of Pups Born to Sedentary and Exercised
   Rats Fed Varying Levels of Dietary Carbohydrate................................. 98
8. Average Birthweight of Pups Born to Sedentary
   and Exercised Rats Fed Varying Levels of Dietary Carbohydrate................................. 99
9. Total Litter Weight of Pups Born to Sedentary
   and Exercised Rats Fed Varying Levels of Dietary Carbohydrate..................................100
10. Neonatal Pup Mortality in Exercised and Sedentary Rats..................101
11. Neonatal Pup Mortality in Rats Fed Varying Levels of Dietary Carbohydrate.................................102
A. Overview of the Physiological Adaptations to Normal Pregnancy.

Many physiological adjustments occur during pregnancy, presumably in order to optimize the environment for the fetus and survival of the female. The process is complex, and involves every organ system of the pregnant female. Because of these physiological changes, the responses to exercise may be altered. This section is intended to provide sufficient background for the upcoming discussions of the effects of exercise and changes in dietary carbohydrate during pregnancy. Very little of the literature identified for this section pertained to animals, thus human data had to be relied upon for much of the information. Unless otherwise indicated, the discussion refers to women, but whenever possible, species comparisons have been included.

1. Weight Gain and General Body Changes

The magnitude and pattern of weight gain during pregnancy is quite variable between species, and individuals within species. For example, in the rat, total body weight increases by about 30% (2), while the pregnant adult woman gains about 10-20% of her body weight (48,49). Some of the
variation in maternal weight gain within a species is caused by voluntary restriction of food intake in women, influence of age, parity, pregravid body weight, and presence or absence of excessive water retention (79).

Average weight gain in the woman is 12kg. Of this 12kg, the conceptus comprises 5kg (fetus 3.5kg, placenta 0.7kg, amniotic fluid 0.8kg), while gain in uterus, breasts, extracellular fluid, and fat and protein stores in the body constitute about 7kg (48,79). The overall average composition of the weight gain in women has been estimated to be more than 6kg water (50%), approximately 3.6kg fat (30%), and less than 2.4kg protein (20%) (79).

In the rat, fat is increased about 50%, while protein increases about 20% and is evenly distributed among the liver, heart, kidneys, and gastrointestinal tract (2).

Maternal weight gain during the first part of pregnancy may be minimal. At the end of the 1st trimester, weight begins to accumulate, and rate of gain is essentially linear throughout the second and third trimesters. Accumulation of maternal tissues such as blood, uterine, and mammary glands, and tissue stores of fat and protein, occurs primarily during the second trimester, as does growth of the placenta, while growth of the fetus and amniotic fluid occur most rapidly during the third trimester (48,79).

The most dramatic changes are those that occur in the reproductive organs. The mammary glands and teats enlarge
due to a large increase in the amount of ductal tissue and deposition of fat (109). The growth of the uterus occurs by growth of individual smooth muscle cells, and there is an enormous increase in the blood vessels that support and nourish this growth of muscle tissue (109).

As the uterus grows in the woman, it becomes an abdominal rather than a pelvic organ, displacing the intestines, pressing on the urinary bladder, and coming into direct contact with the abdominal wall (2).

From early in pregnancy in the woman, there is an increasing relaxation of ligaments due to the release of estrogens and relaxin (2). The softening and stretching of ligaments may serve a useful purpose in the pelvic joints at parturition. However, during pregnancy this results in a widening of the articulations between joints and in increased joint mobility (2,38,109). Loosening of the sacroiliac joints, along with increased lordosis and upper spine extension to compensate for the enlarged abdomen may result in lower back pain in the pregnant woman (2,109).

Food intake may be altered by pregnancy. Rats and other laboratory animals increase their food intake during pregnancy (89). Although it is recommended that pregnant women increase their energy intake to meet their increased energy requirements, recent studies have suggested that, in practice, there is little or no change in energy intake during pregnancy for women in developed countries (30).
2. **Thermoregulation**

The majority of information concerning maternal and fetal thermoregulation has been derived, due to the invasive nature of the tests, from animal studies. The direct application of some findings to humans may not be possible (68). Rodents and sheep rely primarily on panting as the mechanism for heat dissipation, whereas humans rely on sweating and heat radiation through the skin (68). Most of the animals have considerable fur or hair which modifies heat gain or loss. Size is also important because the larger animal has a greater capacity for heat storage.

Compared to the non-pregnant subject, maternal core and skin temperatures in both animals and humans, are higher during pregnancy (68). Under the influence of progesterone, the elevated core temperature peaks at about mid-pregnancy, and then normalizes later in gestation. In both animals and humans, fetal temperature reflects that of maternal temperature except that it remains 0.4°C to 0.6°C higher (40, 65, 68, 69, 113). Thus, a favourable maternal-fetal temperature gradient normally exists for the fetus. Maternal temperature is the primary determinant of fetal temperature, while other influencing factors are fetal metabolic rate and uterine blood flow (68).
3. **Cardiovascular**

The size of the heart increases during pregnancy, due mostly to increased diastolic filling associated with an increase in blood volume (2,48,73).

The cardiovascular clinical state of the woman during pregnancy is one of a throbbing, rapid pulse, increased cardiac output, and decreased peripheral resistance (2). Cardiac output (CO) rises within the first trimester of pregnancy and remains high for the remainder of pregnancy, increasing by a total of 30-50% (2,38,40,48,73,94,109,113). During the third trimester, cardiac output measured in the woman in the supine position may be below non-pregnant levels because the enlarging uterus obstructs the inferior vena cava, impeding venous return, resulting in reduced cardiac output (2,48,73,94,113). In humans, as opposed to rats and other mammals, there is a tendency to an increase in venous pooling as term approaches (65). This is attributed to venous relaxation and the growth of the large venous plexuses in the broad ligaments of the uterus (73).

The increase in CO is the result of increased maternal heart rate and stroke volume (2,38,48,73,94,109,113). Beginning early in the pregnancy, heart rate increases progressively and reaches a plateau at about 37 weeks in the woman. Stroke volume rises proportionately less than heart rate. During the third trimester, stroke volume may decline because of diminished venous return resulting from enhanced
venous compliance and intra-abdominal pressure, as well as blood flow redistribution to the growing fetus.

Systolic arterial blood pressure is relatively stable throughout pregnancy but diastolic arterial pressure is lower than non-pregnant levels from early pregnancy, rising to non-pregnant levels in the last 2 to 3 months of pregnancy (2,40,48,109,113). The peripheral resistance to flow is decreased because CO is increased and arterial blood pressure is not (2,48). Femoral venous pressure increases, and there is slowing of the venous flow in the lower limbs, the latter resulting from an increase of pressure due to obstruction by the uterus when lying down.

Plasma volume, in both women and animals, increases further than red cell volume, to about 40 to 50% above non-pregnant volume (38,40,51,73,79,94,109,113). The greatest increase in plasma volume occurs in the second trimester, while red cell volume reaches its maximum in the 3rd trimester. This results in lower blood haemoglobin and haematocrit concentration, commonly referred to as the haemodilutional anaemia of pregnancy. These changes result in a decline in the oxygen-carrying capacity of a volume of blood. Blood osmolality falls abruptly in the first 2 months of pregnancy, and remains low. Total protein in the serum falls within the first trimester and reaches a plateau at mid-pregnancy. The fall in plasma albumin is associated with a decrease in plasma colloid osmotic pressure (48).
4. **Respiration and Blood Gases**

The changes in the respiratory system during pregnancy are extensive. They begin early under hormonal influence, mainly progesterone (2,113).

As pregnancy progresses in the woman, the uterus rises in the abdominal cavity elevating the diaphragm, and the entire thoracic cavity compensates by increasing its dimensions so that more air can be inspired (109). The anatomic changes increase the energetic cost of breathing because of the greater diaphragmatic work required to displace the enlarging uterus downward with each inspiration (94). The anatomic changes also result in a reduction in functional residual capacity which is effectively offset by an increase in inspiratory capacity. Therefore there is little or no change in vital capacity and only a modest decrease in total lung capacity (2,38,40,94,109). Tidal volume increases throughout pregnancy, while respiration rate does not change, resulting in an increase in minute ventilation (the volume of gas ventilated per minute) (TV * RR) of more than 40% (2,40,94,109,113).

5. **Maternal Metabolism and Endocrinology**

Resting oxygen consumption exhibits a biphasic pattern, remaining at pre-pregnancy levels for several weeks, and increasing steadily after 18 weeks, reaching a maximum near term of about 16 to 32% above non-pregnant levels in the
woman (40,94). In the pygmy goat, the near-term value is 21% above pre-pregnancy levels (2). About 50% of the total increase in resting oxygen consumption is required for fetal metabolism, while the remainder is accounted for by the added uterine and mammary tissues and the increased work load of the circulatory and respiratory systems (2,40,73,109).

The main source of pregnancy-sustaining hormones during first 6 to 8 weeks is the corpus luteum (2,78). From conception, it produces human chorionic gonadotropin (hCG), which prevents the corpus luteum from involution. After 6 to 8 weeks, the placenta becomes the main source for hormone production, but the corpus luteum continues to produce steroids (progesterone, estrogens, androgens) and the polypeptide relaxin.

Early pregnancy is marked by high levels of chorionic gonadotropin (hCG), along with a sharp rise in progesterone output. Later in pregnancy, there are much lower levels of hCG, and increasing levels of estrogens and progesterone (78). Human chorionic gonadotropin (hCG) is produced by the trophoblast and plays a role in the maintenance of the corpus luteum (levels rise and peak at 8 to 12 weeks, then decline). Thereafter, the role of hCG is poorly understood (2). Human placental lactogen (hPL) is also produced by the placental trophoblast. It is detectable at 6 weeks gestation, and its concentrations in maternal blood are in
direct proportion to the growth of the placenta. During the second half of pregnancy, in conjunction with hCG, it stimulates the placental production of progesterone (78). Its other functions include promoting breast growth and development in preparation for lactation (2,78), it is involved in lipolysis in adipose tissue, nitrogen retention, and antagonizing the peripheral action of insulin, thus making glucose from maternal tissues available to the growing fetus (2,40). Relaxin, another placental hormone, appears to be involved in the prevention of premature labour, and is involved in the softening of the cervix and relaxation of pelvic joints (2).

Of the steroids, progesterone is the most essential for the maintenance of pregnancy (2). About 90% of its production originates from the placenta. Its serum concentration steadily increases with advancing gestation (109). The main purpose of progesterone is to reduce muscle tone in the uterus, thus protecting the fetus from the natural tendency of the uterus to expel it. Progesterone also induces changes in the vascularity and secretion of the cervix, induces alveolar growth of the breasts, is involved in overall systemic smooth muscle relaxation and is the principle cause for venous dilatation and atony of the bowel (2,48). Progesterone also acts on hypothalamic control centres involved in breathing. The threshold for $\text{paCO}_2$ (partial pressure) is lowered, causing overbreathing and
decreased arterial $pCO_2$ (tension)\(^{(2,95,109,113)}\). There is a change in the control of appetite favouring increasing ingestion of food, a contributing factor to weight gain \(^{(2)}\).

The major site of production of the estrogens is also the placenta. Its precursors originate from the fetoplacental unit. Estriol, an estrogen produced in large quantities by the fetoplacental unit and secreted via the maternal circulation is used as an indicator of fetal well-being \(^{(2)}\). The concentration of estrogens constantly increases during pregnancy. The major function of the estrogens is to promote growth, increased vascularization, and function of the uterus and breasts \(^{(2)}\). The estrogens increase the elastic properties and the contractility of the uterus and influence alveolar growth of the breasts. Estrogens alter the polymerization of mucopolysaccharides and thus cause changes in the ground substance of the connective tissue. This is the cause of the increased laxity of the joints and the water retention in the interstitial space.

