Monitoring Blood Glucose Levels in Female Mink During the Reproductive Cycle: The Prevention and Reversal of Hyperglycemia During the Nursing Period

by

Amber M.J. Hynes

Submitted in partial fulfillment of the requirements
for the degree of Master of Science

at

Nova Scotia Agricultural College
Truro, Nova Scotia

in cooperation with

Dalhousie University
Halifax, Nova Scotia
September 2005

Your file Votre référence
ISBN: 978-0-494-16539-3
Our file Notre référence
ISBN: 978-0-494-16539-3

DALHOUSIE UNIVERSITY

To comply with the Canadian Privacy Act the National Library of Canada has requested that the following pages be removed from this copy of the thesis:

Preliminary Pages

Examiners Signature Page (pii)

Dalhousie Library Copyright Agreement (piii)

Appendices

Copyright Releases (if applicable)

DEDICATION

This thesis is dedicated to my parents, Peter and Carol, who have given me endless encouragement and unwavering support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The impacts of body condition, anti-diabetic supplements and reproductive status on blood glucose levels in female mink during the reproductive cycle were investigated. Firstly, dams from 3 farms were assigned to either herring oil (HerO), chromium picolinate (CrPic), or a control group for 6-wk at the onset of lactation. Hyperglycemia was observed throughout reproduction. Females exhibiting hyperglycemia early in the reproductive cycle tended to remain hyperglycemic, have poorer health and fewer kits. CrPic reduced blood glucose levels. Secondly, females having blood glucose values of <5.5 mmol l^{-1} (Normoglycemic) and ≥ 5.5 mmol l^{-1} (Hyperglycemic, HG) early in lactation were supplemented daily for 1-wk at day 21 of lactation with various combinations of HerO, CrPic and acetyl-salicylic acid (ASA). In HG females, treatments, excluding CrPic and ASA alone, reduced blood glucose levels. Blood glucose levels in lactating mink may be affected by anti-diabetic supplements; however, as hyperglycemia occurs prior to nursing, preventative measures are recommended throughout the year.

LIST OF ABBREVIATIONS AND SYMBOLS USED

AA	Arachidonic acid
ASA	Acetyl-salicylic acid
ATP	Adenosine triphosphate
BCS	Body condition score
CoA	Coenzyme A
Cr	Chromium
CrPic	Chromium picolinate
CTRL	Control
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
G6Pase	Glucose-6-phosphatase
GLUT	Glucose transporter
HbA_{1c}	Glycated hemoglobin
HerO	Herring oil
HG	Hyperglycemic
IKKß	Serine kinase IKß kinase
IL-6	Interleukin-6
LDL	Low-density lipoprotein
ME	Metabolizable energy
NFkB	Nuclear factor kappa B
NG	Normoglycemic
$O_2^{\cdot-}$	Superoxide anion radical
PBGM	Portable blood glucose meters
PEPCK	Phosphoenolpyruvate carboxykinase
PUFA	Polyunsaturated fatty acids
RDA	Recommended dietary allowance
ROS	Reactive oxygen species
T2DM	Type 2 diabetes mellitus
TBARS	Thiobarbituric acid reactive substances
TCA	Tricarboxylic acid
TG	Triacylglycerol
TNF-α	Tumour necrosis factor-α
VLDL	Very low-density lipoprotein
8-OhdG	8-hydroxy-deoxyguanosine

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisor, Dr. Kirsti Rouvinen-Watt. Her guidance was invaluable, her words of encouragement always appreciated. She is both a mentor and a friend. Special thanks are also extended to Dr. Gordon Finley and Dr. Leslie MacLaren for their advice and expertise.

A special thank you to the staff and families of the collaborating mink ranches. They welcomed me into their homes, and their participation and cooperation will not be forgotten. I am grateful to the staff of the Canadian Centre for Fur Animal Research, particularly Rena Currie and Tanya Morse, for taking time out of their schedules to assist me. I would also like to thank Gerry Russell and Shelli Meleck for their assistance in sample collection, and to Renée Garbes for her work with the comet assay. I would also like to thank Dr. Tess Astatkie for his help in statistical analysis.

This research was supported by the Canada Mink Breeders Association, the Nova Scotia Fur Institute and the Natural Sciences and Engineering Research Council (NSERC Discovery Grant to KRW).

CHAPTER 1. Introduction and literature review

1.1 INTRODUCTION

In mammals, glucose is a primary nutrient for both fetal growth and milk synthesis (Bell & Bauman, 1997) and the development of hyperglycemia during gestation and lactation may result in significant health risks for the mother, fetus and newborn (Di Cianni *et al.*, 2003). The nursing period is a time of critical importance during the reproductive cycle of the mink dam; as lactation progresses, the ability of the mink female to meet increasing energy demands is influenced by various metabolic, nutritional and environmental factors (Clausen *et al.*, 1992; Rouvinen-Watt, 2003). While various studies have investigated glucose metabolism in lactating mink (Børsting & Gade, 2000; Fink & Børsting, 2002; Fink *et al.*, 2001a; Fink *et al.*, 2002a; Fink *et al.*, 2002b; Fink *et al.*, 2004), reports evaluating the impact of disruptions in glucose homeostasis during the reproductive cycle of mink females are sparse. Within normal pregnancy a condition of insulin resistance develops, a process favoring glucose supply to the developing fetus and milk production, with glucose homeostasis restored at the cessation of lactation (Di Cianni *et al.*, 2003). However, it is suggested that obesity or lipodystrophy (deficiency of body fat), n-3 polyunsaturated fatty acid (PUFA) fatty acid deficiency and/or oxidative stress may aggravate this metabolic response, resulting in the development of insulin resistance and hyperglycemia in the mink dam (Rouvinen-Watt, 2003).

Insulin resistance is a common underlying abnormality in a number of metabolic conditions (Colagiuri & Brand Miller, 2002) and hyperglycemia (23.4±2.8 mmol l^{-1}) has been observed in mink dams diagnosed with nursing sickness as compared to normal

blood glucose concentrations (5.3±0.3 mmol l^{-1}) in apparently healthy lactating mink (Wamberg *et al.*, 1992a). Nursing sickness is a disorder believed to develop from a complex of metabolic, nutritional and environmental factors, which influence the ability of the mink dam to meet the extreme demands of lactation (Clausen *et al.*, 1992). Mink are a rapidly growing species and while the etiology of nursing sickness remains uncertain, an increased demand for gluconeogenesis due to heavy milk production may be a causative factor (Wamberg *et al.*, 1992a). Børsting and Gade (2000) suggested that the development of the disorder may be associated with a disruption in glucose homeostasis. Increasing dam age, large litter size, and female weight loss have been identified as major determinants for its development (Clausen *et al.*, 1992) and clinical signs include lethargy, loss of appetite, emaciation, and extreme dehydration (Seimiya *et al.*, 1988; Schneider & Hunter, 1992). Females affected with nursing sickness have been shown to lose approximately 31% of their body weight during lactation, as compared to 14% in unaffected dams (Clausen *et al.*, 1992). With morbidity as high as 14-15% and mortality up to 8% (Clausen *et al.*, 1992), differences in incidence rates observed among individual ranches demonstrate the importance of ranch-level factors in the development of the disease (Schneider & Hunter, 1992).

Generally viewed as a symptom of the disease, little research has been performed to investigate the role of high blood glucose in the development of nursing sickness, although pilot studies conducted suggest a relationship between the two (Hynes *et al.*, 2004; Rouvinen-Watt & Hynes, 2004). The research presented investigates glycemic

control and supplemental strategies designed to prevent or reverse hyperglycemia in the mink female throughout the reproductive cycle.

1.2 LITERATURE REVIEW

1.2.1 GLUCOSE REGULATION

In mammals, glucose is a key fuel and an important metabolic substrate (Wood & Trayhurn, 2003). The regulation of glucose metabolism is a key aspect of metabolic homeostasis and various hormones influence this regulatory system (Olefsky, 1999). These include insulin, which decreases blood glucose, and counter-regulatory hormones, such as glucagon, epinephrine and cortisol, which increase blood glucose concentrations.

1.2.1.1 *Absorptive phase*

Insulin is a protein hormone secreted by the β-cells of the pancreatic islets during the absorptive phase of digestion to reduce blood glucose levels. One of the most important actions of insulin is to enhance the uptake of both dietary glucose and glucose synthesized within the body from circulation into target peripheral tissues, i.e. skeletal muscle and adipose cells (Olefsky, 1999; Holman & Sandoval, 2001; Wood & Trayhurn, 2003; DeFronzo, 2004). Additionally, insulin promotes the conversion of excess glucose into storage as glycogen in liver and muscle (glycogenesis) or triacylglycerol (TG) in adipose tissue, inhibits lipolysis and, thereby, reduces circulating free fatty acid (FFA) concentrations, and inhibits hepatic glucose production (Nelson & Cox, 2000; DeFronzo, 2004).

Post-prandially, the rapid stimulation of glucose transport into the target peripheral tissues is essential for the maintenance of glucose homeostasis (Kahn, 1994; Kahn & Pessin, 2002). Glucose transporters (GLUT) are a group of membrane proteins that are integral in this process (Holman & Sandoval, 2001; Wood & Trayhurn, 2003). Confined mainly to insulin-sensitive fat and skeletal muscle tissues, GLUT-4 facilitates insulin-responsive glucose transport into the adipocytes and myocytes of these target tissues (Kahn, 1994; Holman & Sandoval, 2001; Khayat *et al.*, 2002; Wood & Trayhurn, 2003). GLUT-1 has a minor role in enhancing glucose transport in response to insulin in these tissues (Kahn, 1994). When insulin levels are low, glucose transporters are located within the membranes of cell cytoplasmic vesicles. As plasma glucose levels rise, insulin binds to its receptor and stimulates tyrosine kinase activity, initiating a signaling cascade that draws GLUT to the surface membrane of insulin-responsive cells (Kahn, 1994; Olefsky, 1999; Holman & Sandoval, 2001; DeFronzo, 2004). Here GLUT become incorporated into the plasma membrane and facilitate the removal of glucose from the blood via the passive diffusion of glucose down a concentration gradient (Kahn, 1994; Karp, 1996; Holman & Sandoval, 2001; Wood & Trayhurn, 2003).

After absorption into the cells, glucose can be used immediately for the release of energy to the cells or converted to glycogen or fat. During carbohydrate metabolism (glycolysis), in the absence of oxygen, pyruvate is reduced to lactate, and during aerobic oxidation, pyruvate is decarboxylated and linked to coenzyme A (CoA), to form acetyl-CoA. This complex passes through the tricarboxylic acid (TCA) cycle, releasing energy through electron transport to be used in adenosine triphosphate (ATP) formation (Karp, 1996).

These pathways act to remove glucose from the blood, restoring normal blood glucose concentrations.

1.2.1.2 *Post-absorptive phase*

When blood glucose levels are low, pancreatic α-cells release the hormone glucagon, an action that mediates the glycogenolysis pathway; the breakdown of stored glycogen to yield glucose for fuel (Kahn & Pessin, 2002). Glucagon also increases lipolysis in adipose tissue, releasing FFA into the bloodstream. However, when these fuel reserves are low, or when amino acids are in abundance, glucose may be synthesized from non-carbohydrate precursors, i.e. amino acids from the breakdown of protein and the glycerol of triacylglycerol (TG), through the process of gluconeogenesis. More costly from an energy standpoint, the gluconeogenesis pathway is essentially the reverse of glycolysis (the break down of glucose), however separate enzymes, i.e. pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose bisphosphatase, and glucose-6-phosphatase (G6Pase), are involved (Bender, 1997).

Glucagon, epinephrine, and cortisol may interact to increase circulating blood glucose levels (Eigler *et al.*, 1979). Acute stress increases epinephrine production, releasing glucose for energy. Secreted by the adrenal medulla, epinephrine stimulates hepatic glucose production and stimulates the adipose cells to release FFA, decreasing the use of glucose for fuel and thereby increasing blood glucose levels (Sherwin *et al.*, 1980; Nelson & Cox, 2000). Stevenson *et al.* (1991) reported increased stimulation of both glycogenolysis and gluconeogenesis in dogs infused with increasing concentrations of

epinephrine. It also stimulates glycogen breakdown in skeletal muscle, producing ATP, in order to facilitate violent muscular action (Elliott & Elliott, 2001). In humans, increases in muscle glycogen utilization observed after epinephrine infusion were associated with a reduction in muscle glucose uptake (Watt *et al.,* 2001). Similarly, epinephrine has reduced both basal and insulin-stimulated glucose uptake in rat skeletal muscle (Chaisson *et al.*, 1981; Jensen *et al.*, 1987). The reduction in glucose uptake is suggested to be due to inhibited glucose phosphorylation resulting from enhanced muscle glycogenolysis (Chaisson *et al.*, 1981; Watt *et al.*, 2001; Hunt & Ivy, 2002).

Under conditions of chronic stress, cortisol is secreted by the adrenal cortex, stimulating gluconeogenesis and increasing the breakdown of protein and fat (De Leon *et al.*, 1997). Handling and restraint stress have resulted in significant increases in both plasma cortisol and glucose levels in captive bats; cortisol levels rose by approximately 800% after two hours of restraint (Widmaier & Kunz, 1993). Similarly, elevated cortisol concentrations were observed in the plasma of cubs from pregnant blue fox vixens handled during late gestation (Osadchuk *et al.*, 2004). In an earlier study, cortisol levels were consistently lower in men fed a high carbohydrate diet in comparison to those fed a diet high in protein (Anderson *et al.*, 1987). In lactating mink, plasma cortisol levels were lowered one week after weaning, indicating that weaning is a source of stress, with a reduced strain on the dams after weaning and separation from the kits (Sørensen *et al.*, 2001). In the skeletal muscle of rats, cortisol excess induced insulin resistance by directly inhibiting GLUT-4 translocation (Dimitriadis *et al.*, 1997).

Glucose regulation in the mink

In the wild, the mink is a carnivore with a diet high in animal protein and fat and relatively low in carbohydrates (Fink *et al.,* 2001a). For carnivores, gluconeogenesis is an important factor in the maintenance of glucose homeostasis (Kettlehut *et al.*, 1980). When compared to omnivores, carnivores have a higher capacity for glucose synthesis due to increased gluconeogenic enzyme activities (Børsting & Gade, 2000). For example, liver G6Pase and PEPCK activities were shown to be very high in the mink (Sørensen *et al.,* 1995). Nursing mink females rely heavily on gluconeogenesis to meet glucose demands. In a study by Fink and Børsting (2002), mink dams fed carbohydrate-free diets synthesized approximately 13g of glucose per day via gluconeogenesis, this was in the same range as those fed 17% of metabolizable energy (ME) from carbohydrates. It has been shown that the ability of the mink to synthesize glucose, when fed a carbohydrate-free diet, is dependant upon the availability of sufficient precursors in the form of amino acids (Fink & Børsting, 2002; Damgaard *et al.*, 2003). In addition to having the ability to synthesize large amounts of glucose via gluconeogenesis, the mink is able to utilize high levels of dietary digestible carbohydrates without critically elevating plasma glucose concentrations (Fink & Børsting, 2002; Fink *et al.*, 2002b). When fed significant amounts of carbohydrates, the mink has the ability to store excess glucose as glycogen (Børsting & Gade, 2000). Fink and Børsting (2002) suggest decreased *de novo* glucose synthesis in dams fed a high carbohydrate supply, possibly due to decreased activity of gluconeogenic enzymes.

1.2.1.3 *Glucose metabolism during gestation and lactation*

In order to maintain glucose supply to the gravid uterus and lactating mammary gland, metabolic adaptations act to buffer against variations in maternal nutrition and other environmental factors (Bell & Bauman, 1997). The endogenous production of glucose, particularly through gluconeogenesis, is amplified in order to meet the increased energy demands associated with the developing fetus (Di Cianni *et al.,* 2003). Additionally, an adaptive reduction of insulin sensitivity occurs in glucose metabolizing tissues during late gestation and lactation, facilitating the uptake of glucose by the fetus and mammary gland (Bell and Bauman, 1997; Butte *et al.*, 1999; Di Cianni *et al.*, 2003; Rand *et al.,* 2004). Possibly elicited by placental hormones, an increase in insulin secretion by the β-cells offsets the adaptive decrease in insulin sensitivity in order to maintain glucose homeostasis (Di Cianni *et al.,* 2003). In late gestation and into lactation, increases in circulating levels of hormones, i.e. prolactin, cortisol, and glucagon, promote greater utilization of alternative fuels, particularly fatty acids, by peripheral tissues (Buchanan, 1991; Butte *et al.*, 1999; Sivan *et al.*, 1999). The pregnancy-induced mobilization of adipose tissue results in elevated non-esterified or FFA concentrations (Regnault *et al.*, 2004). These FFA serve as an alternative energy source, conserving glucose for both fetal development and milk production (Di Cianni *et al.,* 2003).

Milk synthesis

An adequate supply of glucose is essential as a precursor in lactose synthesis (Annison *et al.*, 1968). Luick *et al.* (1962) reported that in lactating dogs, 68–100% of the carbon required for the synthesis of lactose is derived from plasma glucose. Lactose is the major

carbohydrate in the milk of most species; however a detailed profile of the carbohydrate fraction of mink milk has not yet been established (Fink *et al.,* 2004). In most species, lactose synthesis is the primary determinant of milk volume (Bell & Bauman, 1997). The triglyceride fraction of milk is derived from two sources: the *de novo* synthesis of fatty acids and their subsequent esterification within the mammary gland and the uptake of plasma lipids by the mammary gland (Del Prado *et al.*, 1999). However, it was reported that the mink mammary gland has a low capacity for *de novo* fatty acid synthesis and lacks the capability for desaturation and chain elongation (Wamberg *et al.*, 1992b). As lactation progresses the mammary gland may uptake FFA derived from the hydrolysis of TG stored in adipose tissue or TG from very low-density lipoprotein (VLDL) (Del Prado *et al.*, 1999).

Lactation demands in the mink

At peak lactation, approximately four weeks postpartum, the female mink often sustains a litter mass exceeding her own body mass (Fink *et al.*, 2002a). Findings indicate that increasing litter size places very high energetic demands on the mink dam during lactation (Børsting & Damgaard, 1995; Tauson, 1997; Wamberg & Tauson, 1998). Tauson *et al.* (1998) calculated a daily milk yield of 29.7 g per kit at 4 weeks postpartum for lactating mink dams. The yield of lactose and other milk sugars is around 9 g per day in dams nursing a litter of 8 kits; this is within the same range as the total absorption of glucose (Børsting & Damgaard, 1995). When fed a diet with 12% of ME derived from carbohydrates, approximately 75% of the glucose requirement for lactating mink is obtained through gluconeogenesis (Børsting & Damgaard, 1995). As increased glucose

production occurs in females nursing 8 kits in comparison to those nursing only 4, it is evident that greater demands for gluconeogenesis are placed on females with large litters (Børsting & Damgaard, 1995). It is suggested that as lactation progresses, dams are often unable to cover their total energy requirement by dietary intake, resulting in the acute mobilization of body reserves (Tauson, 1997). Clausen *et al.* (1992) found that the total biomass of kits raised and weaned by dams developing nursing sickness was significantly larger than that of apparently healthy dams. In support of these findings, a field survey conducted by Rouvinen-Watt and Hynes (2004) indicated that mink farms reporting problems with nursing sickness had higher numbers of kits born and weaned when compared to those not afflicted with the disease.

