Fatty Liver Syndrome in Mink - Causes and Metabolic Consequences

by

Morag F. Dick

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ABSTRACT

This study investigated the effects of feeding intensity and dietary fat source on the development of fasting-induced fatty liver in mink. Mink were fed diets, containing either herring oil (n-3 PUFA), soya oil, (n-6 PUFA), or canola oil (n-9 MUFA) as the dietary fat source, at either 80% RDA or 120% RDA for a period of 10 weeks, followed by an overnight or 5-day fast. Fasting caused the mobilization of body fat reserves, increasing the hepatic delivery of fatty acids and resulting in the development of fatty liver. Increasing adiposity increased the severity of fatty liver, and is likely a result of increased visceral lipolysis. Increasing n-3 PUFA intake decreased the liver lipids in the mink fed at 120% RDA. Increasing n-3 PUFA content of the liver and mesenteric fat may help prevent the excessive accumulation of liver lipids by promoting hepatic fatty acid oxidation.

LIST OF ABBREVIATIONS AND SYMBOLS USED

A:G	Albumin: Globulin
AA	Arachidonic acid
AKPH	Alkaline phosphatase
ALA	Alpha linolenic acid
ALB	Albumin
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCS	Body condition score
BMI	Body mass index
CP	Crude protein
CF	Crude fat
CHO	Carbohydrate
DHA	Docosahexaenoic acid
DM	Dry matter
EPA	Eicosapentaenoic acid
F	Fasted
FA	Fatty acid
FAME	Fatty acid methyl ester
FI	Feeding intensity
FID	Flame ionization dectector
FHL	Feline hepatic lipidosis
FLS	Fatty liver syndrome
GC	Gas chromatograph
Glob	Globulin
GRA	Granulocyte
HCT	Haematocrit
HDL	High density lipoprotein
HGB	Haemoglobin
HIS	Hepatosomatic index
HOMA	Homeostasis model assessment
HSL	Hormone sensitive lipase
IU	International unit
LCPUFA	Long chain polyunsaturated fatty acids
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LYM	Lymphocyte
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
ME	Metabolizable energy
MON	Monocyte
MPV	Mean platelet volume
MUFA	Monounsaturated fatty acid
MS	Mass spectrometer

NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NEFA	Nonesterified fatty acid
NF	Non-Fasted
NH_3	Ammonia
PLT	Platelet
PPAR-α	Peroxisome proliferator activated receptor alpha
Pro: Pre	Product: Precursor
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
RDA	Recommended daily allowance
RDW	Red blood cell distribution width
ROS	Reactive oxygen species
SFA	Saturated fatty acid
T_3	Triiodothyronine
T_4	Thyroxine
TAG	Triacylglyceride
TAS	Total antioxidant status
TProt	Total protein
TNF-α	Tumor necrosis factor alpha
VLDL	Very low density lipoprotein
WBC	White blood cell

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CHAPTER 1. Introduction

Fatty liver syndrome (FLS) is a metabolic disorder in mink (*Neovison vison*), and has been a common pathological finding in ranched mink since the 1960's (Hunter and Barker 1996; Hunter 2008). The development of fatty liver (hepatic lipidosis) is typically asymptomatic and can occur quickly in mink (Hunter and Barker 1996; Bjornvad et al. 2004), leaving little time for intervention or treatment to occur. In addition, FLS tends to occur before the pelage development has finished resulting in a pelt that has little or no monetary value and a decrease in profits. Thus, the prevention of FLS is of considerable concern to mink producers.

Studying the similarities between mink FLS and other diseases involving fatty liver may help in better understanding the etiology and the development of effective methods to prevent fatty liver in mink. A strong resemblance between FLS in the mink and other mustelid species is observed with feline hepatic lipidosis (FHL) (Cornelius and Jacobs 1989) and non-alcoholic fatty liver disease (NAFLD) in humans (Adams 2005; Nieminen et al. 2009). Fatty liver has also been identified as a key contributor to the development of nursing sickness in mink (Rouvinen-Watt 2003). Excessive body condition is a common feature of mink with FLS (Hunter and Barker 1996), as well as in humans with NAFLD characterized by hepatic steatosis and cats with FHL (Marceau et al. 1999; Armstrong and Blanchard 2009). Furthermore, low levels of n-3 polyunsaturated fatty acids (PUFAs) or a higher n-6: n-3 PUFA ratio in humans and diets with insufficient essential fatty acids in cats have been shown to be involved in the development of hepatic lipidosis/steatosis (Szabo et al. 2000; Araya et al. 2004). Additionally, it is suggested that preventing excessive fat deposition and increasing dietary n-3 PUFAs to mink during the fall will help prevent the development of nursing sickness (Rouvinen-Watt 2003). This suggests that body condition and fatty acid nutrition may play a role in the development of fatty liver in mink.

Fasting has been shown to induce the accumulation of fat in mink liver and can be used as an experimental model to study the causes and consequences of fatty liver disease (Bjornvad et al. 2004; Mustonen et al. 2005a). In this model it has been shown that mink readily mobilize intra-abdominal fat depots (Mustonen et al. 2005a; Rouvinen-Watt et al. 2010), and preferentially mobilize n-3 PUFAs from the fat depots (Nieminen et al. 2006;

Rouvinen-Watt et al. 2010). Decreasing body fat and increasing n-3 PUFA reserves may aid in preventing excessive accumulation of lipid during fasting periods in mink.

The development of nutritional and management strategies to prevent the development of FLS would be of significant value to the mink industry. In addition, the mink may provide a potentially useful animal model for the study of etiological factors in fatty liver disease in humans and companion and zoo animals. To date, no research has been conducted to directly determine how previous dietary history in terms of feeding intensity and fatty acid nutrition affects the development of fatty liver in mink. As such, the purpose of this research is to determine the effects and role of feeding intensity and dietary fat source in the development of fasting-induced fatty liver in the mink.

CHAPTER 2. Literature Review

2.1 Structure and Function of the Liver

The liver is a vital organ in the body playing large roles in the metabolism of protein, glucose and lipids (Sjaastad et al. 2003). It also contributes to other functions in the body including the storage of iron and vitamins A, D, B_{12}, and K, degrading or conjugating toxic substances for excretion from the body, the urea cycle, and synthesizing and secreting bile (Cederbaum et al. 2004; Ross and Pawlina 2006).

Six distinct lobes are found in mink livers (Hunter and Barker 1996). Within these lobes, the liver is organized into liver lobules which are the classic functional unit of the liver (Figure 2.1). The lobules are hexagonal in shape with the points of the lobules containing portal triads, and the centre of the lobule containing the central vein. The portal triad contains the portal vein and hepatic artery which disperse blood throughout the liver and the bile ducts (Ross and Pawlina 2006).

The liver is unique in its blood supply compared to other organs; approximately 75% of the blood entering the liver is deoxygenated blood from the portal vein and the remaining 25% of the blood supplied to the liver is oxygenated blood from the hepatic artery (Ross and Pawlina 2006). Blood flows towards the central vein through the sinusoids, which are vascular channels in between the cords of hepatocytes that form the parenchyma of the liver (Ross and Pawlina 2006). The direction of blood flow leads to an oxygen gradient in the liver, with oxygen being the highest in the periportal region compared to the centrilobular area (Jungermann and Kietzmann 2000).

The sinusoidal endothelium is discontinuous with large gaps and fenestrate and also contains Kupffer cells, the liver's resident macrophages, as part of the lining (Wisse et al. 1996). The space of Disse is the area between the sinusoidal endothelium where the exchange of materials occurs between the blood and hepatocytes and hepatic stellate cells. The primary function of the hepatic stellate cells is the storage of vitamin A in the form of retinyl esters; however in certain pathological states these cells can differentiate gaining the characteristics of myofibroblasts (Iredale 2007). These cells play a large role in the development of liver fibrosis generating type I and III collagen in the perisinusoidal space (Iredale 2007).