The thyroid gland increases in size but its function is the same as in the non-pregnant state, probably due to presence of hCG \(^{(40)}\). The concentrations of plasma thyroxine and triiodothyronine (T3 and T4) increase. However, because there is also an elevation of thyroxine binding globulin, the amount of free T3 and T4 remains constant \(^{(2,40,109)}\). Moderate and progressive increase in
ACTH secretion by the adrenal cortex is also observed, with a rise in total and free cortisol concentrations in plasma (40,109). Plasma growth hormone is low, and its secretion in response to stimuli (eg. hypoglycaemia) is blunted (40,109). High levels of estrogens cause a decline in production of follicular stimulating hormone (FSH) and luteinizing hormone (LH), causing an anovulatory state (2). The suppression of FSH and LH is potentiated by the inhibitory influence of high levels of the placental analog to these hormones, hCG. In spite of the stimulatory effect of estrogens on hGH levels in the non-pregnant state, in pregnancy the hGH levels are lower than in the non-pregnant state, due to the prevailing inhibitory effect of placental lactogen (2). Prolactin levels, in spite of the inhibitory effect of its placental analog, hPL, increase in pregnancy to levels 10 to 20 times higher than in the non-pregnant state (2,109). Its functions include preparation of the mammarys for lactation, and possibly osmoregulation in the fetus (2). Parathyroid production of PTH is increased to maintain calcium homeostasis (2). Basal epinephrine and norepinephrine concentration in plasma are not changed during pregnancy (2,40), but increase during labour and parturition. The secretion of aldosterone increases during pregnancy, probably through the increased activity of the renin-angiotensin system. The primary stimulus is thought to be progesterone (2).
Plasma insulin and glucagon both increase, but on a molar basis, circulating insulin increases more than glucagon, resulting in an increased insulin-to-glucagon ratio (40). Because of the marked increase in peripheral insulin resistance during pregnancy, the hyperinsulinemia results in less of a fall in plasma glucose levels than would otherwise be present (40,65). However, in both humans and rodents, resting blood glucose levels gradually fall throughout pregnancy (2,102,105). In sheep, fasting blood glucose levels do not decrease as pregnancy progresses.

In the human, even short-term fasting produces a cascade of metabolic events termed "accelerated starvation". It is characterized by exaggerated hypoglycaemia, hypoinsulinemia, hyperketonemia, and hypoalpininemia. Utilization of glucose and amino acids by the fetus is considered to be responsible for this phenomenon (40).

Circulating concentration of plasma free fatty acid is increased in the third trimester of pregnancy, as are plasma triglycerides (40,109). Plasma ketones are markedly increased in late pregnancy (40).

The gastro-intestinal tract also undergoes changes during pregnancy. The main change in its function is its decline in activity (2,109). Gastric secretion is decreased, and decreased gastric tone and motility are implicated as the primary cause of nausea (109). Relaxation of the cardiac sphincter of the stomach results in a
tendency to regurgitate gastric acid into the lower oesophagus, which may cause heartburn, especially in the third trimester (2,109). There is a decrease in tone and motility of the intestines, resulting in prolongation of gastric emptying and delayed intestinal passage. Constipation may occur due to this and be intensified by increased absorption of water in the colon (2,109).

Digestion of fatty foods may be more difficult (2,109). This may be related to decreased motility of the gall bladder. The urinary losses of most sugars and amino acids are increased. Renal retention of sodium and water are enhanced, aiding in the expansion of total body water and permitting cell growth (109). To some extent, the urinary losses of nutrients may be a consequence of the 30-50% increase in glomerular filtration rates (2,48,109). The increased GFR, however, facilitates the filtration of waste products.

6. Distribution of Blood Flow

The distribution of the increased blood flow is not uniform, and is not thoroughly defined. In both humans and animals, the fetoplacental unit receives much of the increased circulation. In all species, uteroplacental blood flow increases progressively as pregnancy progresses as a result of an increased maternal and fetal CO and a decreased vascular resistance (35,94). At term in the woman, blood
flow to the uterus represents 20 to 25% of her cardiac output (78). Mammary blood flow probably increases (48, 73, 94), as evidenced by increased vascularity. Renal blood flow rises from early on in pregnancy, with the kidneys being the major recipients of the increased cardiac output after the fetoplacental unit (2, 40, 48, 94). Skin blood flow is probably also increased (48, 94), whereas there is no change in hepatic and cerebral blood flow (48, 94). Coronary blood flow increases, and it is uncertain whether there is a change in blood flow to the human gut, however this does appear to increase in other animals (48). The increase of blood flow to the skin and kidneys serves the purpose of dissipation of heat and elimination of waste material.

7. Oxygen and Nutrient Delivery to the Fetus.

The uterine circulation is an essential link in the chain of transport that supplies nutrients and oxygen to the placenta and removes waste products, including heat. Blood flow to the placental villi ultimately determines the amount of oxygen and nutrients delivered to the fetus (2, 48, 78). Fetal blood has a greater affinity for oxygen than the maternal blood. The fetal haemoglobin dissociation curve is shifted to the left compared to that of maternal blood under standard conditions (2, 49). The fetal haemoglobin dissociation curve allows a relatively high oxygen saturation and thus a considerably higher circulating load.
of oxygen to be carried at any given \( \text{paO}_2 \), which becomes more apparent at lower oxygen partial pressures. The avidity of fetal haemoglobin ensures that oxygen reaching the fetal capillaries is captured, but the total oxygen supply to the fetus depends not only on placental diffusing capacity and the amount of oxygen extracted for its own use but also on blood flow between the fetus and the placenta.

Delivery of nutrients for growth of the fetus is also via the placenta. Glucose is the principle energy substrate for the fetus and, as the precursor for fetal lipid and glycogen synthesis, is also a major source of the energy reserves for the newborn (6,35). In all species fetal blood glucose levels are maintained at a lower level than in maternal blood, and when maternal levels change, the fetal changes lag behind by several minutes. Glucose is transferred to the fetus via facilitated diffusion with a specific carrier (48). The fetus has a \( K_m \) six times greater than maternal blood for glucose (78). A large amount of the incoming glucose is used by the placenta, with only one third being transferred to the fetus (48,78,112).

The sheep fetus meets about 46% of its requirements for oxidative metabolism from glucose (78). For the human and rat fetuses, glucose contributes more significantly to fetal metabolism, meeting 50 to 70% of requirements for oxidative metabolism (6).
With the exception of some immunoglobulins no maternal proteins cross the placenta, thus fetal proteins are synthesized from amino acids transferred from maternal blood. This has been documented in the rat, guinea pig, sheep, cow, and human (78). The transfer is an active process with a strong preference for L-amino acids. Fetal plasma levels are higher than those of maternal plasma for every amino acid, while for some others, placental levels are highest (49).

Transfer of lipid is limited to that of maternal free fatty acids. Fatty acids cross the placenta non-selectively, with their transfer depending upon the content of albumin in the plasma. The fetus derives practically all of its fat from maternal fatty acids and the remainder by lipid synthesis from carbohydrate and acetate (49).

Placental transfer of fat in the rat is poorer as compared to the human, guinea pig and rabbit, but is still substantial, whereas fatty acids do not cross the sheep placenta (6, 78).

B. Overview of the Effects of Changes in Dietary Carbohydrate

The effects of changes in dietary carbohydrate (CHO) will be reviewed for two conditions: pregnancy and exercise. They are reviewed separately as no identified study combined
exercise in pregnancy with alterations in maternal dietary carbohydrate composition.

1. **In Exercise**

Studies investigating the interactions of exercise and dietary CHO have focused on either performance or basic physiologic questions about substrate utilization during exercise. In this section, the most common dietary manipulations affecting CHO availability are reviewed.

Diet has an important effect on substrate availability and utilization during exercise, and consequently on exercise performance. Dietary manipulations can be used to maximize hepatic and muscle glycogen stores. For example, while average normal muscle glycogen stores range from 10 to 20 g/kg muscle tissue, dietary manipulation such as a CHO overloading diet can result in muscle glycogen content of 30 to 45 g/kg (83).

The original CHO overloading diet, known as the Scandinavian diet, was popularized in the later 1960's. It consisted of an initial intensive, prolonged, exhaustive exercise session, followed by low CHO intake (<5% of total energy) for three days during which an attempt could be made to continue exercise sessions. This period was regarded as the glycogen depletion phase. The next phase consisted of limited exercise and ingestion of a high CHO diet (>90% of total energy) for three days to maximize glycogen stores.
This diet proved to be effective because muscle glycogen content was increased by as much as two to three fold. Carbohydrate overloading is based on the fact that glycogen synthase activity of skeletal muscle is inversely related to the level of the muscle glycogen stores (83). Thus to maximize muscle glycogen stores, these must be depleted and maintained at a low level in order to enhance muscle glycogenic activity. Secondly, providing large amounts of CHO maximizes the saturation of the glycogenic potential at the level of the skeletal muscle. More recent observations however, suggest that as long as glycogen depletion is completed through strenuous exercise and one day of low CHO intake, the subsequent CHO restriction phase may not be necessary. In fact, results have confirmed an increase in muscle glycogen stores without the high fat, high protein, low CHO diet (97). Considering the unpalatability and difficulty in following the low CHO diet, the more recent approach to CHO overloading is more popular. Recent investigations indicate that it is probably just as effective for improving endurance (24,47). A confounding factor however is that most of these studies have used trained subjects. Because training per se induces an increase in glycogen synthase activity, it is difficult to separate the effects of diet and endurance training.

Dietary manipulations have also been done to examine the effect on substrate utilization during exercise. In
man, consumption of a high fat diet for at least one week was seen to result in a greater fat oxidation as evidenced by lower exercise respiratory exchange ratio (85). Similarly, results from animal studies indicate greater muscle citrate synthase and 3-hydroxyacyl-CoA-dehydrogenase activity in rats exercising at high intensity (35m/min) after consuming a high fat diet (78% fat, 1% CHO) for 5 weeks (71). Furthermore, an increase in intramuscular triglyceride has also been reported (22). Despite lower initial muscle and liver glycogen stores with the high fat diets, endurance time to exhaustion in these rats was not decreased (22,71). In humans, similar findings were reported (85). This may be attributed to the glycogen sparing effect associated with these high fat diets. In fact, muscle glycogen utilization during exercise was reduced in humans and rats (71,85). Concomitantly, glucose oxidation during exercise was also decreased (85).

Similar metabolic adaptations can be seen with endurance training. Training results in a greater fat oxidation due to enhanced muscle oxidative enzyme content and activity (36,37,44,47,95). The result is a muscle glycogen sparing effect that may be associated with improved endurance (23,36,37). In addition, endurance exercise training increases glycogen synthetase activity which results in a greater potential for skeletal muscle glycogen storage (37).
2. **In Pregnancy**

Nutrition during pregnancy covers an extensive range of issues. This section will focus on how changes in amount of carbohydrate (CHO) in the diet can affect substrate availability and utilization by the mother and fetus, the course of pregnancy, and pregnancy outcome. Few studies have been conducted on this subject. The majority of the identified studies have been conducted in rats (33, 60, 61, 55, 56, 57, 58, 100), along with one in dogs (92), and one in humans (53).

Carbohydrate (CHO) is the principle metabolic fuel for the growing embryo and fetus (6, 35, 78). It is essential during pregnancy. The absence of dietary CHO in the maternal diet of the rat during pregnancy results in complete resorption of implanted embryos by mid-gestation (58, 100). With over 4% CHO added to the diet, fetal resorption rate was not increased and number of pups born was normal (33, 57, 58, 61). Romsos (92) fed CHO-free, lipid-based diets to dogs during the last 2 trimesters of gestation, and found that the number of pups born was unaffected, but that there was an increased number of stillbirths.

Severe restrictions of CHO result in significantly decreased maternal feed intake (33, 58), weight gain (33, 57, 58), and liver glycogen stores (33, 57), delayed parturition (55, 56, 58), reduced fetal liver glycogen stores
(33,57), reduced birthweight in rats (33,57,58), and increased neonatal mortality in rats (58) and dogs (92). Reduced birthweight has also been reported in humans following maternal consumption of low CHO diets during pregnancy (53).

The effects of CHO restriction are dose-dependent, with the greatest perturbations occurring with the more severe CHO restrictions. Thus, as the amount of CHO in the diet increases, all these parameters approach normal. With restriction of CHO to 12% of total energy, feed intake did not decrease significantly (33,57,58). However, another group reported decreased maternal feed intake with restriction of CHO to 19% of total energy (61). The same group reported that maternal weight gain was not reduced (61). At 19% CHO (by energy) and 24% CHO (by weight), birthweight was unaffected in rats (60,61). At 12% CHO, neonatal mortality in rats was 2%, not different than for the 62% glucose-fed controls (57). In dogs, early neonatal mortality was increased in pups whose dams had been fed CHO-free diets during pregnancy, such that at 3 days of age, only 35% of the pups whose dams had been fed this diet were alive (92).