1.2.2 HYPERGLYCEMIA AND ACQUIRED INSULIN RESISTANCE

During gestation, predisposing genetic and environmental factors can lead to an exaggerated response in the normal physiologic reduction in insulin sensitivity and, in turn, a decline in β-cell function and the development of hyperglycemia (Di Cianni *et al.*, 2003). Colagiuri and Brand Miller (2002) define insulin resistance as a state in which "greater than normal insulin levels are required to elicit a quantitatively normal glucose response in the whole body, a tissue or at the cellular level." Type 2 diabetes mellitus (T2DM) is a disorder characterized by hyperglycemia and is associated with impaired carbohydrate, lipid and protein metabolism (Parillo & Riccardi, 2004). These abnormalities can result from insulin resistance in peripheral tissues and/or abnormal insulin secretion by pancreatic β-cells and elevated hepatic gluconeogenesis (Zimmet *et al.*, 2001; Lenhard & Gottschalk, 2002). In states associated with marked insulin

resistance, such as T2DM, the ability of insulin to stimulate GLUT-4 translocation, and therefore glucose uptake, in muscle and adipose cells is abnormally diminished (Khayat *et al.,* 2002). Insulin output must then be further increased in order to achieve glucose utilization in peripheral tissues and decrease hepatic gluconeogenesis (Lenhard & Gottschalk, 2002). Within T2DM, a combination of peripheral cell insulin resistance and β-cell dysfunction, leads to postprandial hyperglycemia (Bell & Polansky, 2001). Fasting hyperglycemia develops as β-cell glucose sensitivity further declines (Palumbo, 2001). Furthermore, insulin resistance is closely linked to impaired lipid metabolism; normally increases in circulating insulin levels stimulate lipid synthesis in adipose tissue and inhibit lipid mobilization (Howard, 1999). However, in insulin resistant states, the ability of insulin to inhibit the rate of lipolysis and to reduce plasma FFA is markedly impaired (DeFronzo, 2004). The elevated levels of FFA, generally observed in insulin resistance, are reported to further disrupt the insulin secretory response of pancreatic β-cell to glucose, as observed *in vivo* in rats (Mason *et al.*, 1999).

Although not documented in mink, acquired insulin resistance is a common disorder observed in another carnivore, the cat (Bennett, 2002; Behrend, 2002). Major abnormalities found in feline diabetes include impaired insulin secretion, peripheral insulin resistance and increased basal hepatic glucose production (Behrend, 2002). Similar findings have been reported in another member of the *Mustelidae* family, a black-footed ferret that exhibited, among other symptoms, weight loss and hyperglycemia (Fox & Marini, 1998). Clinical signs in companion animals include polydipsia, polyuria, weight loss, rear-limb weakness, unkempt coats, dehydration and lethargy (Plotnick &

Greco, 1995, Rand & Martin, 2001). Insulin resistance may result from various physiological, pathological and genetic factors. In both humans and companion animals, key factors for the development of T2DM include increasing age and obesity (Hoenig, 1995; Scarlett & Donoghue, 1998). In a proposed hypothesis for the pathogenesis of nursing sickness, Rouvinen-Watt (2003) suggests history of obesity or lipodystrophy, n-3 PUFA deficiency, and/or high protein oxidation rate as key components in the development of insulin resistance in the mink female.

1.2.2.1 ***Risk factors in the development of insulin resistance***

Obesity and Lipodystrophy

Adipose tissue, a major source of metabolic fuel stored in the form of TG (Samra, 2000; Morgan & Mercer, 2001), takes up glucose and releases lactate, as well as glycerol, alanine, and glutamine, which can act as substrates for gluconeogenesis (Samra, 2000). Obesity results when dietary energy intake exceeds the energy dissipated through either metabolism or exercise (Morgan & Mercer, 2001). Surplus energy is converted into TG that can be broken down (lipolysis) following hormonal stimulation to release FFA and glycerol (Samra, 2000; Reidy & Weber, 2000). Oxidation of fat generates twice as much energy as that of carbohydrate or protein (Samra, 2000). FFA are stimulators of hepatic glucose production and elevated concentrations will augment glucose-stimulated insulin secretion and contribute to insulin resistance (Frayn, 2001). As fatty acids compete with glucose for substrate oxidation, increases in fat oxidation may also interfere with insulin-signaling and glucose metabolism (Brownwyn *et al.*, 2000; Perseghin *et al.*, 2003).

Adipose tissue also releases cytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Samra, 2000), concentrations of which are increased in the insulin resistant states of obesity and T2DM (Trayhurn & Beattie, 2001; Dandona *et al.*, 2004). In a study by Lang and associates (1992), TNF-α produced both hepatic and peripheral insulin resistance and may have reduced the number or activity of GLUT-4 isomers. Insulin and cytokines, such as TNF-α, stimulate the production and secretion of the hormone leptin by adipocytes (Cunningham *et al.*, 1999; Trayhurn & Beattie, 2001). Identified as an important regulator in reproduction and lipid metabolism, circulating levels of leptin are directly correlated to body fat mass (Reidy & Weber, 2000; Frayn, 2001). Tauson and Forsberg (2002) found that plasma leptin concentrations strongly reflected plasma insulin levels and changes in body condition in female mink. Leptin changes the fuel source from which ATP is generated; with preference altered from glucose to lipid (fatty acids) (Reidy & Weber, 2000). When circulating levels of leptin are high, insulin action is inhibited in both adipocytes and muscle, TG synthesis is decreased, and lipid oxidation rates are increased (Reidy & Weber, 2000).

Numerous studies have identified obesity as a key risk factor in the development of insulin resistance and T2DM in both animals (Nadler *et al.*, 2000; Hoenig, 2002) and humans (Bray, 1992; Kahn & Flier, 2000; Frayn, 2001; Felber & Golay, 2002; Parillo & Ricardi, 2004). In healthy individuals, insulin inhibits the release of FFA from adipocytes through suppression of hormone-sensitive lipase (DeFronzo, 2004). However, chronically elevated levels of circulating FFA have been reported in both lean and obese individuals (Groop *et al.*, 1991; Gavrilova *et al.*, 2000; Frayn, 2001, Felber & Golay, 2002;

DeFronzo, 2004). These findings indicate an impaired ability of insulin to suppress the rate of lipolysis when the normal action of adipose tissue is compromised. Obesity is characterized by the excess accumulation of TG in adipocytes, causing these cells to fail in their normal role of protecting other glucose metabolizing tissues from the daily influx of dietary fatty acids (Frayn, 2001). Similarly, in the case of lipodystrophy, there is a deficiency of adipose tissue resulting in TG deposition in skeletal muscle, liver and pancreatic β-cells and leading to the impairment of glucose metabolism (Flier, 2001; Frayn, 2001). Recent data indicate that prolonged elevation of FFA levels inhibit insulin-signaling, leading to reduction in insulin-stimulated glucose transport, mediated by a decrease in GLUT-4 translocation (Boden & Shulman, 2002). Lipodystrophy is associated with leptin deficiency; when administered leptin replacement, glycated hemoglobin (HbA_{1c}) and TG levels were decreased in lipodystrophic patients (Oral *et al.*, 2002).

Studies demonstrate that not only the degree of adiposity but the distribution of body fat is an important factor in the development of T2DM (Parillo & Riccardi, 2004). Central distribution of body fat has consistently been associated with increased risk of developing the disorder (Hartz *et al.*, 1983; Bjorntorp, 1991; Chan *et al.*, 1994; Parillo & Riccardi, 2004; Raz *et al.*, 2004). Research conducted by Cassano *et al.* (1992) showed a positive correlation between blood glucose levels and abdominal fat accumulation, independent of total body adiposity. It is suggested that these centrally located adipocytes have greater metabolic activity, with elevated rates of lipolysis, increased sensitivity to hormonal stimulation and decreased sensitivity to insulin (Raz *et al.*, 2004). Parillo and Riccardi

(2004) implied that proximity of these adipocytes to the liver may result in escalated entry of FFA to the liver and, in turn, interfere with glucose oxidation and hepatic removal of insulin.

n-3 PUFA deficiency

It has been shown that both the amount and type of fatty acids ingested alter insulin sensitivity in glucose metabolizing tissues (Storlien *et al.,* 1991; Taouis *et al.*, 2002). Linoleic and ∝-linolenic acid are essential to mammals and are precursors for n-6 and n-3 PUFA, respectively (Allen & Harris, 2001; Hansen *et al.*, 2004). Docosahexaenoic acid (DHA, C22:6 n-3) and eicosapentaenoic acid (EPA, C20:5 n-3) may be formed by the elongation and desaturation of ∝-linolenic acid (Allen & Harris, 2001). However it was reported that the mink mammary gland has a low capacity for *de novo* fatty acid synthesis and lacks the capability for desaturation and chain elongation (Wamberg *et al.*, 1992b). The fatty acid composition of milk lipids is highly variable and depends on dietary lipid composition, maternal energy balance and the maternal dietary carbohydrate-lipid ratio (Del Prado *et al.*, 1999). PUFA make up a 14-32 % of the TG fraction of mink milk (Wamberg *et al.*, 1992b). As lactation progresses, there is increased demand for milk production placed on mink females (Børsting & Damgaard, 1995) and conceivably lowered n-3 PUFA status resulting from increased amounts of n-3 fatty acids being secreted in the milk (Rouvinen-Watt, 2003). EPA and DHA are found in high proportions in oily fish and fish oils (Belzung *et al.* 1993; Takahashi & Ide, 2000; Calder, 2002; Grimm *et al.*, 2002) and the increased dietary intake of these preformed n-3 PUFA may help the mink dam meet the elevated n-3 PUFA requirements during lactation.

Oxidative stress

It has been suggested that oxidative stress may be a contributing factor in the development of insulin resistance and diabetes (Bonnefont-Rousselot *et al.,* 2000; Lenhard & Gottschalk, 2002; Quilley, 2002; Piconi *et al.,* 2003). Under normal physiological conditions, there is a balance maintained between endogenous oxidants and antioxidants; reactive oxygen species (ROS), including superoxide anion radical (O_2·-) and hydrogen peroxide, are continuously formed within cells as a consequence of metabolic reactions (Piconi *et al.*, 2003; Wu *et al.*, 2004). When an imbalance occurs, such as with the chronic intake of high dietary protein or amino acids in excess of requirements, oxidation is increased in order to maintain homeostasis (Petzke *et al.*, 2000).

Oxidative stress results from the excessive production of ROS or from a reduced antioxidant reserve, which can cause oxidative damage to nucleic acids, cholesterol, lipids, carbohydrates, proteins and antioxidants (Bonnefont-Rousselot *et al.*, 2000; El Midaoui *et al.,* 2002; Wu *et al.*, 2004; Davies, 2005). Increases in the production of ROS or decreases in their rate of scavenging will increase the oxidative modification of these cellular molecules (Stadtman & Levine, 2000); as a result, deoxyribonucleic acid (DNA) is constantly being damaged or oxidatively modified (Wu *et al.*, 2004). Östling and Johanson (1984) described a method, referred to as the comet assay, for measuring DNA damage to individual cells, based on the technique of single cell gel electrophoresis and the identification of strand breaks in the supercoiled DNA structure. Oxidative free radicals have been found to activate nuclear factor kappa B (NFkB) cellular transcription

factor, which signals activation of the protein complex serine kinase IKβ kinase (IKKβ) (Calder, 2002; Dandona *et al.*, 2004); IKKβ plays a key role in tissue inflammation (Hundal *et al.,* 2002).

Elevated extra- and intracellular glucose concentrations have been shown to result in oxidative stress (Wolff *et al.*, 1991; Bonnefont-Rousselot *et al.*, 2000). Wu *et al.* (2004) reported that in diabetes, oxidative free radicals are excessively formed through glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Increased blood concentrations of thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation, have been observed in patients with diabetes (Cheng *et al.*, 2004). Similarly DNA damage, indicated by the comet assay, has been found to be significantly higher in diabetic patients when compared to a control (Anderson *et al.*, 1997b; Collins *et al.*, 1998; Sardas *et al.*, 2001). Persistent hyperglycemia may be an important factor, as Kayali *et al.* (2003) observed increases in plasma oxidative protein damage and lipid peroxidation in chronic but not in acute diabetic rats.

Increases in plasma FFA are associated with insulin resistance, obesity and T2DM (Frayn, 2001; Perseghin *et al.*, 2003). Fatty acids compete with glucose for substrate oxidation and increased fat oxidation may cause the insulin resistance associated with obesity (Perseghin *et al.*, 2003). Collins *et al.* (1998) reported a significant correlation between DNA strand breaks and body mass index in diabetic subjects. There is a positive correlation between adiposity and circulating levels of cytokines IL-6 and TNF-α, with

an emphasis on accumulation of abdominal fat (Browning, 2003). Elevated plasma levels of IL-6 and TNF-α are markers of inflammation (Dandona *et al.*, 2004; Das, 2004). Proteins have been identified as major targets for oxidants (Davies, 2005) and dietary protein content has a significant influence on protein oxidation rate, with elevated levels resulting in increased oxidative stress and reduced antioxidant status (Griffiths, 2002). Research indicates that dietary protein restriction may inhibit these effects and in some cases, reduce oxidative damage to proteins while under oxidative stress (Youngman *et al.*, 1992). In a study by Petzke *et al.* (2000), long-term intake of high protein diets did not lead to increased oxidative stress in rats when diets were adequate in antioxidants. Fink *et al.* (2001a

) demonstrated a 20% increase in protein oxidation in lactating mink fed a high protein diet (58% of metabolizable energy [ME]) when compared to dams fed 39% of ME derived from protein. Rouvinen-Watt (2003) proposed that through the traditional feeding of diets high in protein during the breeding and lactation periods, mink dams may be subjected to increased levels of oxidative stress.

1.2.2.2 ***Monitoring glycemic control***

An important factor in achieving glycemic control in animals with acquired insulin resistance is the appropriate selection and interpretation of analytic monitoring tests (Bennett, 2002). The use of portable blood glucose meters (PBGM) has become common in veterinary medicine as a quick and relatively inexpensive means of monitoring animals' blood glucose concentration, using only a small amount of blood (Stein & Greco, 2002). The convenience, ease and accessibility of PBGM make their use a feasible

option in ranch-level monitoring of glycemic control. In a study by Hynes *et al.* (2004), PBGM were found to be an effective means for blood glucose analysis in mink dams, giving blood glucose values comparable to previously reported clinical values (Wamberg *et al.*, 1992a).

Plasma glucose

Glucose can be measured in whole blood, serum, or plasma, but plasma is recommended for diagnosis (Sacks *et al.*, 2002). Although PBGM use whole blood in analysis, most meters are programmed to report plasma glucose concentrations (Sacks *et al.*, 2002). It is suggested that postprandial hyperglycemia may be a better index of glycemic control than fasting basal glucose (Palumbo, 2001; Fonseca, 2003) and Bennett (2002) reported that fasting may act to further interfere with regulation. Wess and Reusch (2000) concluded that PBGM provided sufficiently accurate measurement of blood glucose in dogs to warrant their use in clinical practice. However, Hoenig and Ferguson (1999) report a single glucose measurement or glucose measurements from one day may not adequately reflect the status of the animal during the preceding weeks.

Glycated hemoglobin

While high plasma glucose concentrations have been linked to symptoms of diabetes, more information is available that directly correlates increased HbA_{1c} levels with other variables used to assess glycemic control, e.g. clinical signs, changes in body weight, serum glucose concentrations (Elliott *et al.*, 1999). HbA_{1c} is produced from an irreversible, non-enzymatic insulin-dependent binding of glucose to hemoglobin in red

blood cells and is directly related to blood glucose concentration and erythrocyte lifespan (Elliott *et al.,* 1999; Loste & Marca, 2001; Behrend, 2002). A change of 1.9 mmol l^{-1} in mean plasma glucose per increase of 1 % HbA_{1c} was determined in human subjects, with 4 % HbA_{1c} indicating an estimated mean plasma glucose of 3.6 mmol l^{-1} (Rohlfing *et al.,* 2002).

Used for the assessment of long-term glycemic control, Ononogbu and Echeta (1987) found that HbA_{1c} measurement gave an estimation of mean blood glucose over the preceding 2 or 3 months, and ranged from 5 to 8 % in rats. Normal circulating levels of HbA_{1c} are reported to fall between 2.4-3.4 % in healthy dogs and between 2.0-2.9 % in healthy cats (Haberer & Reusch, 1998; Bennett, 2002). Measurement of HbA_{1c} has been found to be an effective means of assessing glycemic control in canine (Davison *et al.,* 2002) and feline (Elliott *et al.,* 1997) diabetic patients and a median level of 3.8 % was determined for cats with newly diagnosed diabetes mellitus (Haberer & Reusch, 1998). Research by Little *et al.* (1988) indicated that while elevated HbA_{1c} concentrations usually indicated diabetes mellitus or impaired glucose tolerance, a normal HbA_{1c} did not exclude its diagnosis. In a study by Marca *et al.* (2000), acute and transient hyperglycemia was not found to significantly affect glycated hemoglobin concentrations in healthy dogs; similar findings were reported in cats (Elliott *et al.*, 1997).

1.2.3 EXPERIMENTAL TREATMENTS FOR THE PREVENTION AND REVERSAL OF HYPERGLYCEMIA

Various pharmacological approaches can be used to improve glucose tolerance and insulin sensitivity via different mechanisms, i.e. by stimulating insulin secretion, promoting glucose utilization and reducing hepatic-glucose production, and enhancing cellular insulin action on glucose and lipid metabolism (Scheen & Lefebvre, 1998; McCarty, 2005). In this regard, the use of various anti-diabetic agents may be effective in the prevention and treatment of hyperglycemia in the mink female during lactation.

1.2.3.1 ***Fish oil***

Dietary fatty acids have been found to strongly influence the fatty acid composition of various adipose tissue depots in the mink (Rouvinen & Kiiskinen, 1989; Rouvinen *et al.*, 1992). Adipocyte plasma membrane phospholipids typically contain high proportions of the n-6 PUFA arachidonic acid (AA, C20:4) and low proportions of n-3 PUFA (Tebbey *et al.*, 1994; Calder, 2002). In cases of obesity and diabetes, serum levels of AA may be significantly elevated in comparison to controls (Tebbey *et al.,* 1994). Through feeding fish oils, n-6 PUFA contained within cell phospholipids may be replaced by n-3 PUFA, as these fatty acids compete directly for incorporation into cells, from where they are able to influence cell function (Carbonell *et al.*, 1997; Sen *et al.*, 1997; Calder, 2002; Grimm *et al.*, 2002; Browning, 2003). In addition to having roles in lipid biosynthesis and energy production, fatty acids are physiological regulators of the adipocyte glucose transport system (Long & Pekala, 1996). Research has shown that n-6 PUFA (e.g. AA and linoleic acid) are strong negative modulators of glucose uptake (Tebbey *et al.*, 1994).

Furthermore, AA is the precursor of 2-series prostaglandins and 4-series leukotrienes, which are highly active mediators of inflammation (Calder, 2002).

The fatty acid composition of the diet strongly influences glucose transport into insulin responsive adipocyte cells and muscle tissues (Ezaki *et al.,* 1992; Riccardi *et al.*, 2004). When substituted for other types of lipids in the diet, fish oils high in n-3 PUFA have been shown to have beneficial effects on insulin-stimulated glucose transport and metabolism in peripheral tissues (Ezaki *et al.*, 1992; Long & Pekala, 1996; Luo *et al.*, 1996; Jen *et al.,* 2003). In a study by Ezaki *et al.* (1992), cells from high-fish oil-fed rats showed increases in cellular GLUT-4 and GLUT-1 and improved insulin-stimulated glucose activity at 1.7 times that of high-safflower oil-fed rats after 1-week of feeding. Delarue and colleagues (2004) reported that in rodents *in vivo*, n-3 PUFA protected against high fat induced insulin resistance by preventing the depletion of GLUT-4 in muscle and the decreased expression of GLUT-4 in adipose tissue. Luo *et al.* (1996) noted a significant improvement in glucose incorporation into lipids, and glucose transport and oxidation, after feeding a diet containing 30% fat from fish oil over a 6-week period. Jucker *et al.* (1999) concluded that fish oil feeding spares the muscle from becoming insulin resistant in part by lowering intramuscular TG availability for oxidation and increasing pyruvate dehydrogenase versus TCA flux. It has also been suggested that n-3 PUFA act to down regulate the activity of NFkB (Calder, 2002).