Hepatocytes comprise 80% of the cell population within the liver, and are organized into plates one cell thick (Ross and Pawlina 2006). The average life span of a hepatocyte is 5 months, and rapid regeneration of these cells is seen during disturbances caused by disease, surgery or toxins (Ross and Pawlina 2006). Forming the parenchyma of the liver, hepatocytes are responsible for function such as lipid and glucose metabolism and bile formation. The bile produced by the hepatocytes is transferred from the cell to the canaliculus which two sides of hepatocytes face (Ross and Pawlina 2006). The secreted bile flows in the opposite direction of the blood, with the canaliculi joining to form intrahepatic ducts before joining the portal ducts located in the bile duct in the hepatic triad (Ross and Pawlina 2006).

Another structural unit seen when examining the liver lobule is the liver acinus. The acinus is a functional interpretation of the liver lobules that provides a better representation of blood perfusion, metabolic activity and pathology than the classic liver lobule (Ross and Pawlina 2006). The acinus is divided into three semi-circular zones by the distance from the adjoining hepatic triads to the central vein. Zone 1 is located in the first third of the acinus, which is located closest to the two triads, and receives the most oxygenated blood compared to zone 3 which is the furthest from the portal triads (Jungermann and Kietzmann 2000).

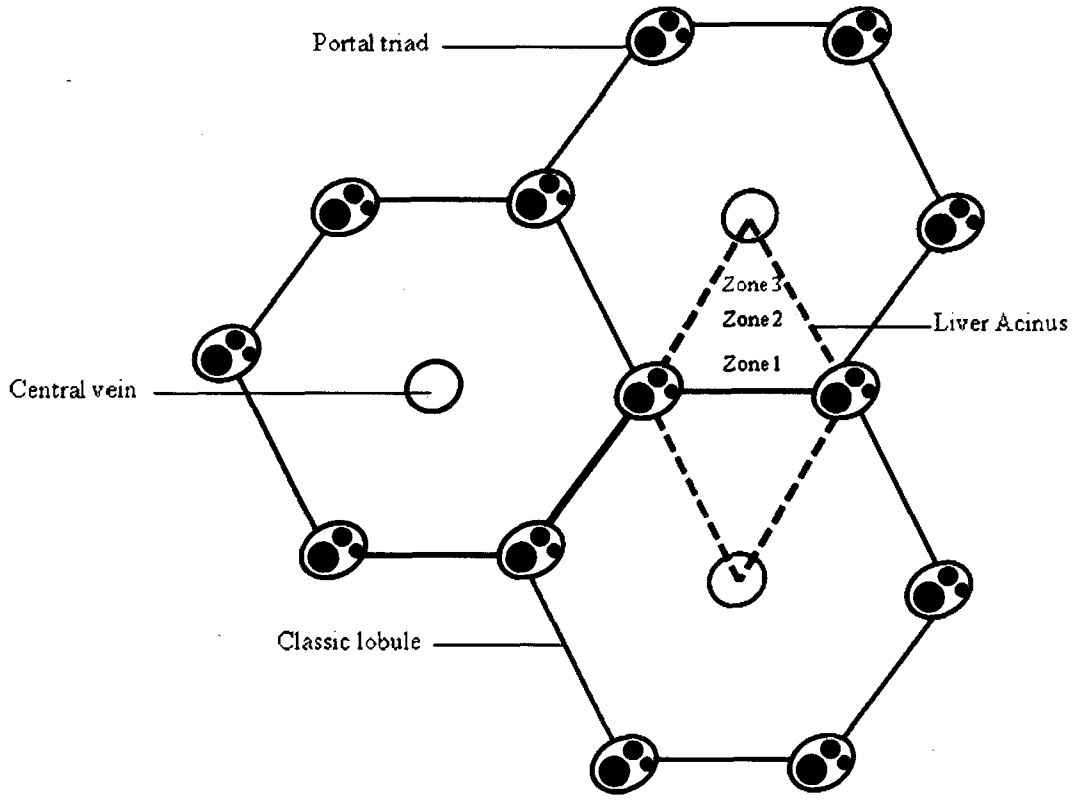

Figure 2.1. Classic lobule and liver acinus in relation to the central vein and portal triads.

2.2 Fatty Liver Disease

2.2.1 Stages of Fatty Liver Disease

Simple fatty liver, synonymous with hepatic steatosis or hepatic lipidosis, is the first stage of fatty liver disease where fat accumulates within the hepatocytes and no inflammation is present at this stage (Cullen et al. 2006). The fat accumulated is in the form of triacylglycerols (TAG) and is present in vacuoles within the hepatocytes (Hall et al. 1997). Two types of vacuoles can be present. The first type is macrovesicular steatosis, where one large vacuole of lipid is present and displaces the nucleus to the side of the cell (Day and James 1998a). The second type is microvesicular, where many small vacuoles of lipid are present but do not displace the nucleus from the centre of the cell (Jevon and Dimmick 1998). In humans, hepatic steatosis can be benign, and may or may not progress to the more chronic stages of fatty liver disease (Dam-Larsen et al. 2004). The accumulation of fat in the liver causes enlargement of the hepatocytes and compression of the sinusoids, which will decrease the efficiency of oxygen and nutrient delivery from the blood and may stimulate the development of inflammation (McCuskey et al. 2004).

Clinically, in carnivore species such as the mink and cats, the evaluation of fatty liver disease typically includes only the presence of lipid vacuoles or increased lipid content. Little information is available in these species with regards to the presence of the advanced stages of fatty liver disease, such as steatohepatitis, fibrosis and cirrhosis that is seen in humans.

Steatohepatitis is the progression of hepatic steatosis where hepatocyte death and inflammation are seen in combination with the already present hepatic steatosis (Clark et al. 2002). Ballooned hepatocytes and Mallory bodies are key characteristics in this stage. Hepatocyte ballooning is a structural manifestation of microtubular disruption and severe cell injury (Burt et al. 1998). Ballooned hepatocytes are enlarged and swollen and may contain Mallory bodies (Brunt, 2004). Mallory bodies are intracellular inclusions of protein aggregates within hepatocytes and are more commonly found in the advanced stages of steatohepatitis (Brunt, 2004).

Fibrosis and cirrhosis characterize the final stages of liver disease in humans and are present when the development of perisinusoidal, perivenular and bridging fibrosis occurs (Clark et al. 2002). The development of fibrosis can result in increased portal hypertension and liver failure (Iredale, 2007). The presence of inflammation within the liver from hepatocyte injury leads to the development of fibrosis which serves in the wound healing process (Iredale 2007). Hepatic stellate cells can be activated which results in the production of collagen, proliferation, extra-cellular matrix degradation and chemotaxis and leukocyte chemoattraction (Friedman 2000).

2.2.2 Fatty Liver Disease in Mustelids

FLS is a metabolic disease in mink caused by the accumulation of excessive TAG within hepatocytes making the liver friable and yellow in colour (Hunter and Barker 1996). Normal mink livers contain between 2 to 6.4 % fat, however in cases of FLS liver fat content may exceed 50% (Kannan et al. 2002; Koskinen and Lassén 2006; Mitchell and Rouvinen-Watt 2008). It has been reported that the frequency of FLS in autopsies performed on mink mortalities ranged from 2 to 11% (Koskinen and Lassén 2006). The true prevalence of FLS in mink herds is unknown as it can only be diagnosed post mortem and therefore may be present in mink that appear otherwise healthy. Weakness can sometimes be observed in mink; however FLS is typically asymptomatic (Hunter and Barker 1996). Fatty liver can be observed during periods of high stress such as whelping and weaning in the female mink and the furring out period in the fall (Hunter and Barker 1996). During these periods the animal may show a loss of appetite for a period of a few days causing rapid weight loss and resulting in accumulation of fat within the liver.

Liver lipid accumulation is also reported in lactating mink dams, with increased severity in dams affected with nursing sickness, a metabolic disorder which occurs during the latter part of lactation or shortly after weaning (Wamberg et al. 1992). Nursing sickness may be due to the collective physiological demands of milk production, excessive body tissue catabolism, insufficient feed intake and environmental stressors (Clausen et al. 1992). Affected dams show weight loss, lethargy, inappetance, and dehydration (Schneider and Hunter 1992). Examination of the livers revealed mild fatty

changes, but this was deemed a normal physiological phenomenon of lactation rather than pathological feature of nursing disease as it was seen in dams without nursing sickness as well (Clausen et al. 1992). Dysregulation of normal glucose metabolism is noted with affected dams having abnormally high blood glucose and insulin levels (Wamberg et al. 1992) suggesting that these dams have acquired insulin resistance (Rouvinen-Watt 2003).