When perturbations in fetal CHO homeostasis occur due to restriction of maternal dietary CHO, optimal accumulation of fetal liver glycogen does not occur (33,58). This results in high neonatal mortality, most of it occurring
within the first 2 days after birth (55,58). However, the timing of the CHO restriction during pregnancy is critical for neonatal survival. Using a cross-over design, Koski & Hill (55) showed that the quantity of CHO in the maternal diet in the last 2 days of gestation had a profound effect on postnatal survival. The magnitude of the neonatal mortality 1 day after birth was similar between dams fed the CHO-restricted diet throughout pregnancy or for only the last 2 days of pregnancy, implying that there was clearly no effect of a CHO-rich diet from day 0 to 19 of pregnancy and showing that feeding the low CHO diets beginning on gestation day 20, when fetal liver glycogen accumulation was maximal, contributed to high postnatal mortality.

C. Interactions of Pregnancy and Exercise

The studies of pregnancy and exercise interactions have generally set out to answer one or both of the following two questions: In what way does pregnancy itself alter the ability to exercise? and In what way does exercise influence physiology and metabolism during pregnancy, the course, and ultimately, the outcome of pregnancy? These questions have been investigated in the pregnant woman, sheep, pygmy goat, guinea pig, mouse, and rat using various experimental designs depending on the parameters being examined. In examining how pregnancy affects exercise, the pregnant exercising subjects are either compared to exercising non-
pregnant controls, or to themselves pre or postpartum. For physiological responses to exercise in pregnancy, the design either involves sedentary and exercising pregnant groups, exercising pregnant and non-pregnant groups, or a combination of these, depending on what comparisons are to be made. Studies which examined uterine, placental, and fetal responses to the exercise compare exercised and sedentary subjects. Measurements of the outcome variables are done prior to the exercise bout, during if possible, and for a period of time afterwards. Finally, for studies of repeated exercise on pregnancy outcome, most studies used exercising and sedentary pregnant subjects. In human research examining the effects of repeated exercise or physical training programs on pregnancy outcome, cross-sectional, retrospective, as well as prospective studies have been used. Very few have used rigorous design or statistical analysis to control for possible confounders. Because of variations across studies of species, age, parity, stage of gestation, physical fitness and inclusion or exclusion of training in the protocol, intensity, duration, frequency, and type of exercise, it is often difficult to compare studies or to generalize to other populations.
1. **Physical Working Capacity During Pregnancy**

Physical working capacity as well as indirect estimates of maximal aerobic power have been obtained in pregnant animals, but this has been done very infrequently in pregnant women out of concern for their safety. Instead, most have attempted to predict VO\textsubscript{2}max from heart rate responses to submaximal exercise. Data from these investigations generally indicate that maximal physical working capacity of women remains unchanged or slightly decreased during pregnancy (2,40,94,109). Recent studies have shown that when VO\textsubscript{2}max was determined in women using cycling exercise, there was no difference between measurements at 25, 35 weeks gestation and 2 and 7 months postpartum (93,108). However, when swimming was used to do the measurements, VO\textsubscript{2}max was significantly greater between 25 and 35 weeks gestation, and was smaller than than determined on the cycle (108). There is, however, general agreement that a pregnant woman will reach her maximal cardiac output at a lower level of work than will a non-pregnant woman, that is, during exercise, cardiac output at the same workload increases above values in the non-pregnant woman (38,41,77,94,109,113). Weight-dependent exercise requires increasing oxygen uptake as pregnancy progresses, but this increased energy cost is closely related to the increased body weight. When expressed on a per kilogram body weight basis, oxygen consumption during walking or
running increases moderately or not at all (2, 18, 40, 65, 73, 77, 94, 106, 109, 113).

It is generally agreed that a pregnant subject (animal or human) can increase her physical fitness, and thus physical working capacity, with regular, individually prescribed aerobic exercise during pregnancy as can a non-pregnant subject (2, 40, 94, 99, 113).

2. Weight Gain, Body Composition, and Food Intake

Because a single bout of exercise does not affect overall maternal weight gain, feed intake and body changes during pregnancy, the studies which have measured these parameters have done so over the course of part or all of the pregnancy. Most studies have been conducted in humans and rodents. In rats and mice, parameters such as body weight and feed intake are usually measured every day or few days, whereas body composition is done when the animals are sacrificed. In contrast, in women, weight may not be measured at a regular frequency over the course of pregnancy, but is simply calculated from prepartum weight and the last weight before parturition. Food intake is also measured infrequently, as will be discussed below.

Most studies have shown that regular maternal exercise during most of the pregnancy results in reduced maternal weight gain in humans (15, 16, 19, 63), rats (75, 76, 103, 105, 111), and mice (101).
There have been some reports of no reduction in maternal weight gain. In mice, Boehnke et al. (9) reported no decrease in maternal weight gain with exercise during pregnancy. A similar study in mice by Terada (101) reported a decrease in weight gain, and it is unclear why these results differed because similar protocols were used. Two studies in which rats swam prior to and during pregnancy reported no significant reduction in maternal weight gain (96, 104). However, one used the Duncan multiple range test procedure, which has been criticized as not being a valid statistic (110), and did not provide a sample size in order to duplicate the tests (96). The other found that exercised rats gained 35g less weight than sedentary controls (104), which although not statistically significant due to inadequate power in the study, is considered an important reduction. One study of running rats reported that exercised animals consistently weighed more than sedentary controls (25). Examination of their data indicated that the exercised rats gained as much weight as did the sedentary rats. It is unclear why these results differ from the bulk of the literature. In humans, two studies reported no decrease in maternal weight gain with exercise during pregnancy (32, 62). However, in the former (32), it is possible that exercise was not frequent or intense enough to affect weight gain because it was not quantified. In the latter (62), women only exercised from weeks 22-30 of.
gestation, compared to longer periods in the studies reporting decreased maternal weight gain.

More interesting perhaps than body weight changes however, is how, if at all, is body composition or the components of weight gain altered with exercise during pregnancy. Only three identified studies examined this issue in animals. In rats, Mottola et al (75) examined the components of weight gain at the end of gestation following a strenuous exercise program. The pregnant exercised group had a smaller skin component, which included subcutaneous fat and mammary tissue, as compared to sedentary pregnant rats. This suggested that either the mammary gland was underdeveloped and/or the deposition of fat was reduced in the exercised groups. Courant & Barr (25) measured body composition at mating and parturition in rats trained prior to and during pregnancy. The exercised groups consistently had less body fat than sedentary controls, and it appears that exercise during pregnancy suppressed fat accumulation, although the change in fat mass was not statistically analyzed. Savard et al (96) examined regional fat deposits. In pregnant rats which swam prior to and during pregnancy, there was an increase in the adipose tissue weight of the subcutaneous inguinal fat pad, but the abdominal parametrial and retroperitoneal fat pads did not increase. The increases in the abdominal fat pads in the exercised dams were less than they were in sedentary pregnant rats. The
authors suggested that subcutaneous fat is preserved for lactational needs. However, as mentioned previously, the statistic used was invalid, thus the interpretation of the results is questionable. No identified studies measured components of weight gain or body composition in exercising pregnant women. Further information is needed on this issue.

Food or feed intake has been measured very infrequently in studies of exercise during pregnancy in both humans and animals. Energy intake could modify the relationship between exercise and weight gain in pregnancy, and ultimately could affect birth outcome, therefore it should be controlled for. In laboratory animals, the time available for feeding may affect total feed intake. Thus, because exercising animals do not have access to food for the duration of their exercise sessions, the sedentary animals should also have their food removed so that additional time for feeding can be ruled out as having an effect on food intake or weight gain. Of the studies that measured feed intake in rats, only Courant & Barr (25) did this. They reported increased feed intake in the rats exercised strenuously prior to and throughout pregnancy in the first and second weeks of pregnancy, but not the third. Despite decreased time for feeding in the exercised groups, mean daily feed intake has been reported to increase (104) or remain unchanged (96) with swimming in rats, remain
unchanged with running in mice trained prior to pregnancy (101), or decreased if mice were not trained prior to pregnancy (101). Because time available for feeding was not equivalent in the exercised and control groups, feed spillage may not have been controlled for, different types of exercised were used, and the amount of training prior to pregnancy differed greatly between studies, it is difficult to compare results and explain the inconsistent findings.

In humans, only three studies quantified food intake during pregnancy using food records, questionnaires, and recall (16,29,62), and all reported no difference between exercising and sedentary groups. However, methodological limitations make the validity of these findings questionable. The records were only obtained once at 22 weeks and at 30 weeks by Lewis et al (62), and at the end of each trimester by Dibblee & Graham (29). Clapp & Capeless (16) used only questionnaires (no food records) to obtain what they called a "crude estimate" of intake every 6-8 weeks throughout pregnancy. Assessment of food intake would need to be measured at regular intervals prior to and throughout pregnancy in order to properly assess the changes in food intake with pregnancy due to exercise in humans.

3. **Thermoregulation**

Because elevated maternal temperatures, especially in the first trimester, have been associated with teratogenic
effects (68), there is some concern about changes in body temperature during exercise in pregnancy. In humans, increases in core maternal temperature in response to exercise follows the same trend for pregnant and non-pregnant subjects (68). Significant increases in core temperatures can arise with long distance running, which could have potentially detrimental consequences for fetal well-being. It is not clearly defined how the changes in body temperature affect the fetus, especially when they are minor or are later in pregnancy. Possible consequences include rightward shifts of the maternal and fetal haemoglobin dissociation curves, compromised placental and fetal respiratory gas transport, decreased uterine blood flow due to increased blood flow to the skin for heat dissipation, increased metabolism, and as mentioned previously, teratogenic effects if core temperatures are above 40°C in early pregnancy (46,65,68).

Body temperature increases with intensity and duration of exercise (65,69,113). In sheep, Lotgering et al (66) reported that fetal temperature lagged behind the rapidly increasing maternal temperature at the onset and cessation of exercise. However, during the exercise, the maternal-fetal temperature was reversed. The changes in temperature were more pronounced with more intense and increased duration of exercise. Return of fetal temperature to control values was slow, requiring more than 1 hour after 40
minutes of exhaustive maternal exercise at 70% VO₂max. However, there was no evidence of fetal distress, and a shift in the A-V oxygen difference assisted in maintaining oxygen delivery to the fetus. Thus, this suggests that temperature changes due to exercise are well tolerated by the fetal sheep.

There are only a few studies which have examined the thermoregulatory responses of pregnant women during exercise. Clapp et al (17) reported that rectal temperature rose less during moderate exercise in recreational joggers as pregnancy progressed (+1.5°C pre-pregnancy, +0.6°C at 20 weeks, and +0.4°C at 32 weeks), and decreased rapidly afterwards. The authors suggested that this might be related to the augmented blood volume and increased skin flow during pregnancy. However, the subjects were exercising at a lower percentage of their VO₂max as pregnancy progressed (from 74% pre-pregnancy, to 57% at 20 weeks, to 47% at 32 weeks), and this decrease in intensity may have elicited smaller rises in temperature. It is important to once again note that the method for heat dissipation differs between most animals and humans, the former using primarily panting, and humans dissipating heat by sweating (65). This could possibly explain differences in temperature in response to exercise. The possibility still exists that prolonged exercise during pregnancy, especially in hot or humid environments, could result in
dangerously elevated core temperature, although this would be the exceptional case. The effect of training during pregnancy on body temperature control has not been investigated.

4. **Cardiovascular**

The cardiac output (CO) response to exercise in pregnancy has been studied in humans, sheep, and pygmy goats. Available data are not conclusive (40). It is probable that the exercised-induced increase in CO is essentially normal and adequate for the work demands (40). However, since the CO values throughout gestation are higher than in the non-pregnant subject, the absolute CO during exercise may be slightly greater than normal (26,73,93). In late human pregnancy, the exercise-induced increase in CO is diminished, probably due to partial occlusion of the inferior vena cava by the enlarging uterus (64).

The heart rate (HR) response to exercise in pregnancy has been studied extensively in humans, sheep, and pygmy goats. Both during and after exercise in pregnancy, the HR is slightly higher than in the non-pregnant state (26,73,81), but if one takes into account the increase in resting heart rate that is present in pregnancy, the change in HR due to exercise is the same (64).
The changes in maternal arterial blood pressure during exercise are similar to those in the non-pregnant state (40).