Different types of fatty acids have varying effects on adiposity; in most cases, saturated fatty acids and n-6 PUFA have induced more body fat and weight gain when compared to

dietary n-3 PUFA (Hill *et al.,* 1993; Luo *et al.,* 1996; Jen *et al.*, 2003). Hill *et al.* (1993) reported that rats fed a diet containing fish oil at 45% of total calories had less total body fat, less intra-abdominal fat and less insulin resistance when compared with rats fed lard, corn oil or medium chain TG. In humans, dietary fish oil has resulted in reduced body fat mass, lower basal respiratory quotient and a 20% increase in basal lipid oxidation (Couet *et al.,* 1997). In a study by Fickova *et al.* (1998), rats fed n-3 PUFA for a 1-week period had significantly lower mean body mass increment, less epidydimal adipose tissue and smaller adipocytes than the n-6 diet group. Similar results were found in rats with dietary-induced insulin resistance and hyperglycemia; data showed smaller epidydimal fat pads and limited lipid accumulation in retroperitoneal tissue, without change in body weight, after feeding a diet containing fish oil (Luo *et al.,* 1996). Peyron-Caso and colleagues (2003) reported a significant increase in lipolysis stimulation and lipoprotein lipase activity in the adipose tissue of fish oil-fed rats, a mechanism found to reduce circulating TG levels.

N-3 PUFA have been identified as potentially potent agents in the anti-inflammatory processes (Calder, 2002; Grimm *et al.*, 2002; Mori & Beilin, 2004). By directing fatty acids away from storage and towards oxidation, n-3 fatty acids may improve energy supply to functionally important cells, including immune cells (Grimm *et al.*, 2002). Studies have shown significant increases in fat oxidation after feeding a diet containing n-3 PUFA (Hill *et al.*, 1993; Couet *et al.*, 1997). The highly unsaturated nature of n-3 PUFA makes them vulnerable to the damaging effects of oxygen. High doses of fish oil have increased the susceptibility of cell membranes to the induction of oxidative stress,

expressed as lipid peroxidation (Garrido *et al.,* 1989) and a continuing subject of interest is the relationship between fish oil intake and oxidative stress (Ibrahim *et al.*, 1997; Miret *et al.,* 2003). Many studies suggest that lipid peroxidation mediates DNA damage under oxidative stress. Sen and colleagues (1997) found that fish oil supplementation induced lipid peroxidation but did not cause protein oxidative damage. Research by Carbonell *et al.* (1997) showed reduced O_2·- production, which consequently improved the antioxidant status of the inflammatory leukocytes of rats fed menhaden oil. Research by Kikugawa *et al.* (2003) showed that dietary fish oil supplementation resulted in lower levels of oxidative stress-induced DNA damage than supplementation with safflower oil in rats exposed to oxidative stress. In addition, results indicated that elevated lipid peroxidation in rat liver *in vivo* protected against oxidative-stress induced DNA damage (Kikugawa *et al.,* 2003). Fish oil supplementation has been found to decrease the release of proinflammatory cytokines and inhibit AA metabolism, factors which may directly or indirectly decrease the formation of ROS (Sen *et al.*, 1997).

1.2.3.2 ***Chromium picolinate***

Chromium (Cr) is an essential dietary trace mineral (Morris *et al.*, 1999) involved in carbohydrate and lipid metabolism (Schachter *et al.,* 2001; Kim *et al.,* 2002; Vincent, 2004). The trivalent form of Cr, chromium picolinate (CrPic), is its most stable (Ghosh *et al.* 2002) and most efficiently absorbed form (Anderson *et al.,* 1997b, Speetjens *et al.*, 1999; Schachter *et al.,* 2001). Anderson *et al.* (1997a) demonstrated a lack of toxicity of CrPic in rats at levels up to 100 mg/kg of diet. Conditions that increase circulating insulin and glucose concentrations, including pregnancy (Jovanovic *et al.*, 1999), obesity and

T2DM, increase urinary Cr output (Anderson *et al.*, 1997b; Morris *et al.*, 1999; Vincent, 2004). Cr deficiency is reported to be a contributing factor in the progression of diabetes (Vincent, 1999; Cheng *et al.*, 2004).

Several studies have shown supplemental Cr, as CrPic, to improve blood glucose, insulin, cholesterol and HbA_{1c} in people with T2DM (Anderson, 1998). In women experiencing gestational diabetes mellitus, supplementation with CrPic improved postprandial glucose levels and reduced hyperinsulinemia (Jovanovic *et al.*, 1999). Cefalu *et al.* (2002) demonstrated that CrPic significantly enhanced membrane associated GLUT-4 in obese rats after insulin stimulation, as well as enhanced insulin sensitivity and glucose disappearance. Significantly lower blood glucose concentrations have also been reported in dexamethasone treated rats treated with CrPic as compared to a control group (Kim *et al.,* 2002). The mechanism of Cr action on the control of blood glucose concentrations is increased insulin binding to cells due to increased insulin receptor number and an increase in insulin phosphorylation (Anderson *et al.*, 1997b; Anderson, 1998; Kim *et al.,* 2002). Most scientific evidence indicates that Cr supplementation has no significant effect in healthy individuals with good glucose tolerance (Cefalu *et al.,* 2002; Kim *et al.*, 2002; Vincent, 2003).

With numerous studies reporting conflicting results, no definitive conclusions on the effects of supplemental Cr on body composition can be drawn. Findings by Trent and Thieding-Cancel (1995) indicated no significant effect of CrPic on reduction in either percent body fat or body weight or increase in lean body mass in obese individuals on an

exercise program. In contrast, supplementation with CrPic was found to increase lean body mass in obese patients (Bahadori *et al.*, 1997). In agreement with Anderson (1997b), Pittler *et al.* (2003) concluded the probable effect of CrPic on body composition to be a small effect, achieved with long-term supplementation.

Research conducted by Cheng *et al.* (2004) demonstrated significant effects of Cr after 6 months of supplementation on plasma TBARS in people with T2DM. While long-term chromium supplementation resulted in significant decreases of plasma TBARS in mildly- and severely-hyperglycemic patients, conversely there was a significant increase observed in normoglycemic subjects (Cheng *et al.*, 2004). Recent *in vivo* rat studies propose that CrPic generates oxidative damage of DNA and lipids and is mutagenic (Vincent, 2003). Due to its extreme stability, CrPic has the potential to be incorporated into cells intact (Speetjens *et al.*, 1999; Hepburn *et al.*, 2003). In this form, CrPic may result in the catalytic formation of ROS (Hepburn *et al.*, 2003). Reduction species of CrPic, e.g. ascorbate and thiols, are susceptible to oxidation, resulting in the generation of the potent DNA-damaging agent hydroxyl radical (Speetjens *et al.*, 1999).

1.2.3.3 ***Acetyl-salicylic acid***

Dietary acetyl-salicylic acid (ASA or aspirin) is reported to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia in severely insulin resistant obese rodents (Yuan *et al.*, 2001). Glycation of hemoglobin is also inhibited by ASA (Rendell *et al.*, 1986). Hundal and colleagues (2002) reported significant decreases in hepatic glucose production, fasting plasma glucose, fatty acids and TG and an increase in peripheral

glucose disposal of type 2 diabetic subjects after ASA treatment. While various studies have identified ASA as an effective agent for improved insulin sensitivity in insulin resistant patients, in over-dosage it has been found to trigger transient hyperglycemia in healthy subjects (Ferner, 1992). In early studies, ASA treatment was shown to produce a significant increase in plasma insulin while glucose clearance remained unchanged, indicating impaired glucose metabolism in insulin-sensitive tissues by high-dose ASA treatment (Giugliano *et al.*, 1982; Newman & Brodows, 1983; Bratusch-Marrain *et al.*, 1985).

There are several indications that ASA has antioxidant properties (Sobal *et al.*, 2000; El Midaoui *et al.*, 2002). El Midaoui *et al.* (2002) found that dietary aspirin prevented development of hypertension, hyperglycemia, rise in $O_2^{\cdot-}$ formation and relieved insulin resistance increase in rats fed high glucose. Many studies suggest that high doses of salicylates inhibit IKKβ activity, and consequently, prevent the activation of NFkB genes involved in insulin signaling and the pathogenesis of the inflammatory response (Yin *et al.,* 1998; Yuan *et al.*, 2001; Hundal *et al.*, 2002; Gao *et al.*, 2003). In addition, increases in the susceptibility of low-density lipoprotein (LDL) to oxidation have also been associated with severe hyperglycemia (Bonnefont-Rousselot *et al.*, 2000). Sobal *et al.* (2000) found that ASA, through its transformation into a more potent hydroxyl radical scavenger called salicylic acid, specifically limited the consequences of LDL oxidation.

1.3 OBJECTIVES

The overall objectives of this research were to monitor blood glucose levels in the mink female, and to evaluate the role of body condition, body weight, litter size and litter weight in glycemic regulation during the reproductive cycle. In order to prevent or reverse the occurrence of hyperglycemia at late lactation, the efficacy of dietary anti-diabetic supplements was examined.

Year 1

- To examine the effects of supplemental herring oil and chromium picolinate (administered at the onset of lactation) on blood glucose levels in mink dams at late lactation.

Year 2

- To assess the effects of short-term supplementation with dietary herring oil, chromium picolinate and/or acetyl-salicylic acid (administered at mid-lactation) on blood glucose levels and oxidative stress in mink dams at late lactation.

CHAPTER 2. Monitoring blood glucose levels in female mink during the reproductive cycle: Prevention of hyperglycemia during the nursing period

2.1 INTRODUCTION

Nursing sickness is a disorder that develops from a complex of metabolic, nutritional and environmental factors, which influence the ability of the mink dam to meet the extreme demands of lactation (Clausen *et al.*, 1992). Børsting and Gade (2000) suggested that the etiology of nursing sickness is linked to a disruption in glucose homeostasis. Rouvinen-Watt (2003) proposed that the pathogenesis of the disease exhibits striking similarity to acquired insulin resistance. Glucose is a primary nutrient for conceptus growth and milk synthesis and its provision is a metabolic priority for the pregnant or lactating mammal (Bell & Bauman, 1997). Due to low dietary carbohydrate intake, nursing mink females rely heavily on gluconeogenesis from dietary amino acids to meet glucose demands (Børsting & Gade, 2000).

In most mammals, there is an adaptive reduction of insulin sensitivity in glucose metabolizing tissues during late gestation and lactation, accompanied by a compensatory increase in pancreatic β-cell mass and insulin secretion (Bell & Bauman, 1997; Di Cianni *et al.*, 2003; Rand *et al.*, 2004). Predisposing genetic and environmental factors may result in an exaggerated response and, consequently, the development of hyperglycemia and/or acquired insulin resistance (Di Cianni *et al.*, 2003). Abnormally high levels of plasma glucose were observed in the blood of dams affected by nursing sickness (Wamberg *et al.*, 1992a). Rouvinen-Watt (2003) proposed that acquired insulin resistance in mink females may develop in response to (1) obesity or lipodystrophy (deficiency of

adipose tissue), (2) n-3 polyunsaturated fatty acid (PUFA) deficiency and/or (3) oxidative stress.

Variable glucose levels have been observed in mink females (Wamberg *et al.*, 1992a) but little research has been performed to explore possible connections between blood glucose levels and nursing sickness. We investigated this premise by examining glycemic control in the mink female throughout the reproductive cycle. The purpose of this research was to examine the impacts of body condition, dietary anti-diabetic supplements and reproductive status on glycemic regulation during the reproductive cycle of female mink on commercial farms.

2.2 MATERIALS AND METHODS

2.2.1 ANIMALS

Standard Black yearling mink females (N=107) were selected from three collaborating farms in Nova Scotia. Each farm supplied a minimum of thirty (30) females. The animals were housed in individual cages with free access to water. The experiment was performed from March to July 2003. Experimental procedures and husbandry conditions were approved by the Animal Care and Use Committee of the Nova Scotia Agricultural College and carried out in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

2.2.2 SAMPLE COLLECTION AND ANALYSIS

Blood sampling took place at breeding, mid-gestation and late lactation (at the end of the six-week experimental feeding period). Approximately 90 minutes post-prandially, blood samples were taken from the toenail for glucose analysis with the Accu-Chek™ Compact blood glucose monitor (Roche Diagnostics, Laval, Quebec), according to Hynes *et al.* (2004). At the late lactation sample collection, an additional blood droplet was taken for glycated hemoglobin (HbA_{1c}) measurement with the A_{1c}Now™ Hemoglobin monitor (SelfCare Diagnostics Inc., N. Vancouver, B.C.).

At the time of sample collection, the mink females were weighed (to the nearest 0.1 g) and body condition score (BCS) was determined according to a five-point scale (Rouvinen-Watt & Armstrong, unpublished). The body condition scoring system involved palpating the shoulders, rib cage and rump area. At lactation, whelping date and kit number, as well as the total litter weight, for each nursing female was recorded.

2.2.3 FEEDING TRIAL

One of three dietary treatments was administered daily for a six-week period, beginning near the onset of lactation (May 6-8);

1. the control group (CTRL), fed a non-supplemented wet ration which also served as the basal diet (Figure 2.1),
2. addition of dietary herring oil (HerO) at 1-3% inclusion level per day, or
3. supplementation with chromium picolinate (CrPic) at 200 μg/day.

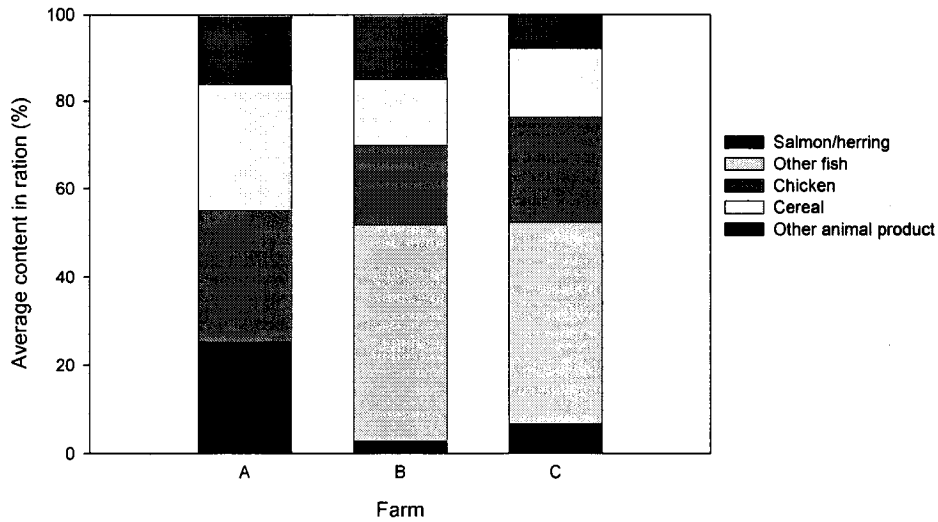

Figure 2.1 Average composition of basal diet fed to mink breeder females throughout the production year for collaborating farms.

The overall composition of the basal diet fed on each farm throughout the production year is presented in Figure 2.1. As shown, farm A fed higher amounts of herring meal and salmon racks (24.7 %) compared to farms B and C (2.9 % and 6.8 %, respectively), whereas farms B (48.9 %) and C (45.5 %) fed high levels of cod. Similarly, differences were also observed in the amount of cereal fed, with farm A feeding higher amounts (28.0 %) than farms B (15.0 %) and C (15.9 %).

2.2.4 STATISTICAL ANALYSIS

The experiment was designed as a randomized block design with a minimum of ten (10) animals allocated for each treatment per farm. For practical reasons, i.e. ease of ranch-level administration, treatments were not completely randomized within the individual animals, but rather in groups of five experimental units. Potential sources of variation between farms, such as air temperature, distance from human disturbance, and cage and nest box size, were identified and measured; no differences were observed in variant data

between farms. Statistical analyses were performed using SAS ® v.8 (SAS Institute Inc., Cary, NC, USA); procedure MIXED was performed using a model with farm (block) and treatment as fixed variables. No significant interaction effects were observed between farm and treatment, therefore the term was removed from the model. Blood glucose, HbA_{1c}, body weight and litter weight at lactation sampling were examined as response variables. Blood glucose levels and dam weights at breeding and gestation, along with kit age and litter weights were analyzed as covariates. Covariates not influencing the response ($P>0.15$) were excluded from the model. Procedure CORR was used to calculate correlations between litter size and litter weight, as well as BCS and body weight. Results are reported as least squares means ± SEM. Categorical data were evaluated using the Fisher's exact test option of procedure FREQ. Statistical significance was set at $P<0.05$.

2.3 RESULTS

2.3.1 FARM LEVEL DIFFERENCES

Table 2.1 Blood glucose levels at breeding and gestation for mink dams on three collaborating farms (least squares means ± SEM)

	Farm			
Variable	A	B	C	P^1
Breeding (n)	40	33	34	
Blood glucose, mmol l^{-1}	5.2±0.1^a	6.5±0.3^b	6.9±0.3^b	<0.001
Gestation (n)	40	30	32	
Blood glucose, mmol l^{-1}	5.3±0.2^a	7.4±0.3^b	6.8±0.3^b	<0.001
Δ Blood glucose[2], mmol l^{-1}	-0.1±0.3^a	1.2±0.3^b	0.2±0.3^a	0.001

[1] Difference between farms.
[2] Change in variable between sampling periods.
[a,b] Values that share common superscripts are not significantly different.

Blood glucose

Significant differences were observed in blood glucose values between the three collaborating mink ranches at the different sampling periods. As shown in Table 2.1, blood glucose values for mink dams on farm A were lower than those observed on farms B and C at breeding and gestation sampling periods. Females from farms B and C were not shown to be significantly different. Dams from farm A showed relatively constant blood glucose values around 5 mmol l^{-1}, whereas farms B and C showed increased variation and a drop in levels after gestation with values ranging from 5.9 to 7.5 mmol l^{-1} throughout the reproductive cycle (Figure 2.2). The overall mean blood glucose value for females on farm A (5.1±0.2 mmol l^{-1}) was lower than those observed on farm B and C (6.7±0.2 mmol l^{-1} and 6.8±0.2 mmol l^{-1}, respectively).

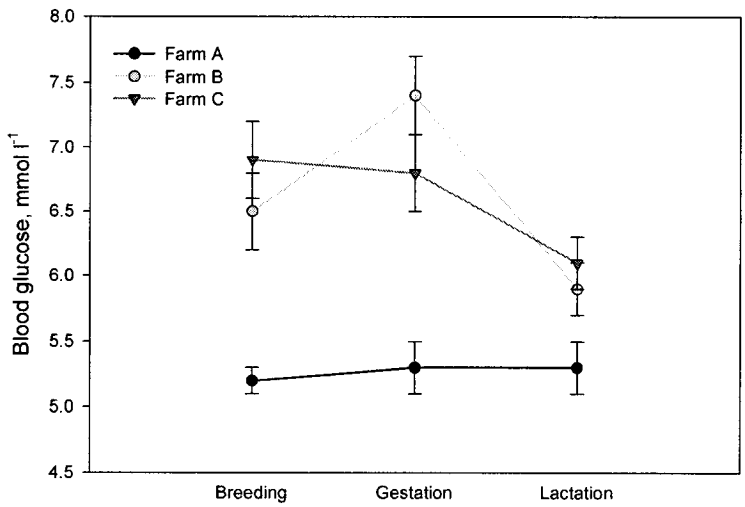

Figure 2.2 Average blood glucose values in mink females measured during the reproductive cycle on three farms.