Fatty liver in mink has been experimentally induced through food deprivation (Bjornvad et al. 2004; Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). Fasting has also been shown to increase fat accumulation within the liver in other mustelid species such as the sable, *Martes zibellina* (Mustonen et al. 2006a), and the European polecat, *Mustela putorius* (Nieminen et al. 2009). The mechanism believed to cause the development of fasting-induced fatty liver is similar to nursing sickness, where extensive mobilization of the body fat reserves occurs to meet energetic needs (Wamberg et al. 1992). However, the liver is unable to maintain sufficient fatty acid oxidation or lipid export to maintain homeostasis (Mustonen et al. 2005a, Nieminen et al. 2009). The regeneration capacity of the liver is also demonstrated in the fasting model. Recovery of the liver, and normalization of lipid content, occurs within one month following a 7-day fasting period (Rouvinen-Watt et al. 2010). The length of food deprivation is key in the accumulation of lipids within the liver and could explain why a short term fast of 48 hours in the American marten, *Martes americana*, did not cause the development of liver dysfunction (Nieminen et al. 2007). In mink, lipid accumulation begins within 2-5 days of fasting (Bjornvad et al. 2004; Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). Another possible distinguishing factor for the development of fasting-induced fatty liver is the species of animal. In the omnivorous raccoon dog, *Nyctereutes procyonoides*, a fasting period of 8 weeks did not lead to the development of liver dysfunction or lipid accumulation (Mustonen et al. 2006b).

Ferrets, a species closely related to mink and polecat, with diabetes mellitus show liver lipid accumulation (Benoit-Biancamano et al. 2005). Other cases of diabetes mellitus in ferrets describe elevated aspartate transaminase levels, indicating liver dysfunction in these animals (Carpenter and Novilla 1977). Both reports characterize the ferrets as emaciated, dehydrated and hyperglycemic, as well as undergoing periods of

anorexia or weight loss (Carpenter and Novilla 1977; Benoit-Biancamano et al. 2005). Lesions in the pancreas show disruption to normal insulin function. Insulin injections are used as the standard treatment and ferrets may respond initially, however this was not successful in the long term (Carpenter and Novilla 1977; Hillyer 1992; Benoit-Biancamano et al. 2005). The difficulty in managing one ferret with diabetes would be multiplied in a commercial mink farming setting, and thus is likely not a viable approach given the low success rate with treatment and labour costs. This further illustrates that long term management of clinical diabetes in ferrets is not an ideal situation nor viable commercially for mink, and that there is a need for preventing the development of the condition in both ferrets and mink.

2.2.3 Fatty Liver Disease in Other Species

Diseases involving fatty liver are also observed in other groups of species including NAFLD in humans (Adams et al. 2005) and FHL in domestic cats (Armstrong and Blanchard 2009), previously referred to as idiopathic feline hepatic lipidosis (Cornelius and Jacobs 1989). In both these cases, obesity has been reported for increasing the risk of development of the disease (Dimski and Taboada 1995; Wasserback York et al. 2009), and indicators of the development of insulin resistance are present (Abdelmalek and Diehl 2007).

FHL is the most common liver diseases in domestic cats (Griffin 2000a) and has been observed in 49% of feline liver biopsies in one study (Gagne et al. 1996). Obese house cats more commonly present with FHL (Dimski and Taboada 1995). The cause of FHL remains unknown, but it has been suggested to involve pathways of protein and lipid metabolism (Griffin 2000b). Sudden periods of anorexia, causing severe negative energy balance, are observed in the development of the condition, and are accompanied by impaired liver function, intrahepatic cholestasis and hepatocyte lipid accumulation (Griffin 2000a). The time frame for the development of lipidosis is likely proportional to the decrease in caloric intake, and may explain why it is often observed clinically in less than 1 week, and experimentally in two weeks (Armstrong and Blanchard 2009). Treatment of the condition involves reestablishing caloric intake, which usually requires

the insertion of feeding tubes (Griffin 2000b). High mortality, close to 100%, has been found in clinical cases for cats that do not receive aggressive nutritional therapy (Jacobs et al. 1989). Experimentally, obese cats under extremely energy restricted diets accumulated the most fat within the liver when fed diets containing low quality protein and corn oil in comparison to higher quality protein and fat diets (Szabo et al. 2000).

Human NAFLD includes a spectrum of 4 types, with simple steatosis as type 1, steatosis and inflammation as type 2, steatosis with hepatocytes injury or ballooning degeneration as type 3 and steatosis with sinusoidal fibrosis and Mallory bodies as type 4 (Matteoni et al. 1999). Nonalcoholic steatohepatitis (NASH) is the most severe form and is composed of type 3 and 4 NAFLD, and is associated with adverse outcomes such as cirrhosis, and advanced liver disease often leading to death (Bugianesi et al. 2002). Development of the disorder is associated with the metabolic syndrome, diabetes mellitus, obesity and diet (Marceau et al. 1999; Toshimitsu et al. 2007). There is currently no specific pharmaceutical treatment for NAFLD although changes in diet, weight loss and lifestyle are recommended (Postic and Girard 2008). The use of insulin sensitizing drugs to restore insulin sensitivity can be effective, but this is limited by weight gain and adiposity (Postic and Girard 2008).

2.3 Diagnosis of Fatty Liver

2.3.1 Histological Findings

A liver biopsy or necropsy is the gold standard for the diagnosis and staging of the progression of fatty liver diseases (Brunt et al. 1999). Fasting-induced fatty liver is characterized by moderate to severe (33-100%) macrovesicular steatosis in mink (Pal et al. 2008) and increases in both macrovesicular and microvesicular steatosis in polecats (Nieminen et al. 2009). It was also noted that lobular inflammation, hepatocellular ballooning, and borderline steatohepatitis was present in a few of the fasted polecats but not enough to reach statistical significance. In humans, NAFLD is predominately macrovesicular steatosis, but may have microvesicular steatosis present as well (Brunt 2004).

The location of lipid accumulation within the liver lobules or acini in mink with fatty liver syndrome or fasting-induced fatty liver has not been reported. In other fatty liver diseases, the location of the lipid accumulation within the liver lobule is variable, with the most common types in adult NAFLD being centrilobular, panacinar and azonal, and less than 1% demonstrate having steatosis located only in the periportal region (Chalasani et al. 2008). This pattern was also observed in the livers of lactating mink with and without nursing sickness, where the mild fatty changes occurred in the centrilobular zone (Clausen et al. 1992), while the histological examination of an adult domestic ferret with diabetes mellitus showed periportal lipid accumulation (Benoit-Biancamano et al. 2005).

2.3.2 Clinical Chemistry Indicators

Changes in the clinical chemistry are often seen with the development of fatty liver. Alterations to lipid metabolism are also observed in humans with elevated serum levels of non-esterified fatty acids (NEFAs), very low density lipoproteins (VLDL), and lower levels of high density lipoprotein (HDL) (Cortez-Pinto et al. 1999). This differs in the mink where fasting models of fatty liver indicate decreases in HDL, but no change in VLDL levels (Mustonen et al. 2005a, 2009). Elevated glucose and insulin levels are observed in obese cats, and cats with FHL, mink dams diagnosed with nursing sickness and human NAFLD, indicating derangement of glucose metabolism (Cornelius and Jacobs 1989; Wamberg et al. 1992; Cortez-Pinto et al. 1999). Indications of decreased insulin sensitivity are not observed in fasting mink or polecats (Mustonen et al. 2005a, 2009; Rouvinen-Watt et al. 2010).