5. **Respiration and Blood Gases**

When examining the changes in respiratory blood gases, body temperature must be taken into account. Blood obtained anaerobically and analyzed for respiratory gases at a temperature below that of the body provide falsely low pCO₂ and pO₂ and falsely high pH (64,65).

During exercise, the efficiency of gas exchange increases to the same extent in both pregnant and non-pregnant subjects as a result of increased pulmonary diffusing capacity and increased alveolar ventilation (64). Hyperventilation during pregnancy results in decreased pCO₂ and buffering capability during and after exercise (64). Maternal arterial blood CO₂ tension decreases as a result of exercise-induced hyperventilation and hemoconcentration (40). Maternal blood O₂ tension and content increases, but not with mild exercise (64). Consequently, the O₂-carrying capacity of the blood is increased, and this enhances the O₂ transport across the placenta (discussed in further detail below) (40). The changes in blood gases are more pronounced in animals, in which hyperventilation has a more important role in heat dissipation than it does in humans. In other
respects, respiration during exercise in pregnancy does not seem to differ from that in the non-pregnant state (64).

6. Metabolism and Endocrinology, and Implications for Nutrient Delivery to the Fetus

Fuel utilization during exercise in pregnancy is a subject of much controversy. Current knowledge is based on comparisons of respiratory exchange ratio (RER) or respiratory quotient (RQ) at similar power outputs in pregnant compared to non-pregnant subjects.

Two studies found no difference in RER with moderate cycle exercise (300-350 kpm/min) in pregnancy as compared to post partum women, suggesting no change in the ratio of fat to CHO utilization at absolute levels of moderate cycle exercise (54,84). With moderate treadmill running (350-380kpm), Knuttgen & Emerson (54) reported that RER was significantly higher during pregnancy compared to 6 weeks postpartum, suggesting greater reliance on CHO as an energy source during exercise in pregnancy. In contrast, Artal et al (1) reported no difference between non-pregnant controls and pregnant women at 29 weeks gestation. In recreational runners at 22 and 32 weeks, Clapp et al (17) reported that despite lower relative workloads (decreased percentage of pre-pregnancy VO2 max determined by heart rate), there was no decrease in RER, suggesting increased CHO utilization by the maternal muscles. Given the inconsistency of results of the
studies to date, no firm conclusions can be made regarding CHO utilization in exercising pregnant subjects. No study measuring RER in animals was located, nor has there been a comparison between trained and untrained individuals in any study. As discussed above, training increases the relative contribution of fat to metabolism in the non-pregnant subject; it remains to be determined whether the same is true for the fit pregnant subject.

Because glucose crosses the placenta by facilitated diffusion, a decrease in maternal blood glucose may result in a decrease in fetal uptake and utilization, therefore repeated exposure to maternal hypoglycemia could possibly result in a decreased birthweight. The effects of exercise on blood glucose are discussed here, while birthweight is discussed in a subsequent section. Of particular concern is that increased CHO utilisation by skeletal muscle during pregnancy could predispose the subject to hypoglycemia.

In pregnant rats, maternal glucose does not appear to be sensitive to exercise. Mild and moderate exercise in late gestation in untrained rats did not result in decreased maternal blood glucose, even when run to exhaustion (13,39). In rats familiarized to treadmill running, plasma glucose was not decreased after a 50 minute treadmill run, on day 19 of gestation, shown to promote maternal muscle and liver glycogen depletion (102). In chronically exercised rats, an
exercise bout of high intensity for 60 minutes on day 20 of
gestation did not result in maternal hypoglycemia (70).

In contrast, even moderate, short term exercise in
women results in a decrease in blood glucose, more than what
is observed in the non-pregnant state (17). The decrease is
more pronounced as pregnancy progresses when hypoglycemia
has been observed with strenuous exercise, and as pregnancy
progresses (40,65). With more prolonged exercise however,
blood glucose levels tend to approach normal (65).

In sheep, who do not have a gradual decline in resting
blood glucose with advancing gestation, blood glucose
increases in proportion to the duration and intensity of
exercise (64).

It is uncertain whether maternal exercise alters fetal
glucose utilization at different stages of gestation. In
rats, Treadway & Young (102) reported that after strenuous
exercise fetal glucose uptake, measured using radioactive
markers, was decreased by 40%, even though maternal
normoglycemia was maintained. The decreased fetal glucose
uptake was attributable to increased glucose uptake during
exercise by maternal skeletal muscle.

In sheep, due to the maternal hyperglycemia during
exercise and the resultant increase in the maternal to fetal
glucose gradient, uterine glucose uptake was increased
during prolonged exercise (65). Despite this, fetal insulin
levels were unchanged until after exercise, when elevated
insulin may have increased fetal glucose utilization (65). After exhaustive exercise in sheep, maternal glucose only partially returned to normal within 20 minutes after the exercise (65).

As in non-pregnant women, plasma lactate is unchanged with short duration and low intensity exercise, but is increased during and after moderate intensity exercise (52), more than in the non-pregnant woman (2, 40, 64). Because pyruvate concentration is only slightly increased in both pregnant and non-pregnant women, the lactate to pyruvate ratio increase is more pronounced in pregnancy during and after exercise (2). In sheep, lactate increases during exercise, but the lactate to pyruvate ratio is unchanged (64).

There may be a slight decrease in free fatty acid (ffa) concentrations during exercise in both pregnant rats and women, which would reflect greater utilization of fat. However, it is not known whether the ffa turnover rate is different (64).

Circulating plasma hormone levels also change with exercise in pregnancy. In sheep, prolonged moderate exercise caused a rapid increase in maternal pancreatic glucagon, an increase in catecholamines, a decrease in growth hormone, and no change in insulin. All hormones returned to normal within 20 minutes following exercise (65). In women, a transient increase in glucagon and

37
catecholamines after mild exercise was reported at 34 weeks gestation, with return to normal within 15 minutes (4,87). With more strenuous exercise, norepinephrine increased further, thus its levels were higher than epinephrine (4). There was no change in plasma insulin or cortisol levels with mild exercise (4,52). However, none of the measurements were done during exercise, and there was no non-pregnant group for comparison. Gorski (40) concludes that there is an increase in plasma catecholamines in pregnant women with mild to moderate exercise, as in the non-pregnant state. Rauramo et al (91) found that after a 10 minute submaximal exercise test at 35 weeks gestation, progesterone and estriol fell slightly, estriol increased immediately after exercise then fell to below pre-exercise levels, and prolactin was markedly elevated for over 1 hour following exercise.

7. Blood Flow Distribution and Oxygen Delivery to the Fetus

The distribution of the cardiac output during exercise in pregnancy is of great importance for the well-being of the fetus. There is little evidence that the regional distribution of blood flow with exercise is altered by pregnancy, except for the uterus. The exercise-induced increase in blood flow to the working muscles and to the skin, and the concomitant decrease to the splanchnic area, raises the possibility that blood flow to the uterus may be
reduced. Because blood flow is the most important determinant of fetal oxygen and nutrient supply, a decrease in uterine blood flow due to exercise could cause fetal hypoxia and/or reduced fetal growth. Studies investigating uterine blood flow have been conducted primarily in sheep, where the animals are usually chronically catheterized, allowing measurements to be made during exercise. Some work has also been done in pygmy goats, rats, and humans. Many of the measurements in humans are done after exercise, and these must be interpreted with caution because blood flow may rapidly return to normal (46).

It is generally agreed that total uterine blood flow decreases with increasing intensity and duration of exercise in sheep, pygmy goats, and rats, and that it usually returns to normal within 20 minutes after exercise (34,38,45,51,64,65,73,109). It may not decrease with mild exercise (14). Some researchers however have reported no change in total uterine blood flow immediately following exercise (26,81), but one used ewes familiarized to treadmill running, and the other included two non-pregnant ewes in the analysis. In rats, training prior to pregnancy did not attenuate the decrease in blood flow to the uterus observed during exercise (51).

Although the decreased blood flow suggests decreased delivery of oxygen and nutrients to the fetus, there are three reasons why this may not be true. Exercise is
associated with maternal hemoconcentration (66,86,108), thus an increased oxygen-carrying capacity. Thus Lotgering et al (66) suggested that the reduction in $O_2$ delivery (oxygen content * flow) to the uterus is much smaller than the decreased flow might suggest. The second reason is that there is increased oxygen extraction from the maternal blood. Finally, the blood flow is redistributed within the uterus. The cotyledons are favoured at the expense of the myometrium (26,64,65,69,73,109). Due to redistribution of blood flow and increased oxygen extraction, a constant oxygen uptake by the fetus may be maintained, and it would thus be protected from hypoxia (66,69). The effects of exercise during pregnancy on blood flow to the fetus is not resolved however, because most of the evidence is in animals, and the application to humans is questionable.

There are few studies in humans and results are inconsistent. Morris et al (72) injected radioactive sodium into the myometrium and its rate of removal, considered to reflect blood flow, was measured. The blood flow decreased by approximately 25% in healthy exercising women in late pregnancy. This result must be interpreted with caution. The exercise used was recumbent cycling, and as discussed in the first section of this review, vena caval obstruction by the gravid uterus in the supine position in late pregnancy could have altered blood flow. Also, as discussed above, decreased myometrial flow may not necessarily mean that
placental flow was compromised. Rauramo & Forss (90) measured placental blood flow before and after 6 minutes of upright cycling at approximately $63\% \text{VO}_2\text{max}$. There was no decrease in placental blood flow 1 minute following exercise. Other studies using Doppler ultrasound techniques have confirmed that placental blood flow is unaltered with mild to moderate maternal exercise, but most measured the flow following exercise (46). The question remains in humans as to whether the flow is decreased during exercise, and whether higher intensity exercise elicits the same response. The effect of training also warrants investigation. Finally, even if there is a decrease in uterine or even placental blood flow, the clinical significance needs to be examined.

8. **Acute Uterine and Fetal Responses**

Because maternal exercise causes an increase in catecholamines, especially norepinephrine, and the uterus increases its activity in response to intravenous injection of this hormone, there has been some concern that maternal exercise could cause increased uterine activity. However, few studies have measured uterine activity following exercise. In late gestation, Veille et al (107) reported no increase in uterine activity following moderate intensity running or cycling for 10 to 30 minutes in moderately active women. In contrast, Durak et al (31) reported that in
previously unfit women in late gestation, there was increased uterine activity in 50% of the sessions of moderate bicycle exercise for 15 minutes, and in 40% of sessions of moderate treadmill exercise. However, they did not analyze the data using means as did Veille et al (107), thus this could have modified interpretation of the results.

Fetal responses to maternal exercise may be affected by exercise-induced changes in uterine blood flow, umbilical blood flow, maternal plasma volume, maternal body temperature, and circulating catecholamine levels (108). Fetal heart rate (FHR) is used as an indicator of the fetal well-being, fetal distress being associated with severe bradycardia (<100 bpm) and tachycardia (>180 bpm). FHR has been measured most often in exercising sheep and women. Doppler ultrasound is the method most frequently used in women, and its validity has been disputed by some, as motion artifacts may be mistaken for FHR (113). Results are variable. FHR has been reported to increase (1,40,65,94,113), decrease (40,52,64,94), or remain unchanged (40,64,94) during exercise. Many studies have compared FHR during a pre-exercise rest period to post-exercise measurements and did not include measurements during exercise. Results are still widely varied, but many have reported increases in FHR immediately following exercise which returns to normal within 20 minutes (1,3,20,40,43,52,108,64,94,113). Of more importance perhaps
than changes in FHR within the normal range is the incidence of bradycardia or tachycardia. Some have reported transient bradycardia after cycling, running or swimming in late pregnancy, the incidence being less with the latter exercise (1,3,108), and unrelated to exercise intensity, but it was usually in less than 10% of subjects and was resolved within 2 minutes. The significance of these transient FHR changes is unknown, but they may have little or no clinical significance in an otherwise normal pregnancy (108).

In rats and mice, exercise prior to and/or during pregnancy did not affect the number of implantation sites (9,103,105). Most studies have reported no difference in fetal resorptions with exercise prior to and during pregnancy (9,34,70,101,105). However in mice with no pre-pregnancy training, exercise during pregnancy resulted in increased fetal resorptions compared to sedentary controls (101). With high intensity running for 8 weeks pre-pregnancy and throughout pregnancy, increased resorptions have also been reported in rats (103).