Demonstrated in Table 2.1, females on farm B experienced a significantly larger change in blood glucose concentration from breeding to gestation (1.2±0.3 mmol l^{-1}) than those

on farm A and C (-0.1±0.3 mmol l^{-1}, P=0.002 and 0.2±0.3 mmol l^{-1}, P=0.001, respectively). Shown in Table 2.2, from gestation to lactation, females on farm A (-0.2±0.3 mmol l^{-1}) experienced significantly less change in mean blood glucose values than farm B (-1.3±0.3 mmol l^{-1}, P=0.008).

Table 2.2 Blood glucose, body weight, litter size and litter weight six weeks *post partum* and reproductive status for mink dams on three collaborating farms (least squares means ± SEM)

Response variable	Farm			P^1
	A (n = 40)	B (n = 29)	C (n = 29)	
Blood glucose, mmol l^{-1}	5.3±0.2^a	5.9±0.2^b	6.1±0.2^b	0.03
Δ Blood glucose[2], mmol l^{-1}	-0.2±0.3^a	-1.3±0.3^b	-0.7±0.3^{ab}	0.03
Dam weight, g	1086.5±18.5^a	990.8±23.1^b	997.0±23.4^b	0.001
Δ Body weight[2], g	-122.5±18.8^a	-230.8±24.0^b	-231.7±24.0^b	<0.001
Δ Body weight[2], %	-8.8±2.1^a	-17.0±2.3^b	-14.4±2.2^b	0.005
Litter size	4.5±0.4^a	4.3±0.4^a	5.5±0.4^b	0.04
Litter weight, g	2430.4±171.3^a	1754.4±201.2^b	2168.3±193.3^{ab}	0.07
Barren females	3	3	2	-
Dam mortalities	0	4	5	-

[1] Difference between farms.
[2] Change in variable over treatment period.
[a,b] Values that share common superscripts are not significantly different.

Body weight and Body Condition Score

Females on farms B and C lost significantly more weight throughout the treatment period than those on farm A (Table 2.2). Dams from farm A experienced a mean loss of 122.5 ± 18.8 g, from gestation to lactation, and females on farms B and C lost 230.8 ± 24.0 g (P<0.001) and 231.7 ± 24.0 g (P<0.001), respectively. Throughout the six-week

treatment period dams on farm A lost 8.8 ± 2.1 % of their body weight from parturition, whereas those on farm B lost 17.0 ± 2.3 % (P<0.001) and farm C lost 14.4 ± 2.2 % (P=0.002).

Table 2.3 Body condition scoring of mink dams on three collaborating farms at breeding, gestation and lactation sampling periods (% of females)

Sample Period	Farm	Body Condition Score					P^1
		1	2	3 (Ideal)	4	5	
Breeding	A	-	10.0	82.5	7.5	-	0.007
	B	-	-	100.0	-	-	
	C	-	-	100.0	-	-	
Gestation	A	-	10.0	77.5	12.5	-	0.61
	B	-	-	73.3	16.7	10.0	
	C	-	6.7	78.1	15.6	-	
Lactation	A	-	5.0	87.5	7.5	-	0.002
	B	-	20.7	51.7	24.1	3.4	
	C	10.3	17.2	69.0	3.4	-	

[1]Table probability.

As shown in Table 2.3, body condition was also found to vary among mink females on individual farms, with differences also observed between sampling periods. Throughout the trial, dams on farm A were categorized as 2 (thin) through 4 (heavy), with approximately 83 % of the females scored as ideal. At breeding, all females on farms B and C were classified as ideal in condition. At lactation, dams on farm B and C experienced more prominent losses and gains in body condition, with only 51.7 % and 69.0 % of females scored as ideal, respectively.

Reproductive parameters

Table 2.4 Reproductive status of mink dams at late lactation on three collaborating farms

Farm	Number of kits weaned (% of females)					P^1
	Dam death	None	1-3	4-6	7-9	
A	0.0	7.5	7.5	82.5	2.5	
B	12.1	9.1	12.2	63.6	3.0	<0.001
C	14.7	5.8	11.8	35.3	32.5	

[1]Table probability.

Shown in Table 2.2, Farm C had a significantly higher litter size (5.5±0.4) in comparison to farms A and B (4.5± 0.4, P=0.05 and 4.3± 0.4, P =0.02, respectively). However, litter weight on farm C was not significantly different than farm A, indicating that farm A weaned larger kits. While 32.5 % of females on farm C weaned litters of 7-9 kits, approximately 15 % mortality of dams was observed (Table 2.4). It should be noted that farm A did not lose any dams during the course of this study.

2.3.2 BLOOD GLUCOSE REGULATION

Glucose history

Blood glucose values observed at breeding were found to marginally affect those observed at gestation (P=0.06). However, the levels observed at breeding significantly affected those observed at lactation, with a modest positive correlation (r = 0.53, P<0.001) between the two. When looking at the ability of the mink dam to regulate blood glucose throughout the reproduction period it was found that changes observed in blood glucose from gestation to lactation were significantly influenced by the changes occurring between the first and second sampling period (r = -0.72, P<0.001). Subjects showing little initial change (close to zero) from breeding to gestation tended to remain

so later on, those showing large fluctuations generally continued the trend at the following sampling.

Body weight and Body Condition Score

Body weight did not have a significant effect on blood glucose levels at any of the time points. While no differences were observed in blood glucose values between body condition scores at breeding or lactation, interesting trends were observed between categories at the gestation sampling, females scored as ideal (6.3±0.2 mmol l^{-1}) had lower blood glucose values than those scored as thin (7.9±0.8 mmol l^{-1}, P=0.06) or heavy (7.2±0.3 mmol l^{-1}, P=0.02) (Figure 2.3). Although fewer subjects were scored as non-ideal in condition, it is interesting to note the increased variation in the blood glucose values of these females throughout reproduction in comparison to data collected from dams scored as ideal.

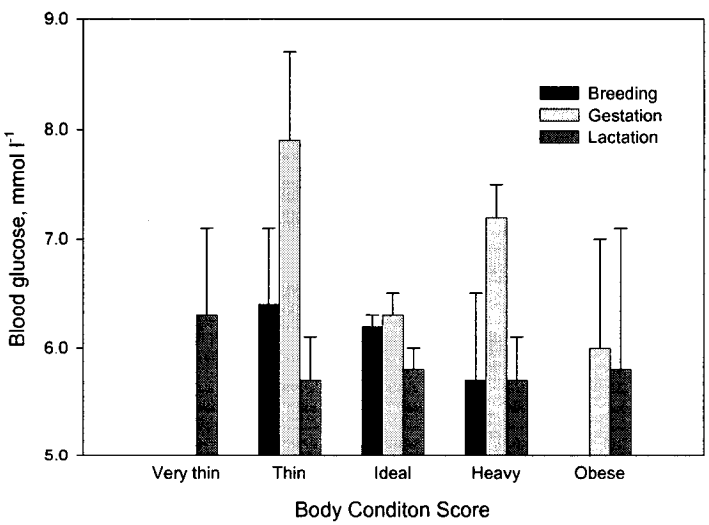

Figure 2.3 Mean blood glucose levels of mink females at breeding (B), gestation (G) and lactation (L) for body condition categories: very thin [B (n=0), G (n=0), L (n=3)], thin [B (n=4), G (n=6), L (n=13)], ideal [B (n=100), G (n=78), L (n=70)], heavy [B (n=3), G (n=15), L (n=11)], and obese [B (n=0), G (n=3), L (n=1)].

In the current study, females in thin body condition during gestation showed an increase in blood glucose levels (0.6±0.7 mmol l^{-1}) during lactation, whereas those in better condition showed decreases in these values (Figure 2.4). Females scored as obese during gestation experienced a larger decrease (-2.5±0.9 mmol l^{-1}) in blood glucose values over the treatment period than those scored as ideal (-0.6±0.1 mmol l^{-1}, P=0.04).

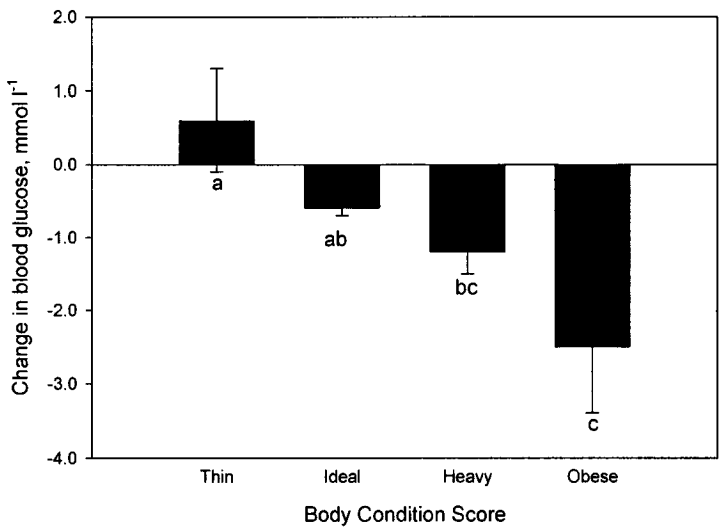

Figure 2.4 Change in blood glucose levels from gestation to lactation sample periods for mink females scored as: thin (n=6), ideal (n=78), heavy (n=15), and obese (n=3) at gestation. Scoring categories with the same letter are not significantly different (P<0.05).

Reproductive parameters

Litter weight and kit age did not have a significant effect on the blood glucose concentration of the dam at the lactation sampling. However, several females showing high blood glucose at one or more stages became sick, died, or did not whelp. As shown in Figure 2.5, dams that died during gestation or lactation had significantly higher blood glucose levels at breeding (8.3±0.5 mmol l^{-1}, P<0.05) than all females that successfully whelped and nursed litters. Females carrying one to three kits were found to have higher blood glucose levels at breeding than those pregnant with four to six kits (6.6±0.5 versus

5.6±0.2 mmol l^{-1}, P=0.05, respectively). Similarly, blood glucose concentrations at breeding were significantly higher in females with seven to nine kits (7.0±0.4 mmol l^{-1}, P=0.002) than those with four to six.

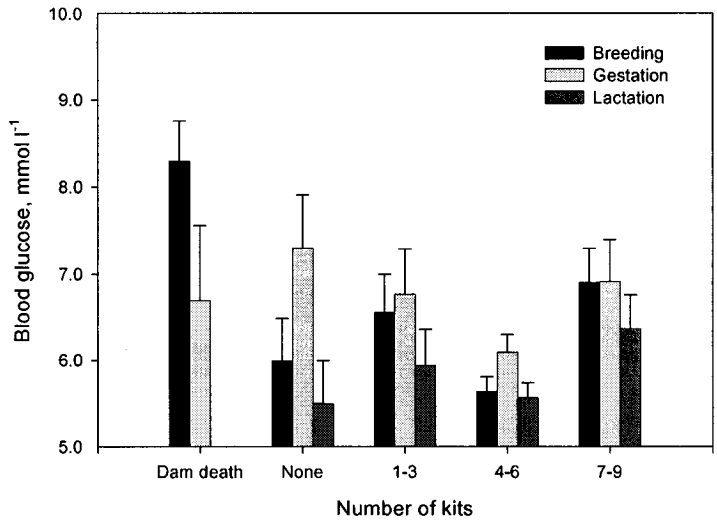

Figure 2.5 Mean blood glucose values measured in mink females at breeding (B), gestation (G) and lactation (L) indicated per number of kits in the litter at late lactation. Number of dams in each category: Dam death [B (n=9), G (n=4), L (n=0)], none [B, G, L (n=8)], 1-3 [B, G, L (n=11)], 4-6 [B, G, L (n=66)], and 7-9 [B, G, L (n=13)].

Barren females had marginally higher blood glucose concentration at the gestation-sampling period than females carrying four to six kits (7.3±0.6 versus 6.1±0.2 mmol l^{-1}, P=0.06, respectively). At lactation sampling, a marginal increase in glucose levels was observed in the blood of dams nursing larger litters (7-9 kits) when compared to those with four to six kits (6.4±0.4 versus 5.6±0.2 mmol l^{-1}, P=0.06, respectively).

Treatment effect

With numerous contributing factors leading to the development of hyperglycemia, easily applicable ranch-level treatments to improve glycemic control in the mink dams were

investigated. Presented in Table 2.5, during late lactation, females supplemented with CrPic showed significantly lower blood glucose levels than either the CRTL or HerO treatment groups. Correspondingly, both CTRL and HerO treatments were found to reduce blood glucose levels less over the treatment period than CrPic, with a decrease of 1.2±0.2 mmol l^{-1}. Effect of dietary herring oil on blood glucose levels at late lactation was not different from the control.

Table 2.5 Blood glucose, body weight and litter weight six weeks *post partum* for mink dams fed a control (CTRL) diet and those supplemented with herring oil (HerO) or chromium picolinate (CrPic) (least squares means ± SEM)

	Dietary treatment groups			
Response variable	CTRL	HerO	CrPic	P^1
Blood glucose, mmol l^{-1}	6.0±0.2^a	6.0±0.2^a	5.3±0.2^b	0.03
Δ Blood glucose[2], mmol l^{-1}	-0.3±0.2^a	-0.6±0.2^a	-1.2±0.2^b	0.01
Dam weight, g	1059.3±19.5	1005.3±20.6	1009.7±20.6	0.11
Δ Body weight[2], g	-162.2±20.2	-219.7±21.2	-203.2±21.3	0.14
Δ Body weight[2], %	-11.0±1.9	-15.7±2.1	-13.5±2.1	0.17
Litter weight, g	2180.5±157.9	2000.1±167.5	2172.4±167.4	0.68

[1] Difference between treatment groups.
[2] Change in response variable over treatment period.
[a,b] Values that share common superscripts are not significantly different.

No significant differences were observed among the effects of the experimental treatments on litter weight, final dam weight or the change in dam body weight incurred over the treatment period (Table 2.5).

2.3.3 HBA_{1C} (GLYCATED HEMOGLOBIN)

Readings with the A_{1c}Now™ Hemoglobin point-of-care analyzer, designed for human diabetics, were obtained in all cases except for 20 of the mink females. Fifteen of the 20 error messages read OR2 indicating an over-sampling of blood (Metrika, Inc., 2002). From the valid readings, 92.3 % of females were determined to have HbA_{1c} levels at less than 3.0 %. Blood glucose concentration, ranch level factors, treatment, body weight, BCS and reproductive parameters were not found to influence the observed HbA_{1c} values. However, a female measuring 3.3 % HbA_{1c} (the highest observed HbA_{1c} value in this study) had considerably higher blood glucose at gestation (12.7 mmol l^{-1}) than females with lower A_{1c} percentages.

2.4 DISCUSSION

2.4.1 FARM LEVEL DIFFERENCES

Our findings indicate that there was a significant farm effect on glycemic control, maintenance of body weight and condition, litter size and weight, and overall health in mink females during the reproductive cycle. The blood glucose concentrations determined for farm A were similar to previously reported values; in healthy lactating females, normal blood glucose has been reported as 5.3±0.3 mmol l^{-1} (Wamberg *et al.*, 1992a). The variation in the glucose values observed between farms demonstrates the importance of ranch-level factors in the regulation of blood sugar levels. These may include the combined effects of the genetics of the herd, animal management and feeding practices (Schneider & Hunter, 1992; Rouvinen-Watt, 2003). It is known that mink on farms B and C may be genetically similar due to frequent exchange of breeding stock.

With regard to feeding practices, significant differences were observed among farms in the dietary constituents fed throughout the production year. Farm A, which showed lower and more consistent blood glucose values throughout the reproductive period, fed significantly higher amounts of herring meal and salmon racks in comparison to farms B and C throughout the year. Fish oil is known to be high in long-chain n-3 PUFA (Belzung *et al.*, 1993; Takahashi & Ide, 2000); however some fish are better sources than others. Farms B and C fed high levels of cod, a low-oil fish that is a poorer source of n-3 PUFA in comparison to the higher levels of salmon and herring (Hearn, 1987). When substituted for other types of lipids in the diet, fish oils high in n-3 PUFA have been shown to have beneficial effects on insulin-stimulated glucose transport and metabolism in peripheral tissues (Ezaki *et al.*, 1992; Long & Pekala, 1996; Luo *et al.*, 1996). Facilitative glucose transporter 4 (GLUT-4) uptakes glucose in response to insulin (Kahn, 1994; Khayat *et al.*, 2002; Wood & Trayhurn, 2003) and increased cellular GLUT-4 content has been identified in the muscle and adipose tissue of high-fish oil-fed rodents (Ezaki *et al.,* 1992; Delarue *et al.*, 2004). The larger amounts of herring and salmon fed throughout the production year on farm A may have contributed to the lower and more stable blood glucose concentrations observed throughout the reproductive cycle.

Similarly, significant dietary differences were also observed in the amount of cereal fed, with farm A feeding significantly higher amounts than farms B and C. When fed significant amounts of carbohydrates, the mink has the ability to store excess glucose as glycogen (Børsting & Gade, 2000). Fink and Børsting (2002) suggested lower *de novo* synthesis of glucose in dams fed a high carbohydrate supply, possibly due to decreased

activity of the gluconeogenic enzymes. It was found that mink were able to utilize high levels of dietary digestible carbohydrates, without critically elevating plasma glucose concentrations (Fink *et al.*, 2002b). In the current study, significantly lower blood glucose levels were observed in the farm feeding increased levels of carbohydrates.

While females on farm A experienced less percent body weight loss during the lactation period than those on farms B and C, the percentage of weight lost by the latter was in agreement with previous findings; Hansen and Berg (1998) found that apparently healthy mink dams lost approximately 15% of their body weight during the first six weeks of lactation, with 10% lost over the last two weeks. Similar differences were shown in the females' abilities to maintain body condition. Previous studies found that readily available carbohydrates helped females maintain their body condition during pre-weaning periods (Pölönen *et al.*, 1993; Fink *et al.*, 2004). By feeding increased levels of dietary carbohydrate, females may be better able to meet the increasing energy demands of lactation (Børsting & Gade, 2000) and, in turn, may be less prone to the mobilization of body reserves that often leads to the development of nursing sickness (Clausen *et al.*, 1992; Wamberg *et al.*, 1992a). The higher levels of dietary carbohydrates fed on farm A may have contributed to the dams' ability to conserve body weight and condition during lactation.

Differences were also observed between farms in both litter size and litter weight at late lactation. While females on farm C nursed larger litters than farms A and B, it was determined that farm A weaned larger kits. Genetic background and nutritional

management are known to influence milk yield in many species. Dietary fat source influences milk and kit tissue fatty acid composition in the mink (Wamberg *et al.*, 1992b; Hansen *et al.*, 2004). It is suggested that variations in milk composition may influence the efficiency of nutrient utilization (Fiorotto *et al.*, 1991); however, the influence of maternal dietary n-3 PUFA supply on kit growth has not been clarified. Conversely, previous findings indicate that mink dam and kit health may be directly affected by level of dietary carbohydrate (Fink *et al.*, 2001b; Fink *et al.*, 2004); in a recent study by Fink *et al.* (2004), mink dams fed a high carbohydrate diet exhibited increased milk production, lower percent weight loss, lower total heat production, and lower protein oxidation than dams fed a diet low in carbohydrates. Feeding a diet high in carbohydrates may allow nursing females to redirect carbohydrates towards milk production by increasing glycogen synthesis and inhibiting gluconeogenesis (Børsting & Gade, 2000). The combination of larger amounts of high quality dietary n-3 PUFA and dietary carbohydrate fed on farm A may have helped the dams to better regulate blood glucose levels, conserve body weight and condition during lactation, and contributed to faster kit growth and better dam health. Further studies on a larger number of farms would clarify the relative importance of diet and genetics on the performance of mink females.