Changes in the clinical chemistry indicators are often reported with the development of fatty liver in mink. In some, but not all cases of hepatic lipidosis, the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are elevated in the plasma (Mofrad et al. 2003). Within the fasting-induced model of fatty liver this has been observed in mink (Mustonen et al. 2005a; Rouvinen-Watt et al. 2010), but not in the polecat (Mustonen et al. 2009). Nor was this observed in calorically restricted obese cats undergoing weight loss (Szabo et al. 2000), but mild to moderate

increases may be observed in cats with FHL (Cornelius and Jacobs 1989). This may be a result of species differences, or as a result of the variability in ALT and AST in predicting liver health (Mofad et al. 2003). Plasma alkaline phosphatase (AKPH) is another indicator of liver health with elevated levels associated with dysfunction in bile production and secretion (Giannini et al. 2005). Observations of fasted mink found increased AKPH after 5 days of fasting (Mustonen et al. 2005a), but the increase was not large enough to be of physiological significance (Giannini et al. 2005). This differs from FHL where an increase of 5 to 15 times normal AKPH levels maybe observed (Cornelius and Jacobs 1989)

2.3.3 Tissue Fatty Acid Composition

Changes in the fatty acid composition of the body are noted in fasted mustelids and humans with NAFLD (Araya et al. 2004; Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). Lower levels of n-3 PUFAs in the livers and fat depots of mink and polecats are observed, while there is an increase in plasma levels of n-3 PUFAs and decrease in n-6 PUFAs during fasting (Nieminen et al. 2006, 2009). This finding is also similar to that seen in humans with NAFLD where these patients have lower levels of n-3 PUFAs in the body and higher levels of n-6 PUFAs (Araya et al. 2004). Increased mobilization of n-3 PUFAs occurs during fasting resulting in increased plasma n-3 PUFA levels (Nieminen et al. 2006, 2009). Changes in the proportion of n-3 PUFA in the liver was found to be the most severe in female mink, which may increase the risk of developing fatty liver or liver inflammation (Rouvinen-Watt et al. 2010). Less is known about changes in fatty acid composition in felines, but a significant decrease in the total n-3 PUFAs is observed in the adipose tissue for cats with FHL, and lower, but insignificant, levels within the liver (Hall et al. 1997).

2.4 Metabolic Changes Associated with Fatty Liver

Mustonen et al. (2005b) found an increase in protein catabolism accompanied by elevated plasma levels of urea, uric acid and ammonia when mink are subjected to food deprivation. Cats, another carnivorous species, do not have as dynamic of an ability to

decrease protein catabolism on low protein diets compared to omnivores and herbivores (Russell et al. 2003). The inability to down regulate protein catabolism during periods of protein scarcity could overpower the functioning of the urea cycle. Failure of the urea cycle can lead to hyperammoneima, a toxic condition that may cause irritability, cognitive impairment, seizures, coma, or death (Cagnon and Braissant 2007). Similarities between these symptoms are also reported in female mink with nursing sickness (Clausen 1992) and cats with FHL (Griffin 2000a).

Arginine is required for the urea cycle and is an essential amino acid for cats (Rogers and Phang 1985). A deficiency in arginine has been suggested to occur during periods of fasting in mink and polecats based on the observations that fasting results in a relative decrease in plasma arginine levels (Mustonen et al. 2005b, 2009). One of the consequences of an arginine deficiency is that orotic acid may accumulate, from an insufficient supply of arginine for the urea cycle (Cornelius and Jacobs 1989). Increased levels of orotic acid may impair the secretion of fat from the liver (Cornelius and Jacobs 1989). It has been suggested that the consequences of protein catabolism in mink and polecats during fasting could negatively impact the liver by limiting an increase in the formation of VLDL and LDL (Nieminen et al. 2009, Rouvinen-Watt et al. 2010).

2.5 Lipid Metabolism in the Body

Under normal metabolic circumstances there is a continuous cycling of fatty acids (FA) between the liver and adipose tissue; an alteration to this cycling could lead to the accumulation of lipid within the liver (Cornelius and Jacobs, 1989), and may increase the severity of the lipid accumulation.

2.5.1 Lipid Transport

Within the liver, NEFAs are esterified into TAG and packaged into VLDLs for export from the liver (Sjaastad et al. 2003). The synthesis, assembly and secretion of VLDLs occurs in the rough endoplasmic reticulum of the liver and requires apolipoprotein B-100 (Mason 1998). Once the VLDLs are released into the blood stream a lipoprotein lipase (LPL) residing in the capillary endothelium of adipose and muscle

tissue hydrolyzes the TAG releasing NEFAs and one glycerol molecule (Wu et al. 2003). This process turns the VLDLs into LDLs (Sjaastad et al. 2003). The NEFAs can then enter the adipocyte where they are repackaged into TAGs for storage.

2.5.2 Lipid Deposition

In addition to serving as energy storage, the white adipose tissue depots in the mink are required for thermal insulation during the winter, especially important for the mink as a long, lean semi-aquatic mammal (Mustonen et al. 2005a). Subcutaneous fat represents 83-86% of the mink's total body fat, while the perirenal fat is only 3-4% of the total. In contrast, the sable, a terrestrial mustelid, has much less (64%) of the total body fat in the subcutaneous depots, and more around the kidneys (15%) (Mustonen et al. 2005a, 2006a).

The FA profiles also differ among the body fat depot. Mustonen et al. (2007) found that in the mink, the proportions of saturated fatty acids (SFA) in the intra-abdominal fat depot were higher and lower proportions of monounsaturated fatty acids (MUFA) were found when compared with other fat depots. Additionally, a higher n-6: n-3 PUFA ratio was observed in the intra-abdominal fat when compared to the subcutaneous fat (Mustonen et al. 2007). The alteration in the n-6: n-3 PUFA ratio was the result of a decreases in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and increase in linolenic acid (LA) in the intra-abdominal fat depots compared to the subcutaneous fat (Mustonen et al. 2007). Dietary fat also plays a role with the fatty acid profile of the diet reflected in the profile of the fat depots (Rouvinen and Kiiskinen 1989; Käkelä and Hyvärinen 1998).

2.5.3 Lipid Mobilization

The mobilization of TAGs from WAT is controlled by hormone sensitive lipase (HSL) which catalyzes the hydrolysis of TAGs (Raclot 2003). Glucagon and epinephrine increase the activation and expression of HSL and thus promote the mobilization of TAG; insulin has the opposite effect, inactivating HSL and activating LPL increasing adipocyte TAG storage (Sjaastad et al. 2003).

Selective mobilization of fatty acids is observed in mink and polecats during fasting (Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). HSL preferentially hydrolyzes fatty acids that are shorter in chain length (Raclot et al. 2001), have a higher degree of unsaturation, or that have the double bond located closer to the terminal methyl end of the fatty acid chain (Raclot and Groscolas 1995). This pattern of fatty acid mobilization was observed in the mink and polecats with the changes most dramatic in the intra-abdominal fat (Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). The effect of fat depot on the mobilization of different fatty acids is also apparent in the subcutaneous fat depot where only minor changes are observed, and this depot is also poorly mobilized (Mustonen et al. 2005a).

The ability to mobilize fat stores during prolonged periods of food deprivation, ranging from 2 to 7 days, in the mink appears to be poorly developed. Mustonen et al. (2005a) found that during a 7-day fast, the weights of the omental and subcutaneous fat depots and lipase activities did not differ between fasted and non-fasted mink. It was observed in this study that the perirenal fat was mobilized more extensively than other fat depots (Mustonen et al. 2005a). Sexual dimorphism in size may also lead to differences in the mobilization of fat depots during food deprivation. In fasted polecats, only females showed a significant reduction in the intra-abdominal reserves compared to non-fasted counterparts (Mustonen et al. 2009). This may be a result of an increase metabolic rate due to smaller body size and higher heat loss in female polecats, which would also be the case for female mink (Korhonen and Niemelä 1998). The poor capacity to withstand fasting is also supported by the mink entering the final phase of starvation after 7 days of fasting as evidenced by pronounced muscle tissue catabolism (Mustonen et al. 2005b).

2.5.4 Lipid Oxidation

Fatty acids are oxidized in the liver and other tissues to yield energy through β-oxidation. For fatty acid oxidation to occur the fatty acid must enter the hepatocyte mitochondria, where it follows a biochemical pathway leading to the formation of acetyl-CoA, and then enter the Krebs cycle (Sjaastad et al. 2003). Long chain PUFAs are too long to enter the mitochondria initially, and undergo peroxisomal β-oxidation to be

shortened to 16 to 18 carbons in length prior to entering the mitochondria (van Roermund et al. 1998). It should be noted that fatty acids of 18 carbons or fewer carbons are likely to be stored in adipose tissue and more likely to be oxidized, due to being the preferred substrate for mitochondrial β-oxidation (Brody 1999).