9. Antepartum Course, Length of Gestation and Course of Labour

As for weight gain and food intake, the studies which have examined length of gestation, antepartum course and the course of labour have involved repeated bouts of exercise
during pregnancy. The same applies for the upcoming sections of this chapter.

Antepartum course, or the course of pregnancy prior to labour, has been examined in women and rodents. In women, the antepartum course is not worsened by exercise. No increases in first and second trimester bleeding, spontaneous abortion, abnormal placentation, congenital abnormalities, premature labour, and premature rupture of the membranes were found in previously fit women who continued to exercise for various time periods throughout pregnancy (15,18a,19). However, there were no reports identified for previously unfit women.

Only one study identified in animals measured the length of gestation. In rats with no pre-pregnancy training or acclimatization to the treadmill who were exercised at a high intensity between days 1 and 21 or 12 and 21 of a 21 day gestation, gestational length was increased 0.5 to 1 day (34). If rats were exercised during only the first half of pregnancy however, the length of gestation was not different from sedentary controls.

In contrast to the report in rats, gestational length has been reported to be decreased (15,19) or unchanged (11,19,21,32,59) in women following exercise during pregnancy. The intensity, duration, time period throughout pregnancy, and the amount of exercise done, as well as the fitness level of participants varied between studies.
Neither Erkkola (32) or Kulpa et al (59) reported the actual amount of exercise that the subjects did during pregnancy, therefore it is difficult to draw any conclusions from these studies. Collings et al (21) and Brenner et al (11) supervised exercise sessions for previously unfit mothers, but they were conducted during only the second and third trimesters of pregnancy, not from early in pregnancy. The subjects in the studies by Clapp & Dickstein (19) and Clapp (15) included previously fit women who chose to continue to exercise during pregnancy. The former was a large retrospective study which compared sedentary women, and fit women who either discontinued exercise by the 28th week of pregnancy or continued until term. Exercise performance was assessed by pre-conception questionnaire, at 28 and again at 36 weeks, and data were stratified according to performance level. Gestational length was decreased by 7 days in the group who continued to exercise to term, but was not different from sedentary women in those who discontinued by week 28. In addition, when stratified for performance level, the reduction in length of gestation increased with increased exercise. In a large prospective study, Clapp (15) compared fit women who either stopped exercising at least 50% of their pre-conceptual level by the end of the first trimester to those who continued to term. There was no increase in incidence of preterm labour, but parturition was 5 days earlier in the group who continued to exercise.
These two studies demonstrated that continued regular exercise to term in previously fit women decreases gestational length, and that the magnitude of the reduction is positively associated with the level of exercise performed. No rigorous studies have examined the effect of exercise to term in previously unfit women.

The course of labour and delivery have been investigated only in women. Exercise during pregnancy does not change the course of labour in women who had not exercised regularly prior to pregnancy, and may improve the intrapartum course in previously active, fit women.

Length of labour has been reported to be unchanged \((11,21,29,32,42,62)\) in previously unfit women who exercised during pregnancy. In fit women, exercise during pregnancy decreased \((15,88)\) or did not change the length of the active phase of labour \((19)\). Use of forceps for delivery, degree of laceration, mode of delivery, and obstetric complications were reported to be unchanged with exercise during pregnancy in previously unfit women \((29,59)\). In fit women who continued to exercise throughout pregnancy, Clapp \((15)\) reported the exercising groups had less obstetric intervention for abnormal labour pattern, 2nd stage arrest, and there was less use of forceps, epidurals, and cesarian sections when compared to sedentary controls. These effects were not attributable to difference in parity between groups. Clapp & Dickstein \((19)\) reported that in fit women
who exercised throughout pregnancy there was no difference in the use of cesarian section, but that in this group, less were due to cephalopelvic disproportion, which was the primary reason in sedentary women. They also reported no differences in use of forceps and incidence of breech births.

10. **In Utero Fetal Growth and Development**

In animals, litter size is an indicator of fetal survival in utero. Most studies in rats and mice have reported no decrease in litter size with various exercise protocols, all of which included some pre-pregnancy training or acclimatization in rats (9,13,25,70,74,75,76,96,101, 104,105,111). However, in rats with no pre-pregnancy training who exercised at a high intensity either throughout pregnancy or in the second half only, litter size was reduced (34). The same group reported that when rats were exercised during the first half of pregnancy only, litter size was unchanged, suggesting that later gestation is a period more vulnerable to exercise than is early pregnancy. There was one other report of decreased litter size, by Treadway et al (103). This group had also reported increased number of fetal resorptions, and as discussed previously, this may be attributable to the high intensity and long time period of the training and exercise protocol. Others have also used high intensity exercise during
pregnancy but they also used shorter pre-pregnancy training periods, and reported no change in litter size (9,70,74,75).

Birthweight is the most commonly used indicator for evaluating the effect of repeated exercise on pregnancy outcome because it reflects in utero growth. It has been measured in humans, rodents, pygmy goats, and guinea pigs whose dams or mothers had exercised repeatedly during pregnancy. Many studies have reported no change in birthweight, however, there may be species differences.

Overall, in utero growth of the rat and mouse fetus appears to be largely unaffected by maternal exercise. In rats and mice, fetal weight on day 19 or 21 of a 21-day gestation, or birthweight, and birth length are usually unchanged by exercise of mild to high intensity of varying durations (9,13,25,34,51,70,74,75,76,96,103,104,105). There was one report of increased birthweight when rats exercised at a high intensity during only the second half of gestation (34). Although the number of pups per litter was decreased by 1, this does not explain the above finding, because for the rats that were exercised throughout pregnancy, litter size was decreased to a greater extent and birthweight was unchanged. One study in mice reported decreased birthweight regardless of whether pre-pregnancy training was present (101). A similar protocol including pre-pregnancy training was used in mice by another group (9), but they did not report decreased birthweight; reasons for these different
results are not apparent. Overall, fetuses of rats and mice do not appear to be vulnerable to maternal exercise in pregnancy as indicated by birthweight.

Although birthweight is normal, there is the possibility that other indicators of fetal growth and development are affected by maternal exercise. This was investigated in several studies. Delayed skeletal ossification in fetuses of exercised dams was reported (9,101,105), but there was no increase in malformations (9,74,101). Neonatal brain, heart, liver, kidney, and lung weights were not altered by strenuous maternal exercise, nor were skeletal muscle weights (74). The development of the diaphragm was unaltered by mild maternal exercise (76). Normal accumulation of fetal liver glycogen, which is important for maintenance of normoglycemia after birth, was either reduced (39) or unchanged (13) by exercise at the end of gestation. However, no neonatal follow up was done. Body composition was reported to be no different in term pups of exercised versus sedentary rats (103).

In pygmy goats exercised at a mild intensity 1-2 days per week from the 80th to the 148th day (term) of pregnancy, birthweight of singlets was unchanged (28,45). In guinea pigs, birthweight was decreased after 60 minutes of exercise per day from day 14 to 63 (term) of pregnancy, but with 45 minutes or less of exercise, there was no difference in birthweight from control animals (80,98).
The effect of maternal exercise on human infant birthweight depends on the maternal fitness level, the timing, intensity and duration of the exercise. In previously inactive women who exercised at mild to moderate intensities during exercise, birthweight, length, and head circumference were unchanged (11,20,27,29,32,42,50,59,62). However, in physically fit women who continued to exercise during pregnancy to term, birthweight has been reported to be decreased by 300-500g compared to unfit women who did not exercise during pregnancy or fit women who stopped exercising by the end of the first trimester (15,16,19). If the women had stopped exercising by the 28th week of gestation, birthweight was not different than those who had not exercised at all during pregnancy, thus it appears that continued exercise into the 3rd trimester is a critical period in the determination of birthweight (19). Clapp & Capeless (16) reported that the birth percentile for weight decreased from 65% to 45%. These women exercised at a higher frequency, intensity, and duration than those in the studies which reported no change in birthweight with maternal exercise. These studies were also the most rigorous in terms of design and statistical analyses of all the studies reviewed. One can thus conclude that the in utero growth of the human fetus could perhaps be vulnerable to regular, moderate to high intensity maternal exercise if it is continued throughout gestation.
However, despite decreased birthweight, birth length and head circumference were unchanged (16). The clinical significance of these decreases in birthweight needs to be assessed. It is unclear whether the incidence of low birth weight increased, although Clapp & Dickstein (19) reported 38% of infants were <10th percentile for weight when mothers had exercised throughout gestation, compared to 11% when women stopped by the 28th week, and only 3% when they did not exercise at all during pregnancy.

The decrease in birthweight appears to be due to a decrease in body fat, as it was reported to be decreased, whereas lean mass was unchanged (16). Clapp & Dickstein (19) reported that the infants of exercising mothers had the same incidence of neonatal complications, and Clapp (15) reported a decreased incidence of meconium in fluid, abnormal heart rate, nuchal cord, and incidence of 1 minute Apgar <7 in the infants of exercising mothers. Thus, at least in the short term, despite decreased body fat and birthweight, the infants' neonatal course does not appear to be compromised.

11. Postnatal Offspring Effects

In rats, a few investigators have followed up on offspring born to dams who exercised during pregnancy. No other animal or human studies which have done this were identified.
Maternal exercise during pregnancy may induce alterations in the conducting properties of the myocardium of the progeny. Primary cell cultures of beating myocardial cells from 5-day-old neonatal offspring of exercised rats were reported to beat at a slower rate, have a greater percentage of contracting cells. However, myocardial cells from offspring of exercised rats had a greater viability compared to those from pups of sedentary dams (10). On the other hand, heart weight was significantly higher in 100-day-old male offspring from mothers who had exercised throughout pregnancy (82). Wilson & Gisolfi (111) reported no influence of maternal exercise on the VO₂max of 45-65 day old offspring.

The offspring of exercised mothers may have decreased postnatal survival. Wilson & Gisolfi (111) reported greater mortality during the 28 days following birth in offspring of dams who had exercised at a high intensity for 50 days prior to pregnancy, and throughout gestation. It is unclear from the results presented whether there was also increased mortality in the offspring of dams who did not exercise prior to pregnancy but who ran at a mild intensity during pregnancy. It is not specified at what point within the 28 days the mortality occurred, that is, whether it was early or late. In addition, dams were exposed to electrical shock during pregnancy, and the influence of this on the offspring may be detrimental.
Two studies reported offspring growth in the first 2 weeks after birth (25,104). Both reported no differences in pup or litter weight in this time period. However, both culled the litters to 8 pups at birth, thus reducing the stress on the dam to support her litter. No studies reported growth in unculled litters of dams who had exercised during pregnancy.
D. References for Chapter I.


57


60. Lanoue, L. & Koski, K.G. (1990) The effects of graded levels of maternal dietary glucose on pre-term glycogen deposition, amniotic fluid composition and glucose homeostasis in pregnant rats dams and fetuses near term. (Submitted)


II. Joint Effects of Exercise and Dietary Carbohydrate on Pregnancy Outcome and Early Neonatal Survival in Rats.

A. INTRODUCTION

Carbohydrate is the principle energy source required by the growing fetus (3,16,34). When dietary carbohydrate (CHO) is restricted during pregnancy, gestation is prolonged, maternal feed intake and weight gain are decreased, and pup birthweight is reduced, and perinatal mortality is increased in rats (14,23,24,25). Maternal intake of dietary CHO in late gestation is essential for the postnatal survival of rat pups (22). The restriction of maternal dietary CHO, even only in the last two days of gestation, prevents optimal glycogen accumulation in fetal liver, resulting in high neonatal mortality within the first two days after birth (22,24).

Exercise during pregnancy may also perturb fetal CHO homeostasis because carbohydrate is also the preferred metabolic fuel during moderate and high intensity exercise of short and moderate duration (7,13). Of particular concern is that, during pregnancy, increased utilization of CHO by maternal skeletal muscle during exercise could result in a decrease in fetal uptake and utilization of glucose. Although maternal blood glucose was not decreased (8,19,40), fetal uptake of glucose was decreased by 40% immediately following exercise in rats in late pregnancy (40). Of the two studies that measured fetal liver glycogen following maternal exercise at the end of gestation, one reported a decrease (19) and the other found no change (8).
Birthweight was not reduced by exercise during pregnancy (11,29,31,32,33,37,42,43,45), but maternal weight gain was reduced (32,33,41,42,43,45). Even though birthweight was normal, mortality in the first 28 days postpartum of pups whose mothers had exercised prior to and throughout pregnancy was increased compared to sedentary controls (45).