2.4.2 BLOOD GLUCOSE REGULATION

Glucose history

With regards to the influence of elevated blood glucose levels early in the nursing period on those observed as lactation progresses, modest but significant correlations were observed. Additionally, dams experiencing large fluctuations in blood glucose

concentration between breeding and gestation were subject to similar changes during the nursing period. Irregularities in glucose homeostasis were previously identified in lactating mink (Wamberg *et al.*, 1992a). Our observations suggest that the occurrence of hyperglycemia in mink females prior to whelping may have significant implications on glycemic control during lactation. Similarly, human subjects that experience gestational and postpartum insulin resistance are believed to have a degree of insulin resistance that precedes their pregnancy (Volk *et al.*, 1999; Eriksson *et al.*, 1999; Homko *et al.*, 2001; Weijers *et al.*, 2002). The implication is that certain females may be predisposed to disruptions in glucose homeostasis during lactation, likely as a consequence of both genetic and environmental factors. Inheritance of glucose intolerance (Holemans *et al.*, 1991; Okauchi *et al.*, 1995; Boloker *et al.*, 2002) has been identified as a risk factor for the development of insulin resistance in rats. Obesity is also a key determinant in the development of gestational insulin resistance (Holemans *et al.*, 2004); increased basal hepatic glucose production and decreased hepatic glucose uptake have been observed in obese rats prior to the development of hyperglycemia (Shiba *et al.*, 1998). We suggest that hyperglycemia is not a transient condition in female mink occurring solely from the demands of lactation and may be, in part, predicted by elevated blood glucose levels early in the reproductive cycle.

Body weight and body condition score

The current data provide support that body condition is linked to the ability of the mink dam to regulate blood glucose during reproduction, particularly during the gestation period. Few studies have demonstrated the relationship between body condition and

glycemic control in the mink female (Hynes *et al.*, 2004; Rouvinen-Watt *et al.*, 2004). However, obesity has been identified as a major risk factor in the development of insulin resistance in both humans (Roden *et al.*, 1996; Zimmet *et al.*, 1996; Forouhi *et al.*, 1999) and companion animals (Plotnick & Greco, 1995; Scarlett & Donoghue, 1998; Rand *et al.*, 2004). Lipodystrophy, a deficiency of adipose tissue, is also associated with this disorder (Frayn, 2001) with pronounced insulin resistance and reduced β-cell insulin secretion observed in lean women during gestation (Kautzky-Willer *et al.*, 1997). In agreement with previous findings (Hynes *et al.*, 2004), evidence of impaired glycemic control was observed in mink females scored as non-ideal in condition during the reproductive cycle. Frayn (2001) proposed that when adipose tissue is overwhelmed or when there is insufficient adipose tissue to absorb dietary fatty acids, other glucose metabolizing tissues (i.e. skeletal muscle, liver and pancreatic β–cell) are exposed to triacylglycerols; ultimately leading to insulin resistance. The normal adaptive reduction of insulin sensitivity occurring during pregnancy and lactation (Di Cianni *et al.*, 2003) may be exaggerated in mink females in non-ideal condition, with an emphasis on the gestation period, leading to a reduced ability to regulate blood glucose levels.

Reproductive parameters

Our results suggest that abnormalities in glucose homeostasis early in the breeding season may be an indicator for reproductive failure and dam illness. In humans, increases in maternal blood sugar are linked to an increased risk of fetal and neonatal morbidity (Farmer *et al.*, 1988; Vambergue *et al.*, 2000; Di Cianni *et al.*, 2003). Elevated blood glucose values were observed early in the reproductive cycle of both barren females and

those with smaller litters. While increased blood glucose levels were observed in dams experiencing decreased reproductive success, elevated levels were also detected in those with larger litters (7-9 kits) during breeding and lactation. Increasing litter size has been identified as a major risk factor for the development of nursing sickness (Clausen *et al.*, 1992; Schneider & Hunter, 1992).

In a study by Børsting and Damgaard (1995) an increased demand for milk production was found in females nursing eight kits, resulting in higher glucose production in comparison to those nursing only four. It is expected that there is a similar increase in glucose demand associated with carrying larger litters. During pregnancy and lactation, a condition of insulin resistance develops, a mechanism favouring glucose supply to the growing fetus and milk production (Di Cianni *et al.*, 2003). The increased demand for glucose associated with larger litters may aggravate underlying insulin resistance in mink females. It is apparent that abnormalities in glucose homeostasis occur throughout the reproductive cycle in mink females; the consequences of which may include reproductive failure and/or the development of dam illness and death.

In both human and animal studies, offspring of gestational diabetic mothers are at increased risk for obesity, glucose intolerance and the development insulin resistance (Boloker *et al.*, 2002; Ramsay *et al.*, 2002; Aerts and Van Assche, 2003; Di Cianni *et al.*, 2003). Boloker *et al.* (2002) found that the altered metabolism of diabetic pregnancy causes permanent defects in glucose homeostasis in the offspring that may lead to the development of diabetes later in life. It is plausible that mink kits born to dams

experiencing impaired gestational glucose regulation may be prone to the development of a similar condition (Boloker *et al.*, 2002). Genetic selection for mink females with large litter size may inadvertently result in the selection of females predisposed to poor glycemic regulation inherited from their mothers. Future studies should be designed to explore this relationship.

Treatment effect

Conditions that increase circulating insulin and glucose concentrations, including pregnancy (Jovanovic *et al.*, 1999) and obesity, increase urinary Chromium (Cr) output (Anderson *et al.*, 1997b; Morris *et al.*, 1999; Vincent, 2004) and Cr deficiency may be an aggravating factor in the progression of insulin resistance (Vincent, 1999; Cheng *et al.*, 2004). Improved glucose tolerance and decreased insulin resistance have previously been observed in rats (Cefalu *et al.,* 2002) and cats (Rand *et al.,* 2004) receiving chromium supplementation. In this regard, our data support these findings with reduced blood glucose levels observed in mink dams supplemented with CrPic in comparison to baseline values. Chromium acts to increase insulin binding to cells through increased insulin receptor number and an increase in insulin receptor phosphorylation (Anderson, 1998; Kim *et al.,* 2002). Within the current study, it is evident that CrPic supplementation effectively decreased blood glucose levels in mink dams during the lactation period when compared to the control and herring oil treatment groups.

Supplementation with dietary herring oil, at the level given in this study, did not affect blood glucose levels in the mink dams during late lactation. This was an unexpected

result as previous studies show dietary n-3 PUFA to improve glycemic regulation (Ezaki *et al.*, 1992; Long & Pekala, 1996; Luo *et al.*, 1996; Jen *et al.*, 2003) and, in the current study, lower and more consistent blood glucose values were shown in mink dams fed higher levels of fish known to be high in n-3 PUFA. Substitution of saturated fat with high quality n-3 PUFA within the diet may be required to significantly improve insulin sensitivity in the mink dam during lactation. This practice may be most beneficial during the fall when body fat reserves are accumulated in preparation for the winter (Korhonen *et al.*, 1989), as mink body fat composition has been shown to be highly responsive to dietary fatty acid profiles (Rouvinen & Kiiskinen, 1989).

2.4.3 HbA_{1c} (GLYCATED HEMOGLOBIN)

HbA_{1c} is produced from an irreversible, non-enzymatic insulin-dependent binding of glucose to hemoglobin in red blood cells and is directly related to blood glucose concentration and erythrocyte lifespan (Elliott *et al.*, 1999; Loste & Marca, 2001; Behrend, 2002). To the best of our knowledge, no research evaluating HbA_{1c} levels in mink has previously been conducted. Measurement of HbA_{1c} has been found to be an effective means of assessing glycemic control in canine (Davison *et al.*, 2002) and feline (Elliott *et al.*, 1997) diabetic patients. Haberer and Reusch (1998) determined that the reference range for HbA_{1c} falls between 2.4-3.4 % in healthy dogs and 2.0-2.9 % in healthy cats. While similar ranges were observed in the current study, for future research we recommend an alternative means for determining HbA_{1c}, as the $A_{1c}Now^{TM}$ Hemoglobin analyzer does not quantify HbA_{1c} levels at less than 3.0%. Due to the large percentage of females within this HbA_{1c} category, conclusive results cannot be drawn.

2.5 CONCLUSIONS

Our findings indicate that in mink breeder females the inability to maintain glucose homeostasis is not a problem occurring solely from the demands of lactation. Significant differences were observed in blood glucose levels between farms, emphasizing the importance of ranch-level factors, i.e. herd genetics, animal and feed management practices, in glycemic regulation. Poor glucose regulation occurs throughout the reproductive cycle and may predispose females to decreased reproductive success and poor dam health. Overall, females demonstrating large changes in glucose levels from breeding to gestation experienced increased glycemic variability while nursing.

Another important factor may be proper conditioning of the females to avoid thin and obese body condition during breeding and gestation. It was observed that females in thin condition at gestation showed an overall increase in blood glucose during the nursing period, whereas those in optimal condition showed decreases in these values. Similarly females classified as obese at gestation experienced a larger drop in blood glucose values during lactation than those scored as ideal. The increased variation observed in blood glucose levels of females in non-ideal condition throughout reproduction indicates an impaired ability to regulate glucose homeostasis.

Blood glucose levels at lactation may be influenced by supplementation with chromium picolinate at the onset of nursing. However, as evidence of poor glucose regulation was shown in the females prior to lactation it appears that preventative measures need to be taken throughout the year. While it is not possible to separate the genetic and

environmental effects on the mink dams' ability to maintain glucose homeostasis and body condition during the nursing period, results from this study suggest that the combination of high quality n-3 PUFA and high levels of dietary carbohydrate fed throughout the production year may be important and warrants further investigation.

CHAPTER 3. Effects of short-term fish oil, chromium picolinate and acetyl-salicylic acid supplementation on blood glucose levels and oxidative stress in female mink at late lactation.

3.1 INTRODUCTION

The nursing period is a time of critical importance during the reproductive cycle of the mink dam. As lactation progresses, the ability of the mink female to meet increasing energy demands is influenced by various metabolic, nutritional and environmental factors (Clausen *et al.*, 1992; Rouvinen-Watt, 2003). Body reserves are frequently mobilized in order to cover production requirements, with excessive mobilization often leading to the development of nursing sickness (Clausen *et al.*, 1992; Wamberg *et al.*, 1992a). Characterized by progressive weight loss, lethargy, loss of appetite, extreme dehydration (Schneider & Hunter, 1992) and high blood glucose and insulin levels (Wamberg *et al.*, 1992a), it has been proposed that the underlying cause of nursing sickness may be acquired insulin resistance (Rouvinen-Watt, 2003).

Within normal pregnancy a condition of insulin resistance develops, a process favoring glucose supply to the developing fetus and milk production, with glucose homeostasis restored at the cessation of lactation (Di Cianni *et al.*, 2003). However, it is suggested that obesity or lipodystrophy (deficiency of body fat), n-3 polyunsaturated fatty acid (PUFA) deficiency and/or oxidative stress may aggravate this metabolic response, resulting in the development of hyperglycemia and possibly acquired insulin resistance in the mink dam (Rouvinen-Watt, 2003). Closer examination of glycemic control in the mink female during lactation may provide further insight into the events leading up to the development of high blood glucose levels. The purpose of this research was to develop a

more complete understanding of the immediate causative components of hyperglycemia and to evaluate short-term administration of potential anti-diabetic treatments for the prevention or reversal of hyperglycemia during late lactation. Oxidative stress was also evaluated in relation to glycemic control and the studied dietary supplements.

3.2 MATERIALS AND METHODS

3.2.1 ANIMALS

Forty-eight (48) yearling female mink were randomly selected from the herd at the Canadian Centre for Fur Animal Research. The animals were housed in individual cages with water provided *ad libitum*. Experimental procedures and husbandry conditions were approved by the Animal Care and Use Committee of the Nova Scotia Agricultural College and carried out in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

3.2.2 SAMPLE COLLECTION AND ANALYSIS

Sampling took place on day 10, 21, 28, 35, and 42 *postpartum* (pp). Blood glucose levels, dam body weights and body conditions scores (BCS), litter size and litter weights were measured according to Chapter 2.2.2. At late lactation (day 42 pp) sampling, an additional capillary blood sample was taken for comet assay analysis, as described by Singh *et al.* (1988).

3.2.3 FEEDING TRIAL

The treatments were based on combinations of established anti-diabetic, antioxidative, and/or anti-inflammatory agents. On day 21 pp, one of eight experimental treatments was administered daily for a one week period;

1. the control group (CTRL), fed a non-supplemented wet ration which also served as the basal diet,
2. addition of dietary herring oil (HerO) at 1-3 % inclusion level per day,
3. supplementation of chromium picolinate (CrPic) at 200 μg/day.
4. HerO and CrPic (Her-Cr),
5. supplementation of acetyl-salicylic acid (ASA) at 100 mg/day,
6. HerO and ASA (Her-A),
7. CrPic and ASA (Cr-A), or
8. HerO, CrPic and ASA (Her-Cr-A).

3.2.4 STATISTICAL ANALYSIS

The experiment was a randomized complete block design with a 2^4 factorial (three replicates) arrangement of treatments with the main effects of day and treatment used to evaluate factors associated with blood glucose and oxidative stress. The mink dams were divided by glycemic status into normoglycemic (NG) (<5.5 mmol l^{-1}) and history of high blood glucose (HG) (≥ 5.5 mmol l^{-1}) (at day 10 or 21 pp) and randomly assigned to one of eight dietary treatments, with 3 NG and 3 HG females per treatment. Blood glucose data were analyzed using MIXED procedures in SAS ® v.8 (SAS Institute Inc., Cary, NC, USA) for repeated measures data. Blood glucose levels, dam weights, and litter

weights at day 10 and 21 and colour phase were analyzed as covariates. An inverse transformation was applied to blood glucose data in order to induce normality. Results are reported for non-transformed blood glucose data, using significance determined for transformed data. Comet Assay score was evaluated using procedure MIXED, with blood glucose levels, dam weights, and litter weights (day 10 through day 42 pp) analyzed as covariates. Covariates not found to influence the variables ($P>0.15$) were not included in the model. Results are reported as least squares means ± SEM. Statistical significance was set at $P<0.05$.

3.3 RESULTS

3.3.1 BLOOD GLUCOSE

A significant HerO x CrPic x ASA x glycemic status interaction effect was found for blood glucose. As shown in Table 3.1, within the control group, NG females were found to have significantly lower mean blood glucose concentration than those with a history of high blood glucose (3.8±0.8 versus 9.9±0.8 mmol l^{-1}, $P<0.001$) during weeks 4-6 of lactation. No other significant differences were observed in blood glucose values from day 28 to 42 pp for NG and HG females receiving the same treatment. Dam weights, litter weights and colour phase did not affect the blood glucose levels observed at late lactation.

Table 3.1 Mean blood glucose, averaged over day 28 to 42 of lactation, for mink dams, with both normoglycemic (NG) (<5.5 mmol l^{-1}) and hyperglycemic (HG) (≥5.5 mmol l^{-1}) history, fed experimental treatments (least squares means ± SEM)

	Treatment	NG females mmol^{-1}	HG females mmol^{-1}	P^1
1	CTRL	3.8±0.8	9.9±0.8	<0.001
2	HerO	4.6±0.7	4.6±1.0	0.734
3	CrPic	5.0±1.0	6.4±0.7	0.277
4	Her-Cr	5.0±0.8	5.4±0.8	0.679
5	ASA	7.9±0.8	6.5±0.8	0.713
6	Her-A	4.2±0.8	5.3±0.8	0.402
7	Cr-A	4.8±0.8	5.3±0.8	0.391
8	Her-Cr-A	5.7±0.8	4.8±0.8	0.224

[1] As determined for transformed data.

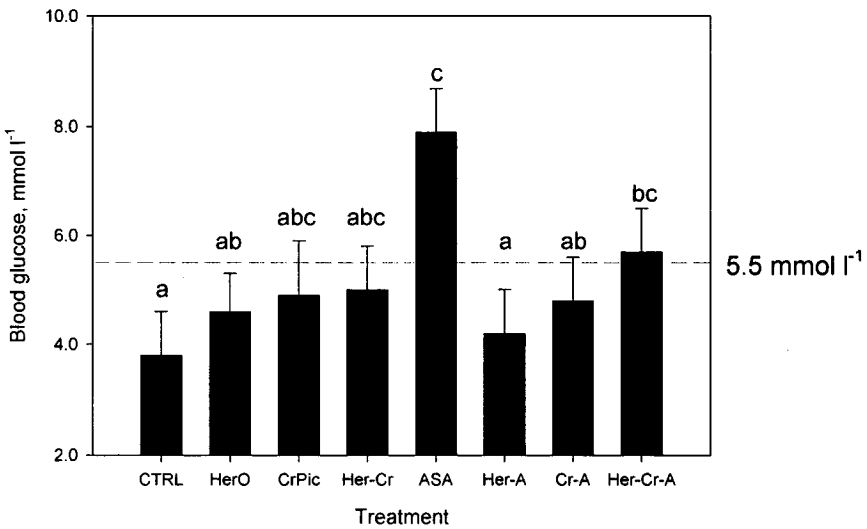

Figure 3.1 Mean blood glucose levels, averaged over day 28 to 42 of lactation, for normoglycemic mink females fed all combinations of herring oil, chromium picolinate and ASA for 1 wk at day 21. Means are least squares means ± SEM; treatments with the same letter are not significantly different as determined for transformed data ($P<0.05$).

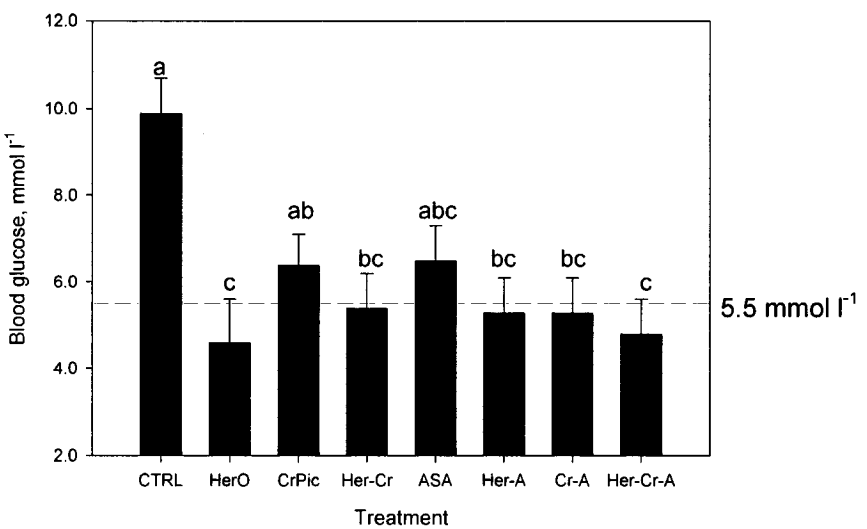

Figure 3.2 Mean blood glucose levels, averaged over day 28 to 42 of lactation, for hyperglycemic mink females fed all combinations of herring oil, chromium picolinate and ASA for 1 wk at day 21. Means are least squares means ± SEM; treatments with the same letter are not significantly different as determined for transformed data ($P<0.05$).