The location of the hepatocyte within the liver lobule also influences its role in fatty acid oxidation. Hepatocytes located in the periportal, zone 1, of the liver have increased mRNA expression of key enzymes involved in fatty acid degradation in comparison to centrilobular, zone 3, hepatocytes (Braeuning et al. 2006). This may help explain observational differences in lipid metabolism in the liver, which results in increased fatty acid oxidation in the periportal zone (Guzman et al. 1995).

During periods of food deprivation up-regulation of β-oxidation is needed to maintain energy levels. Peroxisome proliferator activated receptor-α (PPAR-α) increases the expression of genes involved in fatty acid oxidation resulting in increased fatty acid oxidation and decreased accumulation of fat in the liver (Hashimoto et al., 2000). Ketogenesis is hypothesized to be limited in the mink due to insufficient β-oxidation (Mustonen et al. 2005b). This would have a negative impact on maintaining metabolic fuel for the animal and may lead to further protein catabolism and an accumulation of lipids delivered to the liver for oxidation.

2.6 Glucose Metabolism

Being strict carnivores, mink consume a diet low in carbohydrates compared to omnivore species. When provided with a carbohydrate free diet, blood glucose levels can be maintained provided the mink has enough substrates, such as amino acids from dietary protein, for gluconeogenesis to occur within the liver (Fink and Børsting 2002). Mink also have the ability to maintain glucose levels during fasting (Mustonen et al. 2005a).

Insulin is the primary hormone responsible for the regulation of blood glucose levels in mammals. Insulin stimulates the entry of glucose into cells, and lowers blood glucose levels (Sjaastad et al. 2003). Glucagon opposes the actions of insulin and increases hepatic glucose production and decreases glycogen synthesis (Chibber et al.

2000). Long term stress, such as starvation, can increase cortisol levels which increases protein catabolism to provide amino acids for gluconeogenesis (Sjaastad et al. 2003).

2.7 Regulation of Mink Body Weight

The body condition of mink changes throughout the course of the year, with higher body weights evident during November and December, with a decline just prior to breeding and lowest weights during the summer months (Korhonen and Niemelä 1997, 1998). During the fall months, preparation for winter begins with the deposition of fat in the subcutaneous, mesenteric and inguinal fat depots (Rouvinen and Kiiskinen 1989). The increased deposition of this fat is controlled by rising melatonin levels and ensures that the animal has both insulation and energy reserves for the winter months (Nieminen et al. 2001). Administration of exogenous melatonin increases the levels of thyroxine (T_4) and leptin, which regulates appetite and metabolism (Mustonen et al. 2000). Leptin is a feedback signal that provides information about the body's energy reserves (Friedman and Halaas 1998). Increasing leptin levels are correlated with increasing body mass index (BMI) (Tauson et al. 2002; Maffei et al. 1995).

In wild type mink, natural weight regulation has been observed in farmed conditions through decreased feed intake resulting in ideal body condition for breeding season (Korhonen et al. 1989). However, since obese mink of different colour types are observed during breeding season (Tauson 1985), this suggests that genetic selection pressures may have negatively altered the ability of mink to voluntarily regulate their body weight (Korhonen et al. 1989).

2.8 Development of Fatty Liver: Two Hit Hypothesis

For the development of fatty liver disease in humans, a two-hit model for the progression of hepatic steatosis to steatohepatitis was originally proposed by Day and James (1998b). In this model, the first hit produces simple steatosis, while the second hit is oxidative stress capable of causing lipid peroxidation leading to steatohepatitis (Day and James 1998b). The model has since been updated to include a role for NEFAs in causing both the first and seconds hits (Day 2002). Using this model, we can gain an

understanding of how different factors can come together to cause the development of FLS, and the subsequent progression through the different stages of FLS can be shown. Factors that are known to be involved in FLS include obesity, insulin resistance, and sudden weight loss, the ratio between n-3 and n-6 PUFAs and oxidative stress.

2.8.1 Insulin Resistance and Obesity

The four components of the metabolic syndrome X (fat distribution, impaired glucose tolerance, hypertension and dyslipidemia) have been shown in human to correlate with severity of steatosis (Marceau et al. 1999). In mink, high feeding intensities lead to an increase in body condition and elevated blood glucose levels and the development of hyperinsulinemia (Rouvinen-Watt et al. 2004). Similarly, mink observed to have fatty liver are often obese (Hunter and Barker, 1996). Increasing adiposity can cause an increase in plasma NEFA (Jensen et al. 1989) and tumour necrosis factor-α (TNF-α) (Kern et al. 1995) and a decrease in adiponectin levels (Ryan et al. 2003). TNF-α can modulate insulin responsiveness by reducing the efficiency of insulin receptor binding (Yuan et al. 2001). Impairment of the insulin signalling in adipose tissue will lead to an increase in lipolysis and further cycling of TNF-α with NEFAs. Adiponectin is associated with increased glucose regulation, fatty acid catabolism and decrease in lipogenesis and anti-inflammatory properties (Yamauchi et al. 2007).

Hepatic insulin resistance in the liver may result in an effort to resist further steatosis from enduring prolonged elevated plasma NEFA levels (Day 2002). Increasing accumulation of NEFAs within non-adipose tissues such as the liver, leads to lipotoxicity causing insulin resistance (Kahn and Flier 2000). Thus obesity leads to the development of the first hit, steatosis, whereas the decreasing insulin sensitivity and responsiveness continues the progression of steatosis.

2.8.2 Weight Loss

Sudden weight loss, either from fasting or severe caloric restriction, plays a role in the development of steatosis. FLS in mink is associated with periods of inappetence for one or more days that may result in the mobilization of body fat into the liver (Hunter and

Barker 1996). This is observed experimentally in mink fasted for 5 days and show an accumulation of TAGs within the liver (Bjornvad et al. 2004; Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). Increased hepatic lipid content can also occur in obese cats with restrictive feeding of 25% the maintenance energy requirement if the diet does not include sufficient protein and long chain essential fatty acids (Szabo et al. 2000). This situation could also theoretically occur in mink production if the mink are forced to slim down quickly in preparation for breeding season during the winter months (Damgaard et al. 2004). Fatty acids being mobilized from the visceral fat depots will directly enter the liver via the portal vein (Smith and Shenk 2000). This is of concern, as these depots are readily mobilized in mink and as such may lead to an increase in hepatic lipid content during negative energy balance (Mustonen et al. 2005a). A similar concern is found in FHL. Armstrong and Blanchard (2009) proposed that the liver lipid accumulation may be more the result of dramatic lipolysis caused by weight loss rather than insufficiencies in lipid excretion or oxidation in the liver.

2.8.3 n-3 and n-6 PUFAs

A study comparing the n-3 long chain PUFAs (LCPUFAs), DHA and EPA, content of steatosis and steatohepatitis NAFLD patients found lower levels of DHA and EPA in their tissues compared to healthy controls (Araya et al. 2004). In the same study, it was also shown that NAFLD patients had a significantly higher liver n-6: n-3 PUFA ratio. It is suggested that a deficiency of n-3 PUFAs, particularly EPA, or a high n-6: n-3 PUFA ratio can result in conditions that favour increased hepatic fatty acid synthesis and decreased secretion leading to an accumulation of TAGs within hepatocytes (El-Badry et al. 2007).

Dietary n-3 PUFAs have been shown to prevent the development (Alwayn et al. 2005) or ameliorate hepatic steatosis (Cappani et al. 2006). An increase in insulin sensitivity and decrease in fat deposition in response to dietary n-3 PUFAs has also been shown (Takahashi and Ide 2000). Inconclusive results on the effects of n-3 PUFAs on liver lipid content in mink have been reported. Käkelä et al. (2001) found that when mink were fed diets formulated with capelin oil, containing high levels of n-3 LCPUFAs and

low levels of n-6 PUFAs, higher level of lipids in the liver were observed, while Damgaard et al. (2000) found no difference in liver lipid content with feeding high levels of marine lipids.