No study examining the joint effects of exercise and CHO restriction during pregnancy has been found. The purpose of this study was to examine the interactive effects of diet, specifically varying levels of dietary CHO, and exercise during pregnancy on the mother and offspring. It was hypothesized that moderate maternal aerobic exercise, in combination with a diet containing moderately restricted levels of dietary CHO, would result in a greater reduction in maternal weight gain, fetal growth, and survival of pups in the early neonatal period, than if neither, or only one of these conditions (exercise or reduced CHO diet) were present.

B. METHODS

1. Experimental design

A schematic representation of the study design is shown in Figure 1. A 2*3 factorial which included 2 exercise regimens and 3 dietary concentrations of CHO was used to examine the interaction between exercise and dietary carbohydrate. In the absence of an interaction, this design also permitted the testing of the main effects of exercise and diet. After a 4 to
6d period of adjustment to the laboratory routine and an 18-day 
exercise acclimatization period (see animal care and exercise 
protocol section), half the rats were assigned to exercised (E) 
and half to sedentary (S) groups. This division was based on an 
evaluation of their running performance during the 
acclimatization period. Rats who did not run well were 
automatically designated S animals, as they were highly unlikely 
to be able to complete the exercise protocol during pregnancy. 
Following this group designation, rats were mated, and within 
each activity group (E or S), were randomly assigned to one of 
three dietary CHO treatment subgroups (n=15-20/subgroup). The 
experiment was repeated 3 times, each repetition being 
considered a block, and each having similar numbers of animals.

2. Animal Care And Exercise Protocol

A time line for the experimental protocol is shown in 
Figure 2. Virgin female Sprague-Dawley rats (Charles River 
Canada Inc., St. Constant, Quebec) weighing 175-200g on arrival 
and 245-255g at mating were used for the experiment (n = 113). 
On arrival, rats were housed in individual wire screen cages. 
The rats were acclimatized to a temperature controlled room 
(21±2°C) with automatic fluorescent lighting (12h daily, 0700 - 
1900) for 4-6d (adjustment period). The diet offered was 
standard Purina Rat Chow (Ralston Purina Co., Longueuil, Quebec) 
and water ad libitum until mating.
After the adjustment period, all rats were subjected to an 18d exercise acclimatization period which consisted of running on a motor-driven treadmill, beginning at 10m/min*5min/d and gradually increasing speed and time to 20m/min*45min/d by the end of the 18d. Similar protocols have been used previously by others (31,32,33), and was considered to be the minimum time necessary to sufficiently accustom rats to running in order to be capable of completing the exercise protocol during pregnancy.

Following the exercise acclimatization period, individual female rats were placed at random with one of 13 male breeders of the same strain until impregnation. Mating was confirmed by the presence of a vaginal plug on the cage tray and was designated day 0 (d0) of pregnancy. To prevent detraining during the mating process, E rats continued to run at 20m/min*45min/d until bred.

Exercise (E) rats did not run on d0 of pregnancy; they resumed running on d1. By d3 of pregnancy, these rats were running at 20m/min*60min/d*7d/wk, and continued until d20 of pregnancy. In the non-pregnant rat, this level of exercise corresponds to about 70% VO2 max (4,6,38); thus, this protocol was considered to be of moderate intensity.

A moderate level of exercise was chosen for several reasons. Postnatal pup survival and growth were some of the outcomes that were to be examined. We wanted to avoid a strong independent effect of exercise on these parameters because an interaction between diet and exercise was hypothesized,
otherwise we risked having too few pups alive in later lactation to be able to measure lactational performance of the dams. Increased postnatal mortality had been shown to result from high intensity exercise during pregnancy (45), thus this level of exercise used was judged to be too high for this experiment. A second reason was that a moderate level of exercise was considered to be realistic in terms of being able to have all rats complete the protocol. Previous investigators have reported attrition rates of 20% when rats were run at a high intensity during pregnancy (31,32).

Although an electric shock grid was available on the treadmill, it was not used because this would have represented a stress over and above the exercise which would not have been present in the S animals. Instead of using shock, an observer was present at all times when the rats were on the treadmill, and they were prodded from their hindquarters to keep them running. The only exception was that mild shock was used on the first block of animals for the first and second day of the exercise acclimatization period, but no animals were shocked during pregnancy. Rats were exercised during the day (light cycle). All rats completed the exercise protocol.

While pregnant E rats were running, S rats were placed in the same room to subject them to as similar an environment, except for the exercise itself, as the E rats. Sedentary rats were removed from their feed and water during this time, because the additional 60min/d of food and water available to the S rats
may have influenced cumulative feed intake and weight gain during pregnancy.

All rats remained in the wire screen cages until day 19 of pregnancy when they were transferred to plastic-bottomed maternity cages and provided with cedar chips for nesting. Within 0 to 8h of parturition, pups were counted and the litter weighed as a unit to the nearest 0.1g. Pups were not weighed individually at birth in order to minimize handling of the pups and distress to the dam. Average pup birth weight was calculated as litter weight divided by number of pups born. Neonatal mortality was monitored by examining the litters on a daily basis for dead and/or eaten pups until the end of lactation d2, the vulnerable period for neonatal mortality if CHO homeostasis was perturbed. On d15 of lactation, dams were anaesthetized with Ketamine HCL (CDMV Drugs, Montreal, QC.) injected at a dose of 30mg/kg body weight. After cardiac puncture, the abdomen was opened and the uterus was removed and examined for implantation sites. Number of resorptions was calculated by subtracting the number of pups born from the number of implantation sites on the uterus.

3. **Experimental diets**

The composition of the experimental and control diets is shown in Table 1. The formulation of the 60% CHO control diet and that of the CHO-restricted diets was adapted from work done by Fergusson & Koski (14). Diets were formulated on an equi-
energetic basis, providing 4.15 kcal metabolizable energy per gram dry matter. Dextrose was removed from the 60% control CHO diet and was replaced by an equienergetic amount of soy oil plus a complementary amount of cellulose to make up the weight. These were the only components of the diet to vary; the quantities of protein, vitamins, and minerals were held constant for each diet, and were provided in amounts necessary to meet NRC (35) daily requirements for the pregnant or lactating rat, whichever was higher. Diets were fed ad libitum.

The rationale for the selection of 20% and 40% CHO as the levels for the experimental diets was that postnatal parameters were to be examined, and it was considered undesirable to have a high postnatal pup mortality caused by diet alone, especially because a diet by exercise interaction was hypothesized to further compromise the outcome due to increased utilization of CHO by the dam during exercise. Decreased birthweight and high in utero and postnatal pup mortality have been reported with severe restrictions of dietary CHO (14,24,25), thus a less severe restriction was considered desirable. At 24% CHO, although fetal liver glycogen stores were reduced, birthweight was normal compared to control rats (26), thus a level close to this, considered to be minimally adequate based on birthweight and survival data, was chosen for this experiment. Forty percent CHO was chosen as an intermediate level.

Feed intake was measured every 1 to 2d by weighing the stainless steel feed cups on a Mettler electronic balance
(Fischer Canada, Montreal, QC.) to the nearest 0.1g. Spilled feed was also weighed and taken into account in the calculation of daily and total feed intake. Even though spilled feed was taken into account for feed intake calculations, an additional classification variable (feed spillage) was created for the statistical analysis, separating rats which spilled the majority of their feed during the experiment and those which did not. Rats were weighed individually every 1 to 2d to the nearest gram. Average daily feed intake for each week was divided by the average body weight for that week to obtain the values for weeks 1, 2 and 3. The differences in these values from week to week were also calculated and analyzed.

4. **Data Management and Statistical Analysis**

Data were entered twice and verified before they were stored in a raw data file in spreadsheets in LOTUS version 2.3 (28) on a personal computer. Raw data files were converted to SYSTAT files on a personal computer using DBMS/COPY (12). All calculations were done in double precision.

Statistical analyses for maternal weight gain and feed intake, implantation and resorption sites, length of gestation, number of pups born, litter weight and average pup birthweight were performed using SYSTAT version 4.1 (44) on a personal computer. Multiple regression and ANOVA were used to test the interactions first, followed by the main effects of diet and exercise. Models for all outcomes included exercise, diet,
exercise-by-diet interaction, block and sire. Where appropriate, identified confounders were simultaneously included in the models as covariates. These are listed for each individual outcome in the footnotes for the tables of results and in Appendix 1.

The main effects were considered significant at $p<0.05$. A goodness-of-fit-test was used to test the contribution of variables other than exercise and diet to the model; a cut-off of $p<0.20$ was used. Inclusion of variables with $p<0.20$ served to reduce the error term when examining the effects of exercise and diet. When other variables were dropped from the full model, a reduced model was re-analyzed that still included the exercise by diet interaction. The diet by exercise interaction was considered important at $p<0.20$. After removing other variables, if the p-value for the interaction exceeded this cut-off, it was dropped from the model before a final analysis was done. The a priori hypotheses were then tested. First, exercise rats were compared to sedentary rats. For diet, two orthogonal tests were done. The average of the experimental diets, 20% plus 40%, were compared to the control 60% CHO diet to examine whether CHO restriction affected the outcome. Then, to see whether a difference was present between the two levels of dietary CHO restriction of the experimental diets, 20% CHO was compared to 40% CHO. All tests were 2-tailed.

Postnatal mortality, a non-normally distributed outcome, was analyzed using GLIM (2), a statistical package that analyzes
non-normally distributed categorical data. The analysis controlled for the number of pups born to each dam in addition to exercise, diet, and the exercise by diet interaction. For pup mortality on the 2nd day of lactation, the effect could not be estimated due to the instability of the model (the scaled deviance for the model was less than the degrees of freedom, resulting in a non-asymptotic Chi-squared distribution).

C. RESULTS

1. Feed intake during pregnancy

Unadjusted means for cumulative maternal feed intake during pregnancy are shown in Table 2. After controlling for sire, block, and feed spillage, no significant interaction was found between diet and exercise. The reduced model included feed spillage.

Exercise had no effect on cumulative maternal feed intake, but there was a significant effect of diet on feed intake. Rats fed the 20% CHO diet ate significantly less, a total of 31.4g, than the 40% dams (unadjusted difference: 27.2g). Although rats fed the CHO-restricted diets (20% + 40%) ate 10.1g less (unadjusted difference: 14.7g) than the control rats, this difference was not statistically significant.

Differences in maternal feed intake per gram of body weight are presented in Table 3. The E rats ate more compared to the S rats on a per gram body weight basis because the E animals gained less weight than the S groups (refer to weight gain
section). The effect of exercise was cumulative and increased as pregnancy progressed, becoming statistically significant at week 3. The differences between weeks 1 and 2, and 2 and 3 were slightly less than at week 3 alone, reinforcing that this was a cumulative effect that became significant only at the end of gestation.

The level of CHO in the maternal diet had an effect on feed intake per gram of body weight in the first and second weeks, but not the 3rd week of gestation. During week 1, there was a significantly lower feed intake per gram of body weight in the 20% versus the 40% CHO-fed rats, but 20+40% CHO-fed rats were not different than rats fed 60% CHO. In week 2, the rats fed the 20% CHO diet ate significantly less than the 40% CHO group. During week 3, no difference in feed intake per gram of body weight was found between the different diet groups. The only statistically significant differences when the differences between weeks were examined were between the 20% and 40% CHO-fed groups between week 1 and week 2.

2. Weight gain during pregnancy

Unadjusted means for maternal weight gain during pregnancy are shown in Table 4. After controlling for sire, block, feed spillage, cumulative maternal pregnancy feed intake, number of pups born, and length of gestation, no diet by exercise interaction on weight gain was detected. The reduced model included cumulative feed intake and feed spillage.
Exercised rats gained significantly less weight during pregnancy compared to sedentary rats (10.7g), regardless of diet. The unadjusted difference was 14.3g. Rats fed the CHO-restricted diets (20% + 40%) gained significantly less weight, 15.6g, than the 60% CHO-fed rats (unadjusted difference: 17.1g), regardless of activity level, but there was no significant difference in weight gain between the rats fed 20% and 40% CHO.