As shown in Figure 3.1, blood glucose levels were significantly increased from day 28 to 42 pp in NG females treated with ASA when compared to the control group (7.9±0.8 versus 3.8±0.8 mmol l^{-1}, P=0.001). While HerO and CrPic alone did not significantly alter blood glucose levels, in combination with ASA, treatment of NG females with Her-Cr-A increased blood glucose concentration (5.7±0.8 mmol l^{-1}, P=0.01) in comparison to the control. No statistically significant effects were observed among other treatments, when compared to the control, on blood glucose levels of NG dams during late lactation. In contrast to these findings, as demonstrated in Figure 3.2, HG females in the CTRL group (9.9±0.8 mmol l^{-1}) showed significantly higher blood glucose levels from day 28 to 42 pp than those fed HerO (4.6±1.0 mmol l^{-1}, P=0.01), Her-Cr (5.4±0.8 mmol l^{-1}, P=0.03), Her-A (5.3±0.8 mmol l^{-1}, P=0.03), Cr-A (5.3±0.8 mmol l^{-1}, P=0.003), and Her-Cr-A (4.8±0.8 mmol l^{-1}, P=0.003). In addition, mean blood glucose concentrations for females in these treatment groups were lowered to below the initial hyperglycemic blocking criteria of ≥5.5 mmol l^{-1}.

3.3.2 OXIDATIVE STRESS

Comet score, as a measure of oxidative damage, was not significantly affected by the glycemic status of the mink dam as assessed at day 10 or 21 pp. Shown in Figure 3.3, the levels of oxidative damage associated with each treatment did not significantly differ from the CTRL group. However, evidence of increased oxidative damage was observed in mink dams administered supplemental herring oil, with a comet assay score of 54±6, compared to females treated with Her-Cr (33±5, P=0.01), ASA (31±5, P=0.005), Cr-A (32±5, P=0.01) and Her-Cr-A (37±5, P=0.04). Females supplemented with CrPic (48±6)

had a higher comet assay score than those in the ASA (P=0.04) and Cr-A (P=0.05) treatment groups.

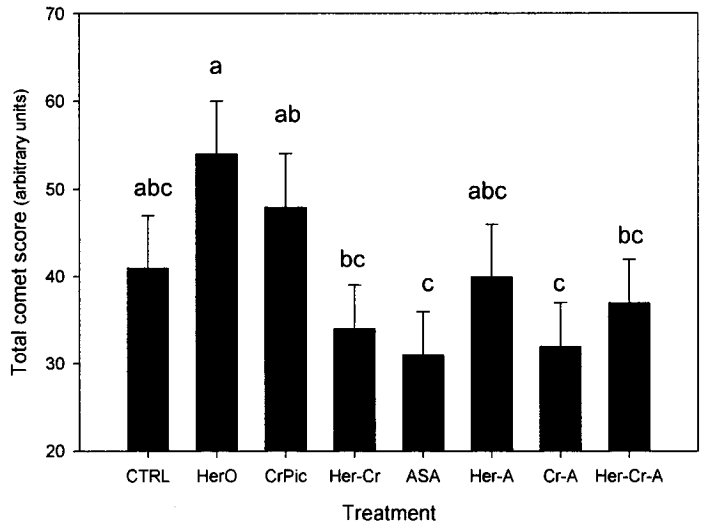

Figure 3.3 Mean comet assay score measured at day 42 of lactation in mink females fed all combinations of herring oil, chromium picolinate and ASA for 1 wk at day 21. Means are least squares means ± SEM; treatments with the same letter are not significantly different (P<0.05).

3.3.3 DAM MORTALITY

At day 42 pp, two HG females displayed typical signs of nursing sickness. Dam A had received the Cr-A treatment, whereas dam B was in the control group. Both dams exhibited sunken eyes and were emaciated and lethargic. Dam A was unresponsive and appeared to be further deteriorated than dam B. At autopsy, low body fat stores were found in both females and their stomachs were contracted and without content. Shown in Table 3.2, severe hyperglycemia was observed in Dam B with blood glucose concentrations of 17.1 mmol l^{-1} and 27.9 mmol l^{-1} measured at days 42 and 43, respectively.

Table 3.2 Blood glucose levels and diagnoses of two mink dams exhibiting typical signs of nursing sickness at day 42 of lactation

		Dam A	Dam B
Treatment group		Cr-A	CTRL
Blood glucose, mmol l^{-1}			
Day 42		5.8	17.1
Day 43		3.4	27.9
Day 44	a.m.	3.4	6.1
	p.m.	4.6	2.0
Diagnosis		Valvular Endocarditis	No diagnosis

3.4 DISCUSSION

3.4.1 BLOOD GLUCOSE

These data indicate that, within the control group, late lactation blood glucose levels are significantly higher among mink females showing elevated blood glucose levels early in the nursing period in comparison to normoglycemic females. During gestation and lactation, in healthy individuals, glucose homeostasis is maintained in spite of the adaptive increase in insulin resistance by a compensatory increase in insulin secretion by pancreatic β-cells (Di Cianni *et al.*, 2003). Deterioration to postprandial hyperglycemia occurs when insulin resistance increases further and/or the compensatory insulin secretion response decreases (Ceriello & Motz, 2004; DeFronzo, 2004). Research suggests that human subjects experiencing gestational and postpartum insulin resistance have a degree of insulin resistance that precedes their pregnancy, a condition that is partially inherited and partially acquired (Homko *et al.*, 2001; Di Cianni *et al.*, 2003). Previous studies have identified genetic transmission of glucose intolerance (Eriksson *et al.*, 1999; Boloker *et al.*, 2002; Ramsay *et al.*, 2002) and pre-gestational obesity (Volk *et al.*, 1999; Weijers *et al.*, 2002) as determinants for the development of hyperglycemia

during gestation. The implication is that hyperglycemia is not a transient condition in nursing female mink and that those experiencing problems with glucose regulation early in lactation may be susceptible to similar problems as lactation progresses.

While dam body weight and condition and litter weight and size were not found to immediately affect the blood glucose levels observed in the mink dams during late lactation, it is possible that the initial defects in glucose homeostasis occur earlier in the production cycle. Previous research shows that autumnal fattening in mink, fed at 120% of the recommended dietary allowance (RDA) of ME over a four month period, resulted in higher blood glucose values in males and females and higher insulin levels in males, in comparison to those fed at less than or equal to the RDA (Rouvinen-Watt *et al.*, 2004). In a study by Børsting and Damgaard (1995) the increased demand for milk production placed on females nursing eight kits resulted in higher glucose production in comparison to those nursing only four. With the development of insulin resistance during pregnancy, a mechanism favouring glucose supply to the growing fetuses (Di Cianni *et al.*, 2003), a similar increase in glucose demand associated with carrying larger litters is to be expected. This increased demand may aggravate underlying insulin resistance in mink females. We suggest that previous non-ideal body condition and increased gestational glucose demands may compromise the mink dam's ability to regulate glucose levels throughout the nursing period.

The elevated blood glucose levels observed in NG females treated with ASA were an unexpected result. Hundal and associates (2002) reported decreased basal hepatic glucose

production, enhanced peripheral insulin sensitivity, and decreased insulin clearance in type 2 diabetic patients treated for 2-weeks with high-dose aspirin. Recent data evaluating the effect of ASA on glycemic control in apparently healthy subjects are sparse. However, while numerous studies have identified acetyl-salicylic acid as an effective agent for improved insulin sensitivity in insulin resistant patients, in over-dosage, it has triggered transient hyperglycemia in healthy subjects (Ferner, 1992). In healthy men, high-dose ASA treatment impaired glucose metabolism in insulin-sensitive tissues (Giugliano *et al.*, 1982; Newman & Brodows, 1983; Bratusch-Marrain *et al.*, 1985). In this regard, we suggest that the selected dosage level of 100 mg/day may have interfered with glucose metabolism in the apparently healthy dams and prompted the development of hyperglycemia. The mechanism for this effect is not clear, however in a recent case study involving salicylate toxicity in a 5-year old girl, hypoglycemia was followed by hyperglycemia. It was suggested that metabolic acidosis lead to aberrations in oxidative phosphorylation, resulting in hyperglycemia due to increased gluconeogenesis (Peña-Alonso *et al.*, 2003).

Her-Cr-A-treated females showed increased blood glucose levels when compared to those in the control and Her-A treatment groups. The differences observed between the Her-A and Her-Cr-A treatment groups indicate an additive effect of chromium picolinate on the combined of effects of HerO and ASA on blood glucose; effectively increasing blood glucose levels in apparently healthy mink dams during lactation. Most scientific evidence indicates that chromium (Cr) supplementation has no significant effect in healthy individuals with good glucose tolerance (Cefalu *et al.*, 2002; Kim *et al.*, 2002;

Vincent, 2003). To the best of our knowledge, no data describe interactions between Cr and fish oil on blood glucose regulation, however ASA has been found to enhance Cr absorption (Davis *et al.*, 1995). The influence of Cr on the control of blood glucose is through increased insulin receptor number and an increase in insulin phosphorylation (Anderson *et al.*, 1997b; Anderson, 1998; Kim *et al.*, 2002). However, with multiple mechanisms of action of anti-diabetic agents on glucose metabolism existing through pancreatic, hepatic, and peripheral effects (Pandit *et al.*, 1993), it is possible that in combination, the studied treatments acted at multiple sites to induce abnormalities in glucose metabolism. In light of potential disruptions in glucose homeostasis associated with ASA and Her-Cr-A, we would recommend against their use in apparently healthy mink dams. Overall, results indicate no beneficial effects of the applied treatments on blood glucose levels during late lactation in female mink demonstrating normoglycemia early in the nursing period.

In contrast to the increases in blood glucose observed in NG dams, when treated with ASA alone and Her-Cr-A, several of the experimental treatments effectively reduced blood glucose levels during late lactation in HG dams exhibiting elevated blood glucose levels early in the nursing period. Furthermore, blood glucose levels in these treatment groups were lowered to below the initial hyperglycemic blocking criteria. In the current study, supplemental herring oil when fed alone, as well as in all combinations with CrPic and ASA, effectively decreased blood glucose levels in HG dams at lactation in comparison to the control. Fish oils high in n-3 PUFA have had beneficial effects on insulin-stimulated glucose transport and metabolism in peripheral tissues (Ezaki *et al.*,

1992; Long & Pekala, 1996; Luo *et al.*, 1996; Jen *et al.*, 2003). If supplemental n-3 PUFA can improve blood glucose levels in female mink during late lactation then we should consider how the dietary fatty acid profile could be changed throughout the production year. A small increase in dietary n-3 PUFA could have a strong effect on glycemic regulation in the mink female throughout the reproductive cycle.

When administered individually, CrPic and ASA did not significantly alter blood glucose levels in HG females in comparison to the control. However, the two in combination significantly reduced blood glucose levels during late lactation. In diabetics, vulnerability to oxidative damage might be partly attributed to a lower antioxidative micronutrient status including trace elements (Cheng *et al.*, 2004). Conditions that increase circulating insulin and glucose concentrations, including pregnancy (Jovanovic *et al.*, 1999) and obesity, increase urinary Cr output (Anderson *et al.*, 1997b; Morris *et al.*, 1999; Vincent, 2004) and Cr deficiency may be an aggravating factor in the progression of diabetes (Vincent, 1999; Cheng *et al.*, 2004). Davis *et al.* (1995) showed that ASA increased absorption, tissue retention and urinary excretion of chromium in adult female rats. Although the mechanism has not been clarified, it was proposed that Cr absorption was increased through aspirin's inhibitory effect on prostaglandin synthesis (Davis *et al.*, 1995). The accumulative effect observed may have resulted from interactions among the insulin-sensitizing effects of chromium (Anderson *et al.*, 1997b; Anderson, 1998; Cefalu *et al.*, 2002; Kim *et al.*, 2002) and the anti-inflammatory (Yin *et al.*, 1998; Yuan *et al.*, 2001; Hundal *et al.*, 2002; Gao *et al.*, 2003) and antioxidant (Sobal *et al.*, 2000; El Midaoui *et al.*, 2002) properties of acetyl-salicylic acid.

3.4.2 OXIDATIVE STRESS

Hyperglycemia induces the overproduction of reactive oxygen species (ROS), particularly superoxide anion, by the mitochondrial electron-transport chain (Ceriello, 2003), which in turn damages cellular deoxyribonucleic acid (DNA). However in the current study, normoglycemic females did not have significantly different comet assay scores than females with elevated blood glucose levels early in the nursing period. It is suggested that oxidative damage is a late event, occurring as an endpoint of hyperglycemia-dependent cellular changes (Baynes & Thorpe, 1999; Mohamed *et al.*, 1999).

When evaluating the effect of short-term supplementations on the degree of oxidative damage observed in nursing mink females, it was found that none differed significantly from the control. Elevated comet assay scores were observed in the blood of HerO-treated females when compared to those in Her-Cr, Cr-A, ASA and Her-Cr-A treatment groups. When exposed to oxidative stress, PUFA can be attacked by free radicals and oxidized into lipid peroxides (Eritsland, 2000). High doses of fish oil fed over a short period of time have increased susceptibility to oxidative stress, expressed as lipid peroxidation, in erythrocytes of rats (Garrido *et al.*, 1989). Cho and associates (1995) suggested that lipid peroxidation may lead to DNA damage as higher levels of 8-hydroxy-deoxyguanosine (8-OhdG), a marker of DNA oxidation, were detected in the liver of fish-oil-fed rats receiving low levels of the antioxidant vitamin E compared to those receiving moderate or high levels. Although the dietary requirement of antioxidants of a PUFA-rich diet has not been defined, data indicate that in order to prevent increased

susceptibility of fish oil to lipid peroxidation, supplementation with larger amounts of antioxidants, greater than those needed to stabilize the oil, may be required.

Females treated with CrPic had a higher mean comet assay score than those treated with ASA and Cr-A. It has been demonstrated that chronic treatment with acetyl-salicylic acid is associated with a reduction of superoxide production in normo- and hypertensive rats (Wu *et al.*, 2002). The mechanisms underlying the reduction in oxidative damage associated with chromium picolinate when combined with ASA may involve the antioxidant properties of the latter (Sobal *et al.*, 2000; El Midaoui *et al.*, 2002). Dietary supplementation with chromium picolinate and acetyl-salicylic acid had neither positive nor negative effects on the total comet assay score, as a measure of oxidative stress, as determined for mink females at late lactation. In light of our results, evaluating the degree of oxidative damage occurring among nursing and non-nursing mink females would be of interest.

3.4.3 DAM MORTALITY

Dam A, in the HG Cr-A treatment group, was diagnosed at autopsy with valvular endocarditis (heart valve infection). Culture showed evidence of *Staphylococcus intermedius* growth; an organism that may originate from disrupted oral, gastrointestinal, or urogenital mucosal surfaces, or from any other localized source of infection (Brown, 2004); this is a significant pathological finding as cause of death was unrelated to other observations. Demonstrating hyperglycemia early in lactation, no blatant abnormalities in glycemic control were observed in Dam A after the treatment period. On the other hand,

Dam B, in the HG control group and receiving no experimental treatment, experienced severe hyperglycemia as measured at days 42 and 43 of lactation. Metabolic diseases are almost impossible to diagnose at autopsy and while there was no diagnosis in this case, there is a clear indication that this female experienced an inability to regulate blood glucose throughout the nursing period with an acute failure occurring in late lactation. It should be noted that while Dam B was nursing a litter of seven, she gave birth to 13 kits. An increased gestational demand for glucose may have exacerbated underlying insulin resistance in this female, resulting in further disruptions in glucose homeostasis throughout lactation.

3.5 CONCLUSIONS

Together, these data indicate that the occurrence of hyperglycemia in the mink female is not a transient condition solely occurring from the demands of lactation. We conclude that there are no advantageous effects of short-term dietary supplementation with all combinations of herring oil, chromium picolinate and acetyl-salicylic acid, at the levels given in this study, on blood glucose levels at late lactation in apparently healthy, normoglycemic female mink. Similarly, the studied experimental treatments had neither positive nor negative effects on the degree of oxidative damage measured at late lactation. However, several of the treatments successfully reduced blood glucose levels during late lactation in dams demonstrating problems with glycemic control early in the nursing period, suggesting that a combined approach of blood glucose monitoring and dietary supplementation may aid in improving glucose regulation in mink females.

CHAPTER 4. Conclusion

Our findings indicate that the occurrence of hyperglycemia in the mink female is not a transient condition solely occurring from the demands of lactation; elevated blood glucose levels were observed early in the reproductive cycle. Overall, females demonstrating large changes in glucose levels from breeding to gestation experienced increased glycemic variability while nursing. It is suggested that certain nursing females may be predisposed to disruptions in glucose homeostasis during lactation. The relative contributions of genetic and environmental factors to this susceptibility remain to be determined.

During the nursing period, body weight and body condition score did not have an immediate effect on blood glucose concentrations observed late in lactation. However, increased variation was observed in the late-lactation blood glucose levels of females scored as non-ideal in condition at gestation. It was observed that females in thin condition at gestation showed an overall increase in blood glucose during the nursing period, whereas those in optimal condition showed decreases in these values. Similarly females classified as obese at gestation experienced a larger drop in blood glucose values during lactation than those scored as ideal. Generally, increased variation was observed in the blood glucose levels of females in non-ideal condition, indicating an impaired ability to regulate glucose homeostasis. The proper conditioning of mink females to avoid thin and obese body condition during breeding and gestation is recommended.

Increased blood glucose levels were observed at breeding and gestation in mink dams experiencing decreased reproductive success. Similarly, several females showing high blood glucose at one or more stages became sick or died. Litter size had a modest effect on blood glucose levels in the mink female during the reproductive cycle; elevated levels were detected in those with larger litters during breeding and lactation. These findings suggest that an increased gestational demand for glucose associated with large litter size may exacerbate underlying insulin resistance in the mink female, resulting in further disruptions in glucose homeostasis as lactation progresses. Poor glucose regulation occurs throughout the reproductive cycle and may predispose mink females to decreased reproductive success and poor health.

With regards to the prevention of hyperglycemia at late lactation, blood glucose levels were significantly reduced by supplementation with chromium picolinate at the onset of nursing. As a preventative treatment, short-term dietary supplementation with all combinations of herring oil, chromium picolinate and acetyl-salicylic acid, at the levels given in this study, had no advantageous effects on blood glucose levels at late lactation. However, in dams demonstrating problems with glycemic control early in the nursing period, short-term supplementation with selected combinations of herring oil, chromium picolinate, and ASA, were beneficial in improving blood glucose regulation during late lactation. As evidence of poor glucose regulation occurs in mink females prior to lactation, it appears that preventative measures need to be taken throughout the year. Blood glucose levels did not influence the degree of oxidative stress observed in lactating mink as indicated by the comet assay, and the experimental treatments had neither

positive nor negative effects on the degree of oxidative damage measured at late lactation.

Significant differences were observed in blood glucose levels between farms, emphasizing the importance of ranch-level factors, i.e. herd genetics and feed management practices, in glycemic regulation in the mink dam. While genetic and dietary contributions could not be differentiated, results from this study suggest that the combination of high quality n-3 PUFA and high levels of dietary carbohydrate, fed throughout the production year, warrants further investigation as a tool to better enable mink dams to maintain glucose homeostasis and body condition during the critical nursing period.

Genetic background, feeding practices, obesity or lipodystrophy and litter size may work together to influence the potential for development of hyperglycemia in the female mink during the reproductive cycle. Further studies must be conducted to better understand the causes of hyperglycemia and its association with metabolic diseases during lactation, such as nursing sickness. Specific areas for future consideration include a more in-depth investigation of the impacts of n-3 fatty acid deficiency, obesity and lipodystrophy and oxidative stress on glucose regulation in the mink female.

REFERENCES

Aerts L and Van Assche FA. 2003. Intra-uterine transmission of disease. Placenta, 24(10):905-11.

Allen KGD and Harris MA. 2001. The role of n-3 fatty acids in gestation and parturition. Exp Biol Med, 226(6): 498-506.

Anderson KE, Rosner W, Khan MS, New MI, Pang SY, Wissel PS, and Kappas A. 1987. Diet-hormone interactions: protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. Life Sci, 40(18):1761-8.