Within the liver, n-3 PUFAs function as transcription factors that upregulate PPAR-α (Levy et al. 2004) and secretion of apolipoprotein B-100, which is required for the structural formation of VLDL (Lindén et al. 2002). An increase in apolipoprotein B-100 secretion can also be observed with n-3 PUFAs (Carlsson et al. 2001). A decrease in lipogenesis is also observed from a decrease in the transcription factor sterol regulatory element binding protein-1, which upregulates lipogenic genes for fatty acid synthetase and stearoyl Co-A desaturase (Levy et al. 2004).

A role in preventing the second hit, the development of steatohepatitis, can also be attributed to n-3 PUFAs. Inflammation caused by chemically induced hepatitis was shown to be decreased with n-3 PUFAs and was associated with a decrease in pro-inflammatory cytokines such as TNF-α, interleukin-1β, interferon-γ and interleukin-6 (Schmocker et al. 2007). Meanwhile, a high intake of n-6 PUFAs has been suggested to shift the body into a pro-inflammatory state with increased vasoconstriction (El-Badry et al. 2007), increased blood glucose levels and poorer glucose tolerance when compared to n-3 PUFAs in rats (Takahashi and Ide 2000).

2.8.4 Oxidative Stress

Oxidative stress is thought to be a potential cause of the second hit in the two-hit hypothesis proposed by Day (2002). Reactive oxygen species (ROS) and lipid peroxidation could lead to apoptosis, the development of megamitochondria, hepatic stellate cell activation and the formation of Mallory bodies (Pessayre et al. 2001). Sources of ROS in steatohepatitis are associated with the metabolic syndrome and include NEFAs and cytokines such as TNF-α and interleukin-6 (Day 2002). Videla et al. (2004) suggested that oxidative stress plays a dual role in FLS. PUFAs are highly vulnerable to oxidative attack due to a high degree of desaturation. Therefore, a deficiency in n-3 LCPUFAs could result from oxidative attack from ROS and would further the progression of FLS (Videla et al. 2004).

2.9 Dietary Influence

2.9.1 Fatty Acid Nutrition

As generalists in the wild, mink prey upon locally available food sources (Eagle and Whitman 1999). As a result of being able to forage in aquatic and terrestrial habitats, fish have an important role in the diet of mink and may contribute up to 50% of the diet (Wise et al. 1981, Chanin and Linn 1980). The diets of wild and feral mink would theoretically be rich in DHA and EPA and have a low n-6: n-3 PUFA ratio as a result of the prominent role fish plays in the diet (Hearn et al. 1987).

Currently there are no guidelines for the fatty acid profile of oils and fats used in mink feeds (Rouvinen-Watt et al. 2005). Analysis of standard diets fed to mink in research settings provides an n-6: n-3 PUFA ratio of 2.85 to 3.3 (Käkelä et al. 2001; Rouvinen-Watt et al. 2010). Many dietary fat sources are available for use in mink diets, which impacts the ultimate fatty acid profile on the diet. Changing the dietary fat source from broiler offal or beef and pork fat to a fish based oil, such a capelin, can result in a dietary n-6: n-3 PUFA ratio change of 3.1 to 1.3 (Käkelä et al. 2001). Maintaining a dietary n-6: n-3 PUFA ratio of 1-4:1 has been suggested for the prevention of n-6: n-3 PUFA related inflammatory disorders in humans (Simopoulos 1991).

The shortest of the n-3 PUFAs, α-linolenic acid (ALA), is an essential fatty acid in all animals and through desaturation and elongation can be synthesized into EPA and DHA. Käkelä et al. (2001) stated that the ability of mink to desaturate and elongate ALA to EPA and DHA is limited. As such, vegetable oils, such as linseed oil, would not provide adequate quantities of EPA and DHA to the mink. The specific ability of the mink to desaturate and elongate linoleic acid and ALA is unknown. Similarities may be present with the cats, which lack the Δ6-desaturase enzyme needed to synthesize both EPA from ALA and arachidonic acid (AA) from linoleic acid (Rivers et al. 1975). Good dietary sources of EPA and DHA for mink are found in marine sources such as fish oil as recommended by Käkelä et al. (2001). The studies to determine the requirements for long chain n-3 PUFA in cats have also not been performed, but an adequate combined intake for DHA and EPA of 0.1 g/kg diet dry matter (DM) for diets of 4000 kcal/kg has been suggested for adult maintenance (NRC 2006).

The n-6 PUFA class of fatty acids is also involved in eicosanoid production. AA and dihomo-γ-linolenic acid are essential for the production of the eicosanoids involved in the inflammatory response such as prostaglandin E_2 (Day 2002). With a large dietary intake of n-6 PUFA an increase in the eicosanoid production from n-6 PUFAs can exceed those formed from EPA, and would promote inflammation. Sources of n-6 PUFAs include corn oil, and soya oil (NRC 2006), AA sources for carnivores may also come from meat products (Li et al. 1998). Diet recommendations for cats have been made for n-6 PUFAs, with a recommended allowance of 0.06 g AA/ kg DM, and safe upper limit of 0.2 g AA/kg DM for diets containing 4000 kcal/kg DM (NRC 2006).

MUFAs are produced in the body through inserting a double bond in the n-7 or n-9 position on 16 and 18 carbon length fatty acids (NRC 2006). The most common form of MUFAs is oleic acid (18:1n-9) (Kris-Etherton 1999). There are no dietary requirements for n-9 MUFAs, but fat sources include canola, olive and high MUFA sunflower oil. The n-9 MUFAs have similar abilities to PUFAs in increasing membrane fluidity, but do not alter the n-6: n-3 PUFA ratio (Vongild et al. 1998; Elliot and Elliot 2005). As such, n-9 MUFAs do not influence the eicosanoid production, and may be considered neutral fatty acids in this context.

2.9.2 Restricted Feeding

After reaching their heaviest body weight in early winter, a decrease in feed intake prior to breeding season occurs naturally under some production circumstances (Korhonen et al. 1989). However, problems arise with high energy feeds and *ad libitum* feeding, resulting in obese animals that require slimming prior to flushing in preparation for breeding (Damgaard et al. 2004). If the weight loss is too excessive before the breeding season it can impair litter size, and similarly if the animals remain too obese a decrease in reproductive success is also observed (Tauson and Alden 1984). The servere caloric restricition needed to reach breeding condition can also have negative repercussions on regulation of an ideal body weight in mink after weight loss (Clausen et al. 2007). A better strategy may be to use a more prolonged and milder restricted feeding regimen, thereby preventing excessive weight gain from occurring during autumn.

Females who were mildly restrictively fed from October till breeding in March have better whelping results than those fed intensively (Wenzel and Schickentanz 1980). This may be the result of preventing excessive weight and the corresponding weight loss needed for breeding. Thus, from a management perspective, preventing excessive autumnal fattening may alleviate problems associated with weight management and the health risks accompanying increased body condition.

The degree to which the feed is restricted should also be taken into consideration to prevent excessive lipolysis in animals. A diet restricted to 25% of the normal energy requirement may induce liver lipid accumulation in cats (Szabo et al. 2000). A more mild restriction of 15-20% RDA may not have negative physiological or reproductive effects (Rouvinen-Watt et al. 2004; Damgaard et al. 2004).

2.10 Conclusions

In conclusion, the inclusion of n-3 PUFAs and prevention of obesity may lower the risk of the development of fasting-induced fatty liver in mink. Prevention of the development of obesity and insulin resistance through control of caloric intake would prevent elevated NEFAs and thus help maintain insulin sensitivity and responsiveness throughout the body. It is also likely that n-3 PUFAs would help maintain normal hepatic fat metabolism and prevent inflammation that a high n-6 PUFA intake would cause. Dietary n-3 PUFAs from fish oil would supply the n-3 LCPUFAs EPA and DHA and lower the n-6: n-3 PUFA ratio and replenish n-3 PUFA that are lost due to peroxidation and increased mobilization during fasting.

2.11 Objectives and Hypotheses

The overall objective of this project is to characterize the development of fasting-induced fatty liver syndrome in the mink and investigate the roles of fatty acid nutrition and body condition in the etiology of fatty liver syndrome. Specifically, the first objective is to determine the effect of diets containing high levels of n-3, n-6 or n-9 PUFA on the development of hepatic lipidosis and fat mobilization during food deprivation. The

second objective is to determine the effect of feeding intensity on the development of hepatic lipidosis and fat mobilization during food deprivation.