3. Patal growth and survival in utero

Unadjusted means for implantations and resorptions sites, length of gestation, number of pups born, litter weight, and average birth weight are shown in Tables 5-10. No significant interaction or main effects of exercise or diet were found for implantations, resorptions, length of gestation, number of pups born, or pup birth weight.

The average length of gestation was 21.5±0.5 days. The implantations per dam averaged 15±2, and the number of fetal resorptions was 1±1 per dam, resulting in an average litter size of 14±2 pups per dam. The average birth weight per pup was 5.3±0.4g.

The litters of dams fed CHO-restricted diets (20% and 40%), however, weighed significantly less, 3.1g, than those of the 60% dams (unadjusted difference: 4.4g). The sire to which the female rat was bred also had a significant effect on litter weight.
4. **Neonatal Mortality**

Neonatal pup mortality for days 0 and 1, and cumulative mortality for d1 and d2 of lactation are shown in Table 11. After controlling for the number of pups per litter, no significant exercise by diet interaction was found for any of the days analyzed, therefore the results are shown separately for exercise and diet groups.

There were no main effects of diet or exercise on neonatal mortality. The sire to which the dam was bred significantly affected mortality for all days examined. The block to which the animal was assigned also significantly affected mortality for d1, and cumulative mortality to postnatal d1 and postnatal d2, with the animals in the third block having the lowest mortality.

D. **DISCUSSION**

This study shows that pregnant rats were able to adapt to the combined stress of moderate exercise and restriction in dietary CHO to bear pups of similar number and size compared to those of control dams, and to support their survival in the early neonatal period. This suggests that neither moderate exercise nor moderate CHO restriction perturbed overall CHO homeostasis. Koski & Hill (25) also reported no increase in neonatal mortality with restriction of dietary CHO to 12%, but no study was identified that reported neonatal survival with less severe CHO restrictions. Carlson (8) reported decreased
fetal liver glycogen stores following maternal exercise in late
gestation, but no neonatal follow-up was done. In addition,
Carlson (8) did not train the rats prior to the late gestation
exercise bouts. As will be discussed below, training may reduce
the reliance on CHO as an energy source during exercise, thus
sparing the available glucose for the fetus. Wilson & Gisolfi
(45) reported increased mortality in the first 28 days post
partum in rat pups whose dams had been trained at a high
intensity for 7 weeks prior to pregnancy and had continued to
run at a high intensity throughout pregnancy. It is unclear
from the results presented by Wilson & Gisolfi (45) whether
running at a mild/moderate intensity during pregnancy without
pre-pregnancy training resulted in a statistically significant
increase in mortality. The mortality was not reported for any
time period less than 28d, thus it is not possible to determine
at what point within these 28d the mortality occurred and
compare it to our results. In addition, several differences
exist between the study by Wilson & Gisolfi and ours. Their
rats exercised at a much higher intensity, and underwent a
longer period of higher intensity pre-pregnancy training.
Exercising rats were subjected to shock if they stopped running
on the treadmill, whereas in our study, shock was not used with
the pregnant rats.

Contrary to the hypothesis, no significant interaction
between exercise and level of dietary CHO was found for maternal
feed intake and weight gain, number of implantations and
resorptions, length of gestation, number of pups born per dam, litter weight, average birth weight, or neonatal mortality. The lack of a diet by exercise interaction may be due to several factors. Exercise during pregnancy could have resulted in an aerobic training effect. In human endurance exercise, training has been shown to increase the activity of muscle glycogen synthase as well as shift the metabolism towards a greater reliance on fat, and less on CHO during exercise (17,18,20,21,36). If this occurs in pregnant rats, the dam may have spared the limited CHO for use by the growing fetus. Alternatively, or in addition, adaptation to the low CHO, high fat diet could have altered metabolism towards a greater use of fat and sparing of CHO. This has been shown to occur in male rats after only one week of being fed a high fat diet (10,30). It has also been suggested, based on measurements of respiratory exchange ratios, that pregnant women may rely less on CHO for fuel during exercise compared to the non-pregnant woman (1,9). However, none of these mechanisms have been investigated in pregnant rats and the interpretation remains speculative.

An alternative explanation for the lack of a diet by exercise interaction in the outcomes reported here is that the adverse effects of exercise and dietary CHO restriction during pregnancy may only manifest themselves on pup survival and growth at a later point post-partum. Previous studies have shown that feeding a severely restricted CHO diet during pregnancy and lactation in rats resulted in decreased milk
lactose, lipid, and metabolizable energy value, and reduced pup growth to d7 of lactation (23,25). In the two studies that examined the effects of exercise in pregnancy and lactation on postnatal pup growth, progressive pup weight was the same among running and sedentary rats throughout lactation to d14 (11), and litter and pup weight were not different between swimming and sedentary groups on d15 of lactation (42). However, in both studies, litters were culled to 8 pups at birth. The dam may have been able to adequately support growth in these pups, but if litters were not culled, the additional requirements for milk production to support the larger litter may not have been met by the dam who was already subjected to the stress of exercise. Treadway & Lederman (42) reported no change in milk yield on day 10 to 11 of lactation. However, there was a reduction in milk lactose content at d15 of lactation, as was found on d6 of lactation after feeding a low CHO diet (23). Thus although no diet-by-exercise interaction was detected during pregnancy or the early neonatal period, the possibility of effects on later postnatal growth and survival must be considered. Wilson & Gisolfi (45) reported increased postnatal mortality to d28 postpartum in pups of exercised dams, and because the timing of this mortality was not specified, it is possible that mortality may have occurred later than the first few days post partum.

Both exercise and diet had independent effects on maternal feed intake and weight gain in spite of the lack of an interaction. Exercise did not alter cumulative feed intake,
but, when expressed on a per weight basis, E rats ate more
during the third week of pregnancy than S controls. Only
Courant & Barr (11) removed feed from sedentary rats from feed
while exercised rats were running as was done in our study.
However, our results do not appear to support their findings.
They reported an increase in total feed intake in the exercised
rats for the first and second weeks of pregnancy, but not the
third. However, they did not report cumulative feed intake nor
feed intake as a proportion of body weight. In addition, their
rats ran at a higher intensity (30m/min) for a longer duration
(2hr/day) during pregnancy, and this could have elicited a
different response than the moderate exercise used in this
study.

Despite increasing their feed intake, E rats still gained
less weight than S controls. This corroborates the findings of
others (32,33,41,43,45), but not those of Courant & Barr (11).
Examination of Courant & Barr's data indicated that exercised
rats did not gain more weight than sedentary controls, but they
were heavier than sedentary controls from the time of mating.

The average effect of restricting CHO, to 20% + 40% did not
result in decreased feed intake, but the intake of the 20% CHO-
fed dams was significantly below that of the 40% CHO-fed dams,
both cumulative and on a per body weight basis. However,
contrary to the effect of exercise, the effect of CHO appeared
to be during early and mid, but not late gestation. Leturque
(27) reported decreased cumulative feed intake when CHO was
restricted to 19% of energy during pregnancy, which our results corroborate.

Although the feed intake of the rats fed the 20% + 40% CHO diets was not decreased compared to the rats fed 60% CHO, their weight gain was reduced. Others have reported decreased maternal weight gain with more severe restrictions of CHO (14,25). With moderate restriction of CHO to 19% of energy, no reduction in weight gain was reported (27), which our results do not support. However, the sources of CHO used by Leturque (27) were starch and sucrose, whereas we used glucose. Additionally, the concentration of dietary protein used in their study was almost twice that used in our study, a different strain of rats was used, and the overall weight gain in the control rats was less than one-half of the weight gain of control rats in our study, suggesting that differences between studies were not only in the effects of diet, but possibly also genetic.

The composition of the maternal weight gain was not measured in this study because rats could not be sacrificed during pregnancy. If the decreased weight gain was due to decreased accumulation of body fat or underdevelopment of the mammary gland, as has been suggested by others (11,32,37), then the ability of the dam to produce an adequate quantity and quality of milk to support the growing pups may be compromised.

The early reproductive period was not vulnerable to either exercise or CHO-restriction. As in other studies, no effect of
exercise (5,41,43) or CHO restriction (14) was found for implantations.

Later reproductive outcome was also not vulnerable to exercise and CHO-restriction. No effect of exercise or CHO restriction on resorptions was found. Others have also reported no effect for exercise (5,15,29,43) and CHO restriction (14) on resorptions. Only one report in the literature showed increased fetal resorptions with exercise in rats (41). However, rats ran at a higher intensity for 8 weeks prior to conception and during pregnancy. This was the longest and one of the more intense pre-pregnancy training periods used for treadmill running in rats, and the treadmill was equipped with a shock stimulus, suggesting a detrimental effect of extensive high intensity exercise and/or shock.

Length of gestation was unaffected by diet, as in other studies (25). Exercise did not affect length of gestation. One study showed a delay of parturition in rats that were not trained prior to pregnancy, but ran at a high intensity during pregnancy (15). The rats in our study were familiarized to treadmill running prior to pregnancy and exercised at only a moderate intensity, possibly explaining the different results.

Because implantations and resorptions were not different between groups, the number of pups born was unaltered, as was reported by others when CHO restriction (25), or exercise was imposed (5,8,11,29,31,32,33,37,39,42,43,45). Two studies did show decreased number of pups born (15,41). The exercise
protocols in these studies were much more severe (higher intensity and duration) than that used in our study. As mentioned previously, Treadway et al (41) exercised rats for long periods at high intensity and used shock. Garris et al (15) did not acclimatize rats to the treadmill prior to pregnancy and ran them at a high intensity during pregnancy.

In our study, exercise did not affect litter weight and birthweight, as has been reported by others (11,15,29,31,32,33,37,41,42,43). However, restriction of dietary CHO significantly decreased litter weight by 3.1g. The number of pups per litter was not significantly different between groups, nor was the average calculated pup birthweight, thus the decrease in litter weight with the 20% + 40% CHO diets, although statistically significant, may not be biologically important. However, had pups been weighed individually, we may have detected a different growth in some of the pups in each litter (runts) which we could not detect using the methods we did. Others have also reported no effect on birthweight of dietary CHO restriction to 24% (26) and with more severe dietary CHO restrictions (14).

The lack of greater detrimental effects of exercise and moderate CHO restriction on the pregnancy outcome may be due to several factors. The rats were not randomized into sedentary and exercised groups. It is possible that those rats which ran were better able to withstand the stress of the exercise than those which would not run. This may have reduced or masked an
effect of the exercise. However, despite this, exercise still resulted in increased maternal feed intake and reduced weight gain. The exercise and CHO restriction may not have been severe enough to result in a great enough stress to alter the overall CHO homeostasis and thus compromise pregnancy outcome. The prepregnancy exercise acclimatization period may also have reduced the stress of exercise during pregnancy. It is possible that exercising rats during the light cycle was an additional stress. Despite this, no detrimental effects of exercise during pregnancy were observed in this study when we exercised rats during the light cycle.

The sire to which the dam was bred affected litter weight and neonatal mortality, suggesting a genetic influence not only on the dam, but on the offspring. This suggests that there is a genetic contribution by the male, independent of the environmental conditions of the dams. Thus sires should be controlled for either in the design (e.g. by breeding control and experimental groups to the same sires) or in the analysis phase in future research in this field. No other study was found that reported this observation.

Block also affected neonatal mortality, indicating that environmental conditions were not identical between blocks. Environmental conditions were kept as stable as possible (e.g. temperature, animal handling, time of day the animals were exercised and weighed). However, fluctuations in room temperature were apparent throughout the experimental period.
Temperature differences may account for the mortality differences between blocks.

In summary, this study shows that the stress of moderate intensity exercise and moderate restriction in dietary CHO had an impact on the dam, shown by alterations in feed intake and reduction in weight gain. The fetuses however, were spared, being born with normal birthweight and not having increased mortality in the early neonatal period. However, as discussed, an impact of the exercise and CHO restriction may manifest itself later in the postpartum period, and is therefore currently under investigation.
E. References for Chapter II.