Anderson RA. 1998. Chromium, glucose intolerance and diabetes. J Am Coll Nutr, 17: 548-555.

Anderson RA, Bryden NA, and Polansky MM. 1997a. Lack of toxicity of chromium chloride and chromium picolinate in rats. J Am Coll Nutr, 16: 273-279.

Anderson RA, Cheng N, Bryden NA, Polansky MM, Cheng N, Chi J, and Feng J. 1997b. Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. Diabetes, 46:1786-91.

Annison EF, Linzell JL, and West CE. 1968. Mammary and whole animal metabolism of glucose and fatty acids in fasting lactating goats. J Physiol, 197: 445-459.

Bahadori B, Wallner S, Schneider H, Wascher TC, and Toplak H. 1997. Effect of chromium yeast and chromium picolinate on body composition of obese, non-diabetic patients during and after a formula diet (Abs). Acta Med Austriaca, 24(5):185-7.

Baynes JW and Thorpe SR. 1999. Role of oxidative stress in diabetic complications. A new perspective on an old paradigm. Diabetes, 48:1-9.

Behrend EN. 2002. Diabetes mellitus: an update on oral hypoglycemic agents and monitoring agents. Vet Med, 743-751.

Bell AW and Bauman DE. 1997. Adaptation of glucose metabolism during pregnancy and lactation. J Mammary Gland Biol, 2(3):265-278.

Bell GI and Polansky KS. 2001. Diabetes mellitus and genetically programmed defects in β-cell function. Nature, 414: 788-791.

Belzung F, Raclot T, and Groscolas R. 1993. Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am J Physiol, 264: R1111-18.

Bender DA. 1997. Introduction to Nutrition and Metabolism, 2nd Ed. Taylor & Francis Ltd., London. pp. 151-155, 175-177.

Bennett N. 2002. Monitoring techniques for diabetes mellitus in the dog and the cat. Clin Tech Small An P, 17: 65-69.

Bjorntorp P. 1991. Metabolic implications of body fat distribution. Diabetes Care, 14(12): 1132-43.

Boden G and Shulman GI. 2002. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction. Eur J Clin Invest, 32(3):14.

Boloker J, Gertz SJ, and Simmons RA. 2002. Gestational diabetes leads to the development of diabetes in adulthood in the rat. Diabetes, 51:1499-1506.

Bonnefont-Rousselot D, Bastard JP, Jaudon MC, and Delattre J. 2000. Consequences of the diabetic status on the oxidant/antioxidant balance. Diabetes Metab, 26:163-76.

Børsting C and Damgaard B. 1995. The intermediate glucose metabolism in the nursing period of the mink. NJF Seminar No. 253, October 4-6, Gothenburg, Sweden.

Børsting CF and Gade A. 2000. Glucose homeostasis in mink (*Mustela vison*): A review based on interspecies comparisons. Scientifur, 24: 9-18.

Bratusch-Marrain PR, Vierhapper H, Komjati M, and Waldhausl WK. 1985. Acetyl-salicylic acid impairs insulin-mediated glucose utilization and reduces insulin clearance in healthy and non-insulin-dependent diabetic man. Diabetologia, 28(9):671-6.

Bray GA. 1992. Obesity increases risk for diabetes. Int J Obes, 16 (Suppl 4):S13-7.

Brown VA. 2004. Aortic valvular endocarditis in a dog. Can Vet J, 45(8):682-84.

Browning LM. 2003. n-3 polyunsaturated fatty acids, inflammation and obesity-related disease. Proc Nutr Soc, 62:447-453.

Brownwyn AE, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, and Cooney GJ. 2000. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. Am J Physiol Endocrinol Metab, 279:E554-E560.

Buchanan TA. 1991. Glucose metabolism during pregnancy: normal physiology and implications for diabetes mellitus. Isr J Med Sci, 27(8-9):432-41.

Butte NF, Hopkinson JM, Mehta N, Moon JK, and O'Brian-Smith E. 1999. Adjustments in energy expenditure and substrate utilization during late pregnancy and lactation. Am J Clin Nutr, 69:299–307.

Calder PC. 2002. Dietary modification of inflammation with lipids. Proc Nutr Soc, 61: 345-58.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals, Vol. 1. Olfert ED, Cross BM, and McWilliam AA. (Eds).

Carbonell T, Rodenas J, Miret S, and Mitjavila MT. 1997. Fish oil and oxidative stress by inflammatory leukocytes. Free Radic Res, 27(6):591-597.

Cassano PA, Rosner B, Vokonas PS, and Weiss ST. 1992. Obesity and body fat distribution in relation to the incidence of non- insulin-dependent diabetes mellitus. A prospective cohort study of men in the normative aging study. Am J Epidemiol, 136 (12):1474-86.

Cefalu WT, Wang ZQ, Zhang XH, Baldor LC, and Russell JC. 2002. Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances skeletal muscle glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. J Nutr, 132(6): 1107-1114.

Ceriello A. 2003. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. Diabetes Care, 26:1589-96.

Ceriello A and Motz E. 2004. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. Arterioscler Thromb Vasc Biol, 24:816-23.

Chaisson J-L, Shikama H, Chu DTW, and Exton JH. 1981. Inhibitory effect of epinephrine on insulin-stimulated glucose uptake by rat skeletal muscle. J Clin Invest, 68:706-713.

Chan JM, Rimm EB, Colditz GA, Stampfer MJ, and Willett WC. 1994. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care, 17(9):961-969.

Cheng HH, Lai MH, Hou WC, and Huang CL. 2004. Antioxidant effects of chromium supplementation with type 2 diabetes mellitus and euglycemic subjects. J Agric Food Chem, 52(5):1385-9.

Cho SH, Im JG, Choi YS, Son YS, and Chung MH. 1995. Lipid peroxidation and 8-hydroxyguanine formation in rats fed fish oil with different levels of vitamin E. J Nutr Sci Vitaminol, 41:61-72.

Clausen TN, Olesen CR, Hansen O, and Wamberg S. 1992. Nursing sickness in lactating mink (*Mustela vison*). I. Epidemiological and pathological observations. Can J Vet Res, 56:89-94.

Colagiuri S and Brand Miller J. 2002. The 'carnivore connection'- evolutionary aspects of insulin resistance. Euro J Clin Nutr, 56 (Suppl 1):S30-S35.

Collins AR, Raslova K, Somorovska M, *et al.* 1998. DNA damage in diabetes: correlation with a clinical marker. Free Radic Biol Med, 25(3):373-7.

Couet C, Delarue J, Ritz P, Antoine JM, and Lamisse F. 1997. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. Int J Obes, 21(8):637-43.

Cunningham MJ, Clifton DK, and Steiner RA. 1999. Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod, 60:216-22.

Damgaard BM, Børsting CF, Ingvartsen KL, and Fink R. 2003. Effects of carbohydrate-free diets on the performance of lactating mink (*Mustela vison*) and the growth performance of suckling kits. Acta Agric Scand - Sect A, Anim Sci, 53(3):127-135.

Dandona P, Aljada A, and Bandyopadhyay A. 2004. Inflammation: The link between insulin resistance, obesity and diabetes. Trends Immunol, 25 (1):4-7.

Das UN. 2004. Metabolic syndrome x: an inflammatory condition? Curr Hypertens Rep, 6(1):66-73.

Davies MJ. 2005. The oxidative environment and protein damage. Biochim Biophys Acta, 1703(2): 93-109.

Davis ML, Seaborn CD, and Stoecker BJ. 1995. Effects of over-the-counter drugs on ^{51}chromium retention and urinary excretion in rats. Nutr Res, 15(2):201-210.

Davison LJ, Podd SL, Ristic JME, Herrtage ME, Parnham A, and Catchpole B. 2002. Evaluation of two point-of-care analysers for measurement of fructosamine or haemoglobin A_{1c} in dogs. J Sm Anim Prac, 43 (12): 526-532.

DeFronzo RA. 2004. Pathogenesis of type 2 diabetes mellitus. Med Clin N Am, 88(4):787-835.

Delarue J, LeFoll C, Corporeau C, and Lucas D. 2004. n-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? Reprod Nutr Dev, 44(3):289-99.

De Leon MJ, McRae T, Rusinek H, *et al.* 1997. Cortisol reduces hippocampal glucose metabolism in normal elderly, but not in Alzheimer's disease. J Clin Endocrinol Metab, 82: 3251–59.

Del Prado M, Villalpando S, Gordillo J, and Hernández-Montes H. 1999. A high dietary lipid intake during pregnancy and lactation enhances mammary gland lipid uptake and lipoprotein lipase activity in rats. J Nutr, 129:1574-1578.

Di Cianni G, Miccoli R, Volpe L, Lencioni C, and Del Prato S. 2003. Intermediate metabolism in normal pregnancy and in gestational diabetes. Diabetes Metab Res, 19:259-270.

Dimitriadis G, Leighton B, Parry-Billings M, *et al.* 1997. Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. Biochem J, 321:707-712.

Eigler N, Saccà L, and Sherwin RS. 1979. Synergistic interactions of physiologic increments of glucagon, epinephrine, and cortisol in the dog: A model for stress-induced hyperglycemia. J Clin Invest, 63(1): 114–123.

Elliott DA, Nelson RW, Feldman EC, and Neal LA. 1997. Glycosylated hemoglobin concentration for assessment of glycemic control in diabetic cats. J Vet Intern Med, 11 (3): 161-165.

Elliott DA, Nelson RW, Reusch CE, Feldman EC, and Neal LA. 1999. Comparison of serum fructosamine and blood glycosylated hemoglobin concentrations for assessment of glycemic control in cats with diabetes mellitus. J Am Vet Med Assoc, 214(12): 1794-1798.

Elliott WH and Elliott DC. 2001. Biochemistry and Molecular Biology, 2nd ed. Oxford University Press, New York. Pp 97-174.

El Midaoui A, Wu R, and de Champlain, J. 2002. Prevention of hypertension, hyperglycemia and vascular oxidative stress by aspirin treatment in chronically glucose-fed rats. J Hypertens, 20(7):1407-12.

Eriksson JW, Smith U, Waagstein F, Wysocki M, and Jansson P-A. 1999. Glucose turnover and adipose tissue lipolysis are insulin-resistant in healthy relatives of type 2 diabetes patients. Is cellular insulin resistance a secondary phenomenon? Diabetes, 48:1572–1578.

Eritsland J. 2000. Safety considerations of polyunsaturated fatty acids. Am J Clin Nutr, 71(suppl):197S-201S.

Ezaki O, Tsuji E, Momomura K, Kasuga M, and Itakura H. 1992. Effects of fish and safflower oil feeding on subcellular glucose transporter distributions in rat adipocytes. Am J Physiol, 263: E94-101.

Farmer G, Russell G, Hamilton-Nicol DR, *et al.* 1988. The influence of maternal glucose metabolism on fetal growth, development and morbidity in 917 singleton pregnancies in nondiabetic women. Diabetologia, 31(3):134-141.

Felber JP and Golay A. 2002. Pathways from obesity to diabetes. Int J Obes Relat Metab Disord, 26(Suppl 2):39-45.

Ferner RE. 1992. Drug-induced diabetes. Baillieres Clin Endocrinol Metab, 6(4):849-66.

Fickova M, Hubert P, Crémel G, and Leray C. 1998. Dietary (n-3) and (n-6) polyunsaturated fatty acids rapidly modify fatty acid composition and insulin effects in rat adipocytes. J Nutr, 128:512–19.

Fink R and Børsting CF. 2002. Quantitative glucose metabolism in lactating mink (*Mustela vison*) – Effects of dietary levels of protein, fat and carbohydrates. Acta Agric Scand - Sect A, Anim Sci, 52:34-42.

Fink R, Børsting CF, and Damgaard BM. 2002a. Glucose homeostasis and regulation in lactating mink (*Mustela vison*): Effects of dietary protein, fat and carbohydrate supply. Acta Agric Scand - Sect A, Anim Sci, 52:102-111.

Fink R, Tauson A-H, and Børsting CF. 2001a. Dietary protein, fat and carbohydrate supply to lactating mink (*Mustela vison*): Effect on glucose homeostasis, energy metabolism and milk production. NJF-seminar No.331, October 1-3, Snekkersten, Denmark.

Fink R, Børsting CF, Damgaard BM, and Rosted AKL. 2002b. Glucose metabolism and regulation in lactating mink (*Mustela vison*) – Effects of low dietary protein supply. Arch Anim Nutr, 56:155-166.

Fink R, Tauson A-H, Hansen KB, Wamberg S, and Kristensen NB. 2001b. Energy intake and milk production in mink (*Mustela vison*) – Effect of litter size. Arch Anim Nutr, 55:221-242.

Fink R, Tauson AH, Chwalibog A, Hansen NE, Kristensen NB, and Wamberg S. 2004. Effects of substitution of dietary protein with carbohydrate on lactation performance in the mink (*Mustela vison).* J Anim Feed Sci, 13:647-664.

Fiorotto ML, Burrin DG, Perez M, and Reeds PJ. 1991. Intake and use of milk nutrients by rats pups suckled in small, medium, or large litters. Am J Physiol, 29:1104-1113.

Flier JS. 2001. Diabetes: The missing link with obesity? Nature, 409:292-293.

Fonseca V. 2003. Clinical significance of targeting postprandial and fasting hyperglycemia in managing type 2 diabetes mellitus. Curr Med Res Opin, 19(7):635-641.

Forouhi NG, Jenkinson G, Thomas EL, *et al.* 1999. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. Diabetologia, 42(8):932-935.

Fox JG and Marini RP. 1998. Diseases of the endocrine system. In Fox, J.G. (Ed) Biology and Diseases of the Ferret, 2nd ed. Williams & Wilkins, Baltimore. Pp. 301.

Frayn KN. 2001. Adipose tissue and the insulin resistance syndrome. Proc Nutr Soc, 60: 375-80.

Gao Z, Zuberi A, Quon MJ, Dong Z, and Ye J. 2003. Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. J Biol Chem, 278(27): 24944-50.

Garrido A, Garrido F, Guerra R, and Valenzuela A. 1989. Ingestion of high doses of fish oil increases the susceptibility of cellular membranes to the induction of oxidative stress. Lipids, 24(9):833-5.

Gavrilova O, Marcus-Samuels B, Graham D, *et al.* 2000. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. J Clin Invest, 105:271-78.

Ghosh D, Bhattacharya B, Mukherjee B, Manna B, Sinha M, Chowdhury J, and Chowdhury S. 2002. Role of chromium supplementation in Indians with type 2 diabetes mellitus. J Nutr Biochem, 13: 690-697.

Giugliano D, Sacca L, Scognamiglio G, Ungaro B, and Torella R. 1982. Influence of acetylsalicylic acid on glucose turnover in normal man. Diabetes Metab, 8(4):279-82.

Griffiths HR. 2002. The influence of diet on protein oxidation. Nutr Res Rev, 15:3-17.

Grimm H, Mayer K, Mayser P, and Eigenbrodt E. 2002. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. Brit J Nutr, 87(Suppl 1):S59-S67.

Groop LC, Saloranta C, Shank M, Bonadonna RC, Ferrannini E and DeFronzo RA. 1991. The role of free fatty acid metabolism in the pathogenesis of insulin resistance in obesity and noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab, 72:96-107.

Haberer B and Reusch CE. 1998. Glycated haemoglobin in various pathological conditions: investigations based on a new, fully automated method. J Sm Anim Prac, 39 (11): 510-517.

Hansen BK and Berg P. 1998. Mink dam weight changes during the lactation period. I. Genetic and environmental effects. Acta Agric Scand - Sect A, Anim Sci, 48:49-57.

Hansen MU, Lassén M, Tauson A-H, Sørensen H, and Clausen T. 2004. Different ratio between n-6 and n-3 fatty acids in diets for lactating mink (*Mustela vison*) dams – effect on milk and kit tissue fatty acid composition. Scientifur, 28(3): 103-108.

Hartz AJ, Rupley DC Jr, Kalkhoff RD, and Rimm AA. 1983. Relationship of obesity to diabetes: influence of obesity level and body fat distribution. Prev Med, 12(2):351-7.

Hearn WE. 1987. Interspecific competition and habitat segregation among stream-dwelling trout and salmon: A review. Fisheries, 12: 24-31.

Hepburn DDD, Burney JM, Woski SA ,and Vincent JB. 2003. The nutritional supplement chromium picolinate generates oxidative DNA damage and peroxidized lipids in vivo. Polyhedron, 22:455-63.

Hill JO, Peters JC, Lin D, Yakubu F, Greene H, and Swift L. 1993. Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. Int J Obes, 17: 223-236.

Hoenig M. 1995. Pathophysiology of canine diabetes. Vet Clin N Am Small, 25(3):553-561.

Hoenig M. 2002. Comparative aspects of diabetes mellitus in dogs and cats. Mol Cell Endocrinol, 197(1): 221-229.

Hoenig M and Ferguson DC. 1999. Diagnostic utility of glycosylated hemoglobin concentrations in the cat. Domest Anim Endocrin, 16(1): 11-17.

Holemans K, Aerts L, and Van Assche FA. 1991. Evidence for an insulin resistance in the adult offspring of pregnant streptozotocin-diabetic rats. Diabetologia, 34(2):81-85.

Holemans K, Caluwaerts A, Poston L, and Van Assche FA. 2004. Diet-induced obesity in the rat: a model for gestational diabetes mellitus. Am J Obstet Gynecol, 190(3):858-865.

Holman GD and Sandoval IV. 2001. Moving the insulin-regulated glucose transporter GLUT4 into and out of storage. Trends Cell Biol, 11(4): 173-179.

Homko C, Sivan E, Chen X, Reece EA, and Boden G. 2001. Insulin secretion during and after pregnancy in patients with gestational diabetes mellitus. J Clin Endoc Metab, 86:568-73.

Howard BV. 1999. Insulin resistance and lipid metabolism. Am J Cardiol, 84:28-32.

Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, and Shulman GI. 2002. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest, 109:1321-6.

Hunt DG and Ivy JL. 2002. Epinephrine inhibits insulin-stimulated muscle glucose transport. J Appl Physiol, 93:1638–43.

Hynes AM, Rouvinen-Watt K, and Armstrong D. 2004. Body condition and glycemic control in mink females during reproduction and lactation. Scientifur, 28(3): 79-86.

Ibrahim W, Lee U-S, Yeh C-C, Szabo J, Bruckner G, and Chow CK. 1997. Oxidative stress and antioxidant status in mouse liver: Effects of dietary lipid, vitamin E and iron. J Nutr, 127:1401-06.

Jen K-LC, Buison A, Pellizzon M, Ordiz F(Jr), Santa Ana L, and Brown J. 2003. Differential effects of fatty acids and exercise on body weight regulation and metabolism in female Wistar rats. Exp Biol Med,228:843-49.

Jensen MD, Haymond MW, Gerich JE, Cryer PE, and Miles JM. 1987. Lipolysis during fasting. Decreased suppression by insulin and increased stimulation by epinephrine. J Clin Invest, 79(1): 207–213.

Jovanovic L, Gutierrez M, and Peterson CM. 1999. Chromium supplementation for women with gestational diabetes mellitus. J Trace Elements Exp Med, 12(2):91-7.

Jucker BM, Cline GW, Barucci N, and Shulman GI. 1999. Differential effects of safflower oil versus fish oil feeding on insulin-stimulated glycogen synthesis, glycolysis, and pyruvate dehydrogenase flux in skeletal muscle. Diabetes, 48: 134-140.

Kahn AH and Pessin JE. 2002. Insulin regulation of glucose uptake: a complex interplay of intracellular signaling pathways. Diabetologica, 45:1475-83.

Kahn BB. 1994. Dietary regulation of glucose transporter gene expression: Tissue specific effects in adipose cells and muscle. J Nutr, 124: 1289S-95S.