It is hypothesized that feeding of a diet high in n-3 PUFA will improve the n-6: n-3 PUFA ratio in the liver tissue and will decrease the severity of the fasting-induced fatty liver syndrome in mink. The fatty acid profiles of the diet will be reflected in the mobilized FAs from the fat depots. Mink that are overfed will show an increase in the severity of fasting-induced fatty liver syndrome when compared to the mink that are fed according to a restricted feeding regime. Overfed mink will show an increase in fat mobilization from intra-abdominal fat depots.

CHAPTER 3. Effect of Dietary Fat Source and Feeding Intensity on the Development of Fasting-Induced Fatty Liver in the Mink (*Neovison vison*)

3.1 Introduction

The development of FLS in mink has been linked to obesity and highly stressful periods of the production season causing mink to go off feed for a period a few days (Hunter and Barker 1996). Experimentally, fatty liver can be induced through fasting resulting in liver lipid accumulation in as little as two days of food deprivation (Bjornvad et al. 2004; Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). The development of fatty liver disease in humans and cats is associated with obesity (Marceau et al. 1999; Armstrong and Blanchard 2009). However, a high n-6: n-3 PUFA ratio and low n-3 PUFA profiles in humans and insufficient dietary supply of essential fatty acids in cats may negatively influence liver metabolism promoting lipid accumulation in the liver (Szabo et al. 2000; Araya et al. 2004). Obesity and a deficiency in n-3 PUFAs has also been suggested to play a role in the development of nursing sickness in lactating female mink (Rouvinen-Watt 2003).

During the autumn months, mink prepare for the winter months by increasing their body fat reserves in both the subcutaneous and intra-abdominal depots (Rouvinen and Kiiskinen 1989). Preventing overconditioning in the mink may help avoid the excessive mobilization of these fat reserves during periods of negative energy balance or inadequate feed intake. Of these fat reserves, the intra-abdominal fat is rapidly mobilized (Mustonen et al. 2005a). Furthermore, prior feeding of diets high in n-3 PUFAs may help maintain normal liver function during periods of over nutrition or food deprivation (Rouvinen-Watt et al. 2010).

This study focuses on the effects of feeding intensity and dietary fat source in fasting-induced fatty liver (hepatic lipidosis) through the evaluation of liver health, clinical chemistry and endocrinology. As well, the study examines the effects of feeding history of non-fasted animals with respect to feeding intensity and dietary fat source. It is hypothesized that mink fed at above the RDA, will become obese, and exhibit more severe hepatic lipid accumulation following fasting than mink fed below the RDA. Also,

it is anticipated that increasing dietary intake of n-3 PUFAs may help reduce the severity of fatty liver in these animals.

3.2 Materials and Methods

3.2.1 Animals and Experimental Treatments

Seventy-two (72) standard dark juvenile mink, half male (36) and half female (36) were used in this study. The mink were housed in breeder style cages at the Canadian Centre for Fur Animal Research located at the Nova Scotia Agricultural College. The trial was conducted from September to December 2007. All procedures and husbandry practices were in accordance with the Canadian Council of Animal Care (CCAC 1993), and were approved by the Animal Care and Use Committee of the Nova Scotia Agricultural College.

The mink were selected from six (6) dam's dams. The average whelping date was used to group the dam's dam by age: early, mid and late whelping. One male and one female from each of the dam's dams groups were randomly allocated to an experimental diet and feeding intensity combination (Figure 3.1).

The experimental diets consisted of a basal diet supplemented with one of three fat sources: herring oil (n-3 PUFA), soya oil (n-6 PUFA), or canola oil (n-9 MUFA) (Table 3.1). Due to the high unsaturated fatty acid content of the diets, the premix was supplemented with an additional 100 IU vitamin E/ tonne wet feed. Each diet was fed at two feeding intensities: a low feeding intensity of 80% of the recommended dietary allowance (RDA) and a high feeding intensity of 120% RDA. The RDA guidelines used were specified for sex and month of the year (Rouvinen-Watt et al. 2005) and further adjusted for the individual metabolic body weight (body weight$^{0.75}$) of the mink. The mink were fed once a day with each individual mink's portion weighed out, and any leftover feed removed the next day prior to feeding.

After being fed the diet regime for a period of 10 weeks, one dam's dams group from each age group (early, mid and late) was selected to undergo an overnight fast of 14 to 16 hours prior to sampling and served as the control (non-fasted, NF). The other dam's dams groups underwent a 5-day fasting period (fasted, F) to induce hepatic lipidosis prior to sampling. One week prior to beginning the fasting period, the F mink were placed in

isolation in a quarantine shed and remained there for the fasting period. While in isolation, the mink were provided with covered nest boxes. Throughout the fasting period the mink had twice daily health monitoring by staff and a daily visual inspection by two licensed veterinarians.

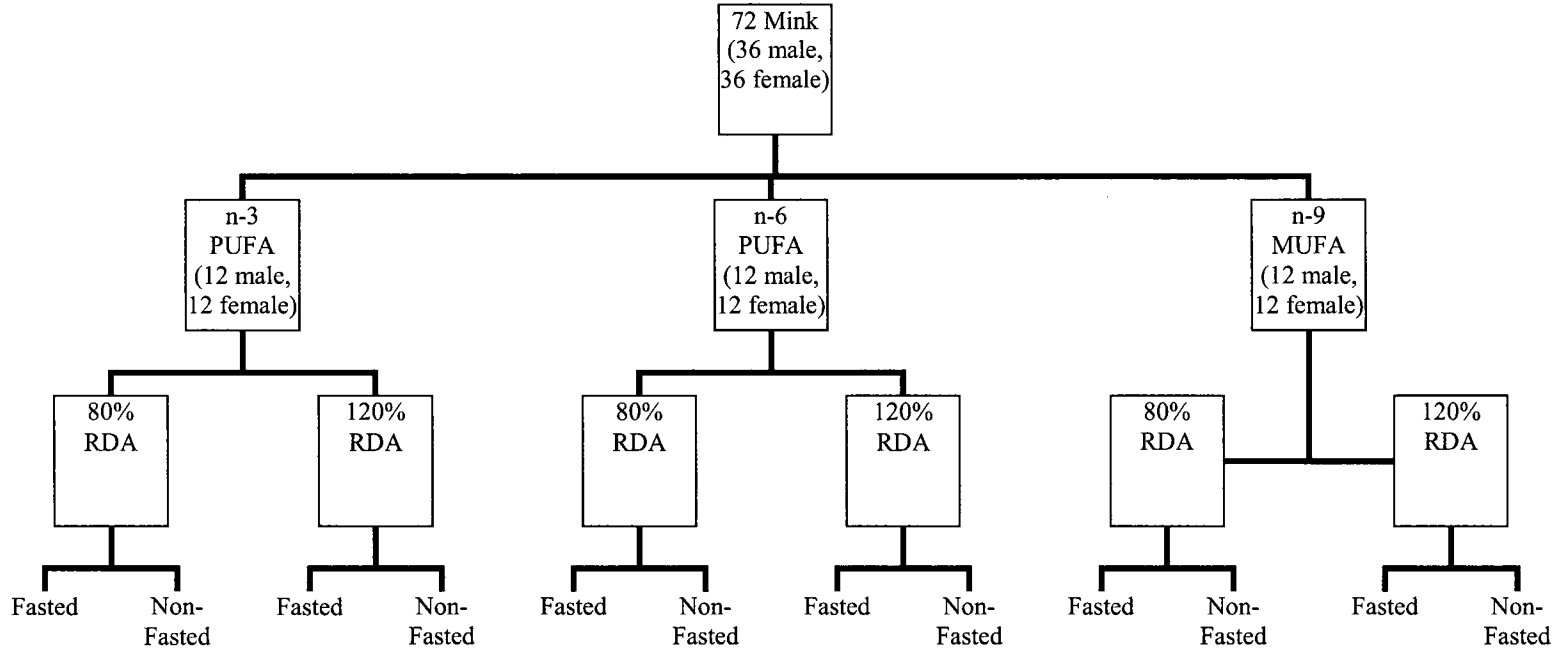

Figure 3.1. Distribution of mink to the diet, feeding intensity and fasting treatments.