88


Figure 1: Experimental Design

All rats: exercise acclimatization (18 days)

Mating

Exercise (E)  Sedentary (S)

Pregnancy day 0

% dietary carbohydrate

20%  40%  60%

Parturition = Lactation day 0
Figure 2: Time line for experimental protocol

- **Adjustment**
- **Exercise**
- **Mating**
- **Pregnancy**
- **Lactation**

<table>
<thead>
<tr>
<th>wk1</th>
<th>wk2</th>
<th>wk3</th>
</tr>
</thead>
<tbody>
<tr>
<td>d0</td>
<td>d4-6</td>
<td>d22-24</td>
</tr>
<tr>
<td>d0</td>
<td>Gestation to d21</td>
<td>d0 to d2</td>
</tr>
</tbody>
</table>

- Teach all rats to run on the treadmill.
- Divide into E and S diet treatments.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Dietary Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose(^2)</td>
<td>60</td>
</tr>
<tr>
<td>Soybean oil(^3)</td>
<td>14.08</td>
</tr>
<tr>
<td>Cellulose(^4)</td>
<td>2.93</td>
</tr>
<tr>
<td>Casein(^5)</td>
<td>15.0</td>
</tr>
<tr>
<td>Vitamin mixture(^6)</td>
<td>1.2</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>5.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.34</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>40</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean oil(^3)</td>
<td>21.96</td>
<td>29.84</td>
</tr>
<tr>
<td>Cellulose(^4)</td>
<td>15.05</td>
<td>27.17</td>
</tr>
<tr>
<td>Casein(^5)</td>
<td>15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Vitamin mixture(^6)</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

1. Dry weight basis (grams).
2. Dextrose (anhydrous), ICN Biochemicals Canada Ltd., Montreal, Quebec.
3. Degummed soybean oil, Canada Packers Inc., Montreal, Quebec.
4. Alphacel, ICN Biochemicals Canada Ltd., Montreal, Quebec.
6. Vitamin and mineral mixture composition as previously reported (14).
<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>447.2±33.2 (18)</td>
<td>475.2±40.6 (19)</td>
<td>462.1±52.4 (16)</td>
<td>461.7±43.1 (53)</td>
<td></td>
</tr>
<tr>
<td>Exercised</td>
<td>436.0±54.9 (18)</td>
<td>461.7±62.4 (17)</td>
<td>477.3±76.6 (17)</td>
<td>457.9±66.0 (52)</td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>441.6±45.1** (36)</td>
<td>468.8±51.7 (36)</td>
<td>469.9±65.4 (33)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.
2. Reduced model included feed spillage.
** Significant difference, p<0.05, between 20% dietary CHO and 40% dietary CHO dams.
TABLE 3. Difference in maternal feed intake per gram body weight between exercised and sedentary rats and rats fed varying levels of dietary carbohydrate¹,²

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Exercise Effect</th>
<th>Diet Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E vs S</td>
<td>20+40 vs 60%CHO</td>
</tr>
<tr>
<td>Week 1</td>
<td>0.12 (0.48)</td>
<td>-0.17 (0.34)</td>
</tr>
<tr>
<td>Week 2</td>
<td>-0.23 (0.14)</td>
<td>-0.34 (0.05)</td>
</tr>
<tr>
<td>Week 3</td>
<td>-0.34 (0.03)*</td>
<td>-0.04 (0.84)</td>
</tr>
<tr>
<td>Difference from week 1 to week 2</td>
<td>-0.27 (0.07)</td>
<td>0.96 (0.07)</td>
</tr>
<tr>
<td>Difference from week 2 to week 3</td>
<td>-0.28 (0.08)</td>
<td>-0.04 (0.50)</td>
</tr>
</tbody>
</table>

¹ Values are mean differences between the groups identified for feed intake (grams) per gram body weight. Numbers in parentheses are p values for the differences.
² For the effects due to the difference from week to week, the earlier week's value was included in the reduced model as a covariate. Reduced models also included feed spillage.
* Significant difference, p<0.05.
TABLE 4. Cumulative maternal weight gain in pregnancy among sedentary and exercised rats fed varying levels of dietary CHO\(^1,2\)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>129.1±24.8</td>
<td>143.4±22.2</td>
<td>153.1±16.7</td>
<td>142.3±22.9**</td>
</tr>
<tr>
<td></td>
<td>(11)</td>
<td>(15)</td>
<td>(12)</td>
<td>(38)</td>
</tr>
<tr>
<td>Exercised</td>
<td>125.2±17.2</td>
<td>121.1±15.1</td>
<td>140.2±28.6</td>
<td>128.0±21.5</td>
</tr>
<tr>
<td></td>
<td>(13)</td>
<td>(14)</td>
<td>(11)</td>
<td>(38)</td>
</tr>
<tr>
<td>All</td>
<td>127.0±20.6</td>
<td>132.6±21.9</td>
<td>146.9±23.5****</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(36)</td>
<td>(36)</td>
<td>(33)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.
2 Reduced model included cumulative feed intake and feed spillage.
** Significant difference, p<0.05, between sedentary and exercised dams.
**** Significant difference, p<0.01, between 20+40% dietary CHO dams and 60% CHO dams.
<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>days</td>
</tr>
<tr>
<td>Sedentary</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.6±0.5</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
</tr>
<tr>
<td>Exercised</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.5±0.5</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
</tr>
<tr>
<td>All</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21.5±0.5</td>
</tr>
<tr>
<td></td>
<td>(36)</td>
</tr>
</tbody>
</table>

1 Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.
2 Number of pups born and maternal weight gain were included as covariates.
### Table 6. Number of implantation sites among sedentary and exercised rats fed varying levels of dietary carbohydrate

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>All</td>
</tr>
<tr>
<td>Sedentary</td>
<td>16±2</td>
<td>15±2</td>
<td>15±2</td>
<td>15±2</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(18)</td>
<td>(15)</td>
<td>(50)</td>
</tr>
<tr>
<td>Exercised</td>
<td>16±2</td>
<td>15±2</td>
<td>15±3</td>
<td>15±2</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
<td>(17)</td>
<td>(16)</td>
<td>(49)</td>
</tr>
<tr>
<td>All</td>
<td>16±2</td>
<td>15±2</td>
<td>15±2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(33)</td>
<td>(35)</td>
<td>(31)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.*
TABLE 7. Number of fetal resorptions among sedentary and exercised rats fed varying levels of dietary carbohydrate\textsuperscript{1,2}

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Sedentary</td>
<td>1±2</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
</tr>
<tr>
<td>Exercised</td>
<td>2±1</td>
</tr>
<tr>
<td></td>
<td>(16)</td>
</tr>
<tr>
<td>All</td>
<td>1±1</td>
</tr>
<tr>
<td></td>
<td>(33)</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.

\textsuperscript{2} Number of implantation sites was included as a covariate.
# TABLE 8. Number of pups born to sedentary and exercised rats fed varying levels of dietary carbohydrate

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14±3</td>
<td>14±2</td>
<td>14±2</td>
<td>14±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18)</td>
<td>(19)</td>
<td>(16)</td>
<td>(53)</td>
</tr>
<tr>
<td>Sedentary</td>
<td></td>
<td>14±2</td>
<td>13±2</td>
<td>14±3</td>
<td>14±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(18)</td>
<td>(17)</td>
<td>(17)</td>
<td>(52)</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>14±2</td>
<td>14±2</td>
<td>14±2</td>
<td>(33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(36)</td>
<td>(36)</td>
<td>(33)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.
2 Covariates included were cumulative maternal feed intake and weight gain, and feed spillage.
Table 9. Average birthweight of pups born to sedentary and exercised rats fed varying levels of dietary carbohydrate

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td></td>
<td>5.2±0.5</td>
<td>5.3±0.4</td>
<td>5.3±0.4</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(19)</td>
<td></td>
<td></td>
<td>(53)</td>
</tr>
<tr>
<td>Exercised</td>
<td></td>
<td>5.1±0.3</td>
<td>5.4±0.5</td>
<td>5.5±0.6</td>
<td>5.3±0.5</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(17)</td>
<td></td>
<td>(17)</td>
<td>(51)</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>5.2±0.4</td>
<td>5.3±0.4</td>
<td>5.4±0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(36)</td>
<td></td>
<td>(33)</td>
<td></td>
</tr>
</tbody>
</table>

1 Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.
TABLE 10. Total litter weight of pups born to sedentary and exercised rats fed varying levels of dietary carbohydrate\(^1,2\)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Dietary CHO Level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Sedentary</td>
<td>73.2±13.2</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
</tr>
<tr>
<td>Exercised</td>
<td>73.1± 9.1</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
</tr>
<tr>
<td>All</td>
<td>73.1±11.2</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
</tr>
</tbody>
</table>

\(^1\) Values are unadjusted means ± SD. Numbers in parentheses are sample sizes.

\(^2\) Covariates included were number of pups born, cumulative feed intake and weight gain, and feed spillage. The reduced model included number of pups born per dam and cumulative maternal feed intake.

** Significant difference, p<0.01, between 20+40% dietary CHO dams and 60% dietary CHO dams.
Table 11. Neonatal Pup Mortality in Exercised and Sedentary Rats Fed Varying Levels of Dietary Carbohydrate

<table>
<thead>
<tr>
<th>% Dietary CHO</th>
<th>Total Births</th>
<th>Daily Mortality</th>
<th>Cumulative Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>day 0</td>
<td>day 1</td>
</tr>
<tr>
<td>Exercised</td>
<td>726</td>
<td>6 (41)</td>
<td>5 (35)</td>
</tr>
<tr>
<td>Sedentary</td>
<td>748</td>
<td>4 (31)</td>
<td>9 (71)</td>
</tr>
<tr>
<td>20</td>
<td>514</td>
<td>5 (28)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>40</td>
<td>488</td>
<td>4 (20)</td>
<td>8 (41)</td>
</tr>
<tr>
<td>60</td>
<td>472</td>
<td>5 (24)</td>
<td>6 (30)</td>
</tr>
</tbody>
</table>
Appendix 1: Summary of Statistical Procedures

For all analyses done in SYSTAT, full models were entered as follows:

category diet=3, exercise=2, block=3, sire=13
predict long
model outcome = constant+ exercise + diet + exercise*diet +
block + sire + covariates
estimate

Sire remained in the reduced model for litter weight and
mortality only. Block was only in the reduced model for
mortality. Covariates other than block and sire varied
according to the outcome, and were the following:

<table>
<thead>
<tr>
<th>outcome</th>
<th>full model</th>
<th>reduced model</th>
</tr>
</thead>
<tbody>
<tr>
<td>weight gain</td>
<td>feed intake</td>
<td>feed intake</td>
</tr>
<tr>
<td></td>
<td>feed spillage</td>
<td>feed spillage</td>
</tr>
<tr>
<td></td>
<td>length of gestation</td>
<td></td>
</tr>
<tr>
<td>total feed intake</td>
<td>feed spillage</td>
<td>feed spillage</td>
</tr>
<tr>
<td>weekly feed intake</td>
<td>previous week's intake</td>
<td>previous week's intake</td>
</tr>
<tr>
<td>per body weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>implantation sites</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>fetal resorptions</td>
<td>implantations</td>
<td>none</td>
</tr>
<tr>
<td>litter weight</td>
<td>number of pups born</td>
<td>number of pups born</td>
</tr>
<tr>
<td></td>
<td>feed spillage</td>
<td>feed intake</td>
</tr>
<tr>
<td></td>
<td>feed intake</td>
<td></td>
</tr>
<tr>
<td>number of pups born</td>
<td>weight gain</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>feed intake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>feed spillage</td>
<td></td>
</tr>
<tr>
<td>average pup birthweight</td>
<td>number of pups born</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>length of gestation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>weight gain</td>
<td></td>
</tr>
<tr>
<td>length of gestation</td>
<td>number of pups born</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td>weight gain</td>
<td></td>
</tr>
<tr>
<td>mortality</td>
<td>number of pups born</td>
<td>number of pups born</td>
</tr>
</tbody>
</table>


103
Hypothesis testing for main effects of diet and exercise was done as follows:

```plaintext
hypothesis
  effect=exercise
  contrast 1 -1
  test
hypothesis
  effect=diet
  contrast .5 .5 -1
  test
hypothesis
  effect=diet
  contrast 1 -1 0
  test
```

The following is an example for the GLIM procedures used for cumulative mortality to d2 (cumd). This procedure was used for mortality d0, d1, d2, and cumulative to days 1 and 2.

```plaintext
input 8 $
fac ex 2 diet 3 bloc 3 sire 13 $
yvar cumd $
error b nopu $
link g $
fit $
fit ex + diet + bloc + sire + ex*diet $
```