Kahn BB and Flier JS. 2000. Obesity and insulin resistance. J Clin Invest, 106(4): 473-481.

Karp G. 1996. Cell and Molecular Biology: Concepts and Experiments. John Wiley & Sons, Inc., New York.

Kautzky-Willer a, Prager R, Waldhausl W, *et al.* 1997. Pronounced insulin resistance and inadequate beta-cell secretion characterize lean gestational diabetes during and after pregnancy. Diabetes Care, 20(11):1717-23.

Kayali R, Telci A, Cakatay U, Karaca C, Akcay T, Sivas A, and Altug T. 2003. Oxidative protein damage parameters in plasma in chronic experimental diabetes in rats. Eur J Med Res, 8(7):307-312.

Kettlehut IC, Foss MC, and Migliorini RH. 1980. Glucose homeostasis in a carnivorous animal (cat) and in rats fed a high-protein diet. Am J Physiol, 239: R437-R444.

Khayat ZA, Patel N and Klip A. 2002. Exercise and insulin stimulated muscle glucose transport: Distinct mechanisms of regulation. Can J Appl Physiol, 27: 129-151.

Kikugawa K, Yasuhara Y, Ando K, Koyama K, Hiramoto K, and Suzuki M. 2003. Protective effect of supplementation of fish oil with high n-3 polyunsaturated fatty acids against oxidative stress-induced DNA damage of rat liver in vivo. J Agric Food Chem, 51(20):6073-9.

Kim DS, Kim TW, Park IK, Kang JS, and Om AS. 2002. Effects of chromium picolinate supplementation on insulin sensitivity, serum lipids, and body weight in dexamethasone-treated rats. Metab Clin Exp, 51(5):589-94.

Korhonen H, Harri M, and Mononen J. 1989. Regulation of weight loss in male farm mink. Comp Biochem Physiol A, 92(3):355-7.

Lang CH, Dobrescu C, and Bagby GJ. 1992. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrin,130:43-52.

Lenhard JM and Gottschalk WK. 2002. Preclinical developments in type 2 diabetes. Adv Drug Deliv Rev, 54(9):1199-1212.

Little RR, England JD, Wiedmeyer HM, McKenzie EM, Pettitt DJ, Knowler WC, and Goldstein DE. 1988. Relationship of glycosylated hemoglobin to oral glucose tolerance. Implications for diabetes screening. Diabetes, 37(1):60-64.

Long SD and Pekala PH. 1996. Regulation of GLUT4 gene expression by arachidonic acid. J Biol Chem, 271(2):1138-44.

Loste A and Marca MC. 2001. Fructosamine and glycated hemoglobin in the assessment of glycaemic control in dogs. Vet Res, 32(1):55-62.

Luick JR, Black AL, Parker HR, and Simesen MG. 1962. Glucose metabolism in the lactating beagle dog. Am J Physiol, 202:329-33.

Luo J, Rizkalla SW, Boillot J, *et al.* 1996. Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. J Nutr, 126: 1951-58.

Marca MC, Loste A, and Ramos JJ. 2000. Effect of acute hyperglycaemia on the serum fructosamine and blood glycated haemoglobin concentrations in canine samples. Vet Res Comm, 24 (1): 11-16.

Mason TM, Goh T, Tchipashvili V, Sandi H, Gupta N, and Lewis GF. 1999. Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. Diabetes, 48:524-530.

McCarty MF. 2005. Nutraceutical resources for diabetes prevention – an update. Med Hypoth, 64(1):151-8.

Metrika, Inc. ©2002. A_{1c}Now Brochure. P/N 90146 Rev.

Miret S, Saiz MP, and Mitjavila MT. 2003. Effects of fish oil- and olive oil-rich diets on iron metabolism and oxidative stress in the rat. Brit J Nutr, 89(1):11-18.

Mohamed AK, Bierhaus A, Schiekofer S, Tritschler, Ziegler R, and Nawroth PP. 1999. The role of oxidative stress and NF-\kappaB activation in late diabetic complications. BioFactors, 10(2-3): 157-67.

Morgan PJ and Mercer JG. 2001. The regulation of body weight: lessons from the seasonal animal. Proc Nutr Soc, 60:127-34.

Mori TA and Beilin LJ. 2004. Omega-3 fatty acids and inflammation. Curr Atheroscler Rep, 6(6):461-7.

Morris BW, MacNeil S, Hardisty CA, Heller S, Burgin C, and Gray TA. 1999. Chromium homeostasis in patients with type II (NIDDM) diabetes. J Trace Elements Med Biol, 13:57-61.

Nadler ST, Stoehr JP, Schueler KL, Tanimoto G, Yandell BS, and Attie AD. 2000. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. PNAS, 97(21):11371-11376.

Nelson DL and Cox MM. 2000. Lehninger Principles of Biochemistry, 3rd ed. Worth Publishers, New York. Pp 527-557, 723-745.

Newman WP and Brodows RG. 1983. Aspirin causes tissue insensitivity to insulin in normal man. J Clin Endocrin Metab, 57:1102-06.

Okauchi N, Mizuno A, Zhu M, Ishida K, Sano T, Noma Y, and Shima K. 1995. Effects of obesity and inheritance on the development of non-insulin-dependent diabetes mellitus in Otsuka-Long-Evans-Tokushima fatty rats. Diabetes Res Clin Pract, 29(1):1-10.

Olefsky JM. 1999. Insulin-stimulated glucose transport minireview series. J Biol Chem, 274 (4):1863.

Ononogbu IC and Echeta NBC. 1987. Hemoglobin glycosylation and erthrocyte free fatty acid concentrations in gari fed rats. Nutr Res, 7: 883-886.

Oral EA, Simha V, Ruiz E, *et al.* 2002. Leptin-replacement therapy for lipodystrophy. New Engl J Med, 346 (8): 570-578.

Osadchuk LV, Braastad BO, and Bakken M. 2004. Effect of prenatal stress on the pituitary-adrenal axis in blue foxes. Russian Journal of Developmental Biology, 35(3):162–167.

Östling O and Johanson KJ. 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem Bioph Res Co, 123: 291-98.

Palumbo PJ. 2001. Glycemic control, mealtime glucose excursions, and diabetic complications in type 2 diabetes mellitus. Mayo Clin Proc, 76: 609-618.

Pandit M, Burke J, Gustafson A, Minocha A, and Peiris A. 1993. Drug-induced disorders of glucose tolerance. Ann Intern Med, 118:529–539.

Parillo M and Riccardi G. 2004. Diet composition and the risk of type 2 diabetes: epidemiological and clinical evidence. Brit J Nutr, 92:7-19.

Peña-Alonso YR, Montoya-Cabrera MA, Bustos-Córdoba E, Marroquín-Yáñez L, and Olivar-López V. 2003. Aspirin intoxication in a child associated with myocardial necrosis: Is this a drug-relation lesion? Pediatr Devel Pathol, 6:342-47.

Perseghin G, Petersen K, and Shulman GI. 2003. Cellular mechanism of insulin resistance: potential links with inflammation. Int J Obes, 27:S6-S11.

Petzke KJ, Elsner A, Proll J, Thielecke F, and Metges CC. 2000. Long-term high protein intake does not increase oxidative stress in rats. J Nutr,130:2889-96.

Peyron-Caso E, Quignard-Boulangé A, Laromiguière M, *et al.* 2003. Dietary fish oil increases lipid mobilization but does not increase lipid storage-related enzyme activities in adipose tissue of insulin-resistant sucrose-fed rats. J Nutr, 133:2239-43.

Piconi L, Quagliaro L and Ceriello A. 2003. Oxidative stress in diabetes. Clin Chem Lab Med, 41(9): 1144-49.

Pittler MH, Stevinson C, and Ernst E. 2003. Chromium picolinate for reducing body weight: Meta-analysis for randomized trials. Int J Obes, 27:522-29.

Plotnick AN and Greco DS. 1995. Diagnosis of diabetes mellitus in dogs and cats: Contrasts and comparisons. Vet Clin N Am Small, 25(3):563-70.

Pölönen I, Scott R, and Oldfield J. 1993. Mink diet energy during pre-weaning and early post-weaning periods. Scientifur, 17:47–51.

Quilley J. 2002. Insulin resistance, oxidative stress and aspirin: therapeutic implications? J Hypertens, 20: 1279-1281.

Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, and Sattar N. 2002. Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. J Clin Endocrin Metab, 87(9):4231-4237.

Rand JS and Martin GJ. 2001. Management of feline diabetes mellitus. Vet Clin N Am – Small. 31(5):881-913.

Rand JS, Fleeman LM, Farrow HA, Appleton DJ, and Lederer R. 2004. Canine and feline diabetes mellitus: nature or nurture? J Nutr, 134:2072S-2080S.

Raz I, Eldor R, Cernea S, and Shafrir E. 2004. Diabetes: insulin resistance and derangements in lipid metabolism. Cure through intervention in fat transport and storage. Diabetes Metab Res, 21(1):3-14.

Regnault TRH, Oddy HV, Nancarrow C, Sriskandarajah N, and Scaramuzzi RJ. 2004. Glucose-stimulated insulin response in pregnant sheep following acute suppression of plasma non-esterified fatty acid concentrations. Reprod Biol Endocrinol, 2:64-74.

Reidy SP and Weber J-M. 2000. Leptin: an essential regulator of lipid metabolism. Comp Biochem Physiol Part A, 125:285-97.

Rendell M, Nierenberg J, Brannan C, *et al.* 1986. Inhibition of glycation of albumin and hemoglobin by acetylation in vitro and in vivo. J Lab Clin Med, 108(4):286-93.

Riccardi G, Giacco R, and Rivellese AA. 2004. Dietary fat, insulin sensitivity and the metabolic syndrome. Clin Nutr, 23:447-56.

Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, and Shulman GI. 1996. Mechanism of free fatty acid–induced insulin resistance in humans. J Clin Invest, 97:2859-2865.

Rohlfing CL, Wiedmeyer HM, Little RR, England JD, Tennill A, and Goldstein DE. 2002. Defining the relationship between plasma glucose and HbA_{1c}. Diabetes Care, 25(2): 275-278.

Rouvinen K and Kiiskinen T. 1989. Influence if dietary fat source on the body fat composition of mink (*Mustela vison*) and blue fox (*Alopex lagopus*). Acta Agric Scand, 39:279-88.

Rouvinen K, Mäkelä J, Kiiskinen T, and Nummela S. 1992. Accumulation of dietary fish fatty acids in the body fat reserves of some carnivorous fur-bearing animals. Agric Sci Finl, 1:483-89.

Rouvinen-Watt K. 2003. Nursing sickness in the mink – a metabolic mystery or a familiar foe? Can J Vet Res, 67:161-168.

Rouvinen-Watt K and Hynes AM. 2004. Mink nursing sickness survey in North America. Scientifur, 28(3): 71-78.

Rouvinen-Watt K, Murphy JP, and Chan C. 2004. Effect of feeding intensity on body condition and glycemic control in mink *Mustela vison*. Scientifur, 28(3): 129-135.

Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, and Parrott M. 2002. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem, 48: 436-472.

Samra JS. 2000. Regulation of lipid metabolism in adipose tissue. Proc Nutr Soc, 59:441-46.

Sardas S, Yilmaz M, Oztok U, Cakir N, and Karakaya AE. 2001. Assessment of DNA strand breakage by comet assay in diabetic patients and the role of antioxidant supplementation. Mutat Res, 490 (2):123-9.

Scarlett JM and Donoghue S. 1998. Associations between body condition and disease in cats. J Am Vet Med Assoc, 212(11):1725-31.

Schachter S, Nelson RW, and Kirk CA. 2001. Oral chromium picolinate and control of glycemia in insulin treated diabetic dogs. J Vet Intern Med, 15(4):379-84.

Scheen AJ and Lefebvre PJ. 1998. Oral anti-diabetic agents. A guide to selection. Drugs, 55(2):225-36.

Schneider RR and Hunter DB. 1992. Nursing disease in the mink. Scientifur, 16:239-242.

Seimiya Y, Kikuchi F, Tanaka S, and Ohshima K. 1988. Pathological observations of nursing sickness in the mink. Jpn J Vet Sci, 50(1):255-257.

Sen CK, Atalay M, Ågren J, Laaksonen DE, Roy S, and Hänninen O. 1997. Fish oils and vitamin E supplementation in oxidative stress at rest and after physical exercise. J Appl Physiol, 83(1):189-95.

Sherwin RS, Shamoon H, Hendler R, Saccà L, Eigler N, and Walesky M. 1980. Epinephrine and the regulation of glucose metabolism: effect of diabetes and hormonal interactions. Metabolism, 29(11):1146-54.

Shiba Y, Yamasaki Y, Kubota M, *et al.* 1998. Increased hepatic glucose production and decreased hepatic glucose uptake at the prediabetic phase in the Otsuka Long-Evans Tokushima fatty rat model. Metabolism, 47(8):908-14.

Singh NP, McCoy MT, Tice RR, and Sneider EL. 1988. A simple technique for the quantitation of low levels of DNA damage in individual cells. Exp Cell Res, 175:184-91.

Sivan E, Homko CJ, Chen X, Reece EA, and Boden G. 1999. Effect of insulin on fat metabolism during and after normal pregnancy. Diabetes, 48:834–838.

Sobal G, Menzel JE, and Sinzinger H. 2000. Influence of acetylsalicylic acid on oxidation of native and glycated low-density lipoprotein. Life Sci, 66(20):1987-98.

Sørensen B, Clausen TN, Wamberg S, and Hansen O. 2001. Physiological changes in mink (Mustela vison) dams subjected to weaning at different times during lactation. Acta Agric Scand - Sect A, Anim Sci, 51(2): 148 – 154.

Sørensen PG, Petersen IM, and Sand O. 1995. Activities of carbohydrate and amino acid metabolizing enzymes from liver of mink (*Mustela vison*) and preliminary observations on steady state kinetics of the enzymes. Comp Biochem Physiol, 112: 59-64.

Speetjens JK, Collins RA, Vincent JB, and Woski SA. 1999. The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. Chem Res Toxicol, 12(6):483-7.

Stadtman ER and Levine RL. 2000. Protein oxidation. Annals New York Acad Sci, 899:191-208.

Stein JE and Greco DS. 2002. Monitoring blood glucose concentrations in dogs and cats with diabetes mellitus. Clin Tech Anim Prac, 17: 70-72.

Stevenson RW, Steiner KE, Connolly CC, Fuchs H, Alberti KG, Williams PE, and Cherrington AD. 1991. Dose-related effects of epinephrine on glucose production in conscious dogs. Am J Physiol Endocrinol Metab, 260:363-370.

Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, and Kraegen EW. 1991. Influence of dietary fat composition on development of insulin resistance in rats: Relationship to muscle triglyceride and ω-3 fatty acids in muscle phospholipids. Diabetes, 40:280-89.

Takahashi Y and Ide T. 2000. Dietary n-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. Brit J Nutr, 84:175-84.

Taouis M, Dagou C, Ster C, Durand G, Pinault M, and Delarue J. 2002. n-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. Am J Physiol Endocrinol Metab, 282:664-671.

Tauson A-H. 1997. Prolactin profiles in pregnant, lactating and non-mated female mink (*Mustela vison*). J Reprod Fert, 51:195–201.

Tauson A-H and Forsberg M. 2002. Body-weight changes are clearly reflected in plasma concentrations of leptin in female mink (*Mustela vison*). Brit J Nutr, 87:101-05.

Tauson A-H, Sørensen HJ, Wamberg S, and Chwalibog A. 1998. Energy metabolism, nutrient oxidation and water turnover in the lactating mink (*Mustela vison*). J Nutr, 128(12):2615-17.

Tebbey PW, McGowan KM, Stephens JM, Buttke TM, and Pekala PH. 1994. Arachidonic acid down-regulates the insulin-dependent glucose transporter gene (GLUT4) in 3T3-L1 adipocytes by inhibiting transcription and enhancing mRNA turnover. J Biol Chem, 269(1):639-44.

Trayhurn P and Beattie JH. 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nutr Soc, 60:329-339.

Trent LK and Thieding-Cancel D. 1995. Effects of chromium picolinate on body composition. J Sports Med Phys Fitness, 35(4):273-80.

Vambergue , Nuttens MC, Verier-Mine O, Dognin C. Cappoen JP, and Fontaine P. 2000. Is mild gestational hyperglycemia associated with maternal and neonatal complications? The Diagest Study. Diabetic Med, 17(3):203-208.

Vincent JB. 1999. Mechanisms of chromium action: Low-molecular-weight chromium-binding substance. J Am Coll Nutr, 18(1):6-12.

Vincent JB. 2003. Recent advances in the biochemistry of chromium (III). J Trace Elem Exp Med, 16(4):227-36.

Vincent JB. 2004. Recent advances in the nutritional biochemistry of trivalent chromium. Proc Nutr Soc, 63:41-47.

Volk A, Renn W, Overkamp D, *et al.* 1999. Insulin action and secretion in healthy, glucose tolerant first degree relatives of patients with type 2 diabetes mellitus. Influence of body weight. Exp Clin Endocrinol Diabetes, 107:140 –147.

Wamberg S and Tauson A-H. 1998. Direct measurements of daily milk intake in suckling mink (*Mustela vison*) kits. J Nutr, 128:2620-22.

Wamberg S, Olesen CR, and Hansen HO. 1992b. Influence of dietary fat sources on lipid synthesis in the mink (*Mustela vison*) mammary tissue. Comp Biochem Physiol, 103A(1):199-204.

Wamberg S, Clausen TN, Olesen CR, and Hansen O. 1992a. Nursing sickness in lactating mink (*Mustela vison*): II. Pathophysiology and changes in body fluid composition. Can J Vet Res, 56:95-101.

Watt MJ, Howlett KF, Febbraio MA, Spriet LL, and Hargreaves M. 2001. Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehydrogenase activation and carbohydrate oxidation during moderate exercise in humans. J Physiol, 534(1):269-278.

Weijers RNM, Bekedam DJ, and Smulders YM. 2002. Determinants of mild gestational hyperglycemia and gestational diabetes mellitus in a large Dutch multiethnic cohort. Diabetes Care, 25:72-77.

Wess G and Reusch C. 2000. Evaluation of five portable blood glucose meters for use in dogs. J Am Vet Med Assoc, 216: 203-209.

Widmaier EP and Kunz TH, 1993. Basal, diurnal, and stress-induced levels of glucose and glucocorticoids in captive bats. J Exp Zool, 265(5):533-40.

Wolff SP, Jiang ZY, and Hunt JV. 1991. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radic Biol Med, 10(5):339-52.

Wood S and Trayhurn P. 2003. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Brit J Nutr, 89:3-9.

Wu LL, Chiuan-Chian C, Chang P-Y, and Wu JT. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta, 339:1-9.

Wu R, Lamontagne D, and de Champlain J. 2002. Antioxidative properties of acetylsalicylic acid on vascular tissues from normotensive and spontaneously hypertensive rats. Circulation, 105(3):387-92.

Yin M-J, Yamamoto Y, and Gaynor RB. 1998. The anti-inflammatory agents aspirin and salicylate inhibit the activity of IκB kinase-β. Nature, 396:77-80.

Youngman LD, Park JYK, and Ames BN. 1992. Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. Med Sci, 89(19): 9112–9116.

Yuan M, Konstantopoulos N, Lee J, Hansen L, Li Z-W, Karin M, and Shoelson SE. 2001. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of *Ikk*β. Science, 293:1673-77.

Zimmet P, Alberti KG, and Shaw J. 2001. Global and societal implications of the diabetes epidemic. Nature, 414: 782-787.

Zimmet P, Hodge A, Nicolson M, *et al.* 1996. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. Brit Med J, 313:965-969.