Table 3.1. Ingredient formulation of the experimental diets.

	Diet		
Ingredient g/ 100g	n-3 PUFA	n-6 PUFA	n-9 MUFA
Cod racks	52.5	52.5	52.5
Pork liver	13.5	13.5	13.5
Pork spleen	7.0	7.0	7.0
Extruded barley	14.4	14.4	14.4
Corn gluten meal	3.1	3.1	3.1
Herring oil	4.7	-	-
Soya oil	-	4.7	-
Canola oil	-	-	4.7
Vitamin mineral premix[a]	0.4	0.4	0.4
Water	4.4	4.4	4.4

[a] Vitamin Mineral Pre-mix growing/furring (g /tonne): vitamin A (600 x10^5 IU/kg), 600 IU; vitamin D (500 x10^5 IU/kg), 600 IU; vitamin E (500 000 IU/kg), 150 IU; vitamin K (33%), 0.66 g; vitamin C (97.5%), 94.6 g; riboflavin (95%), 47.5 g; DL Ca-pantothenate (45%), 6.30 g; niacin (99%), 19.8 g; vitamin B_{12} (1000 mg/kg), 0.02 g; folic acid (3%), 0.45g; biotin (2%), 0.1g; choline chloride (60%), 600g; thiamine (97 x10^4 mg/kg), 3.88 g; pyridoxine (99 x10^4 mg/kg), 4.95 g; manganous oxide (60%), 10.8g; zinc oxide (80%), 30.4 g; cupric sulfate (25%); 5 g; cobalt carbonate (45%), 0.45 g; selenium premix (675 mg/kg), 0.22 g, ferrous sulphate (27%), 99.9 g; ground limestone (38%), 190 g; ethoxyquin (50%), 45 g, wheat middlings 3324 g.

3.2.2 On-Going Physical and Physiological Measurements

At the beginning of the feeding trial and every 4 weeks afterwards, the body weight, body condition and blood glucose of the mink was measured. The mink were manually restrained, and the mink's blood glucose was measured using the Accu-Check Compact™ blood glucose monitor (Roche Diagnositics, Switzerland, Laval, Quebec) from a clipped toe nail. The body condition of the mink was scored on a 5-point scale, with 1 being very thin, 3 being ideal and 5 being obese (Rouvinen-Watt and Armstrong, 2002). To ensure a postprandial blood glucose measurement, the mink were given 50 g of their daily feed allotment 1 to 2 hours before the blood glucose measurement was taken. Finally, the mink were weighed in a tared catch cage (to the nearest 1 g). The average feed intake during each month was measured during the three days before the mink were to be weighed. The feed intake was calculated as the difference in the total DM offered minus the total DM of the collected left over feed each day.

3.2.3 Tissue Sample Collection

The start of the fasting period was staggered to allow sample collection to take place over a period of two weeks in order to be feasible from a handling perspective. During the first week the non-fasted mink were sampled and the fasted mink were sampled the following week. Each day 1 male and 1 female from each diet and feeding intensity were sampled in the NF group during the first week and F group during the second week.

The mink were anesthetised using an intra-muscular injection of xylazine (Rompun, 0.17 mL/kg) and ketamine hydrochloride (Ketalean 100 mg/mL conc., 0.09 mL/kg) for blood sampling. Blood was drawn into 4 mL EDTA Vacutainer® tubes using cardiac puncture with three tubes taken from each female and four from each male. One tube from each mink was used for haematological analysis. The others were spun for 15 minutes at 1000 x G at 4°C, and the isolated plasma stored at -80°C for later analysis. Immediately following the cardiac puncture, the mink were euthanized with an intracardiac injection of pentobarbital (Euthanyl, 0.44 mL/kg). The length of the mink was measured to determine body mass index (BMI) on a measuring board with a V-shaped slot from the base of the tail to the tip of the nose (precision 0.5 cm). BMI was calculated as the body weight (g) over body length cubed (cm^3) (Mustonen et al. 2005a). The body condition score (BCS) was then assessed and the pelt removed.

The livers were removed and weighed. A slice of the left median lobe from each liver was taken and preserved in 10% buffered formalin solution with the remaining liver flash frozen in liquid nitrogen and stored at -80°C.

3.2.4 Analyses

3.2.4.1 Feed Analysis

During the preparation of each three batches of feed, a representative sample was taken for each diet for feed analysis. Feed analysis was performed at the Harlow Institute (Nova Scotia Department of Agriculture, Truro, Nova Scotia) for DM, crude protein (CP), crude fat (CF), and ash content. The carbohydrate (CHO) content of the diets was estimated by the difference from the DM minus CP, CF and ash. The metabolizable

energy (ME) content of the diets was calculated as the sum of the ME contributed by CP, CF, and CHO. The proportions of CP, CF, and CHO in the diets were multiplied by 4.5, 9.5, and 4.2 respectively to estimate the Mcal/kg contributed by these components (Rouvinen-Watt et al. 2005).

Samples of the diets and oils were also taken for fatty acid analysis conducted at the University of Joensuu (Joensuu, Finland), as described by Nieminen et al. (2006). Briefly, the diet and oils were transmethylated according to Christie (1993), by heating samples with 1% methanolic H_2SO_4 in a nitrogen atmosphere. The fatty acid methyl esters (FAMEs) were extracted with hexane. The FAMEs were then analyzed using a gas chromatograph (GC-FID and GC-MS, 6890 N network GC system with autosampler, FID dector and 5983 mass selective detectors, Agilent Technologies Inc, Palo Alto, CA) equipped with two injectors and DB-wax capillary columns (30 m, ID 0.25 mm, film 0.25 μm; J&W Scientific, Folsom, CA). An injection volume of 2 μL was used with a split ratio of 1:20. An injection temperature of 250°C was used, and the initial oven temperature was 180°C, held for 8 minutes then followed by an increase of 3°C/min until a final temperature of 210°C was reached, where it was maintained for 25 minutes. Helium was used as the carrier gas with one injector connected with a flow rate of 1.8 mL/min for the flame ionization detector (FID) lines and 1.0 mL/min for the mass spectrometer (MS) lines. The peaks were manually integrated using the Agilent ChemStation software (Agilent, Technologies Inc). The FAMEs were identified through retention time and mass spectrum in comparison to authentic quantitative standards (Sigma, St. Louis, MO). The FAMEs were quantified through the use of theoretical response factors (Ackman, 1992) and calibrations with authentic standards to convert peak area to mole %.

3.2.4.2 Blood Analyses

Haematological analysis was performed on whole blood at Fundy Veterinary Ltd. (Murray Siding, N.S., Canada). The SCIL Vet abc Animal Blood Counter (Vet Novations Canada Inc., Ontario Canada) was used to perform red blood cell count, white blood cell count, haemoglobin, haematocrit, platelet count, mean corpuscular volume, mean

corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, mean platelet volume, lymphocyte count, monocyte count, and granulocyte count.

Clinical chemistry was performed on previously frozen plasma (stored at -80 °C) at the Atlantic Veterinary College (Charlottetown, P.E.I., Canada) for urea, glucose, ALT, AKPH, total protein (TProt), albumin, globulin, and albumin:globulin (A:G). Further analyses were conducted at the University of Joensuu (Joensuu, Finland) for plasma levels of TAG, LDL cholesterol, HDL cholesterol, total antioxidant status (TAS), uric acid, creatine kinase, ammonia (NH_3), and NEFA as described by Mustonen et al. (2006b, 2009).

Plasma levels of insulin, leptin, cortisol glucagon, triiodothyronine (T_3), and thyroxine (T_4) were determined at the University of Joensuu (Joensuu, Finland). Levels were measured using Human Insulin Specific kit (Linco Research, St. Charles, MO, USA; intra-assay variation 2.8-3.6% CV), leptin using the Multi-Species kits (Linco Reasearch; 2.2-4.4% CV), cortisol using the Spectria Cortisol[^{125}I] Coated Tube Radioimmunoassay (Orion Diagnostica, Espoo, Finland; 2.6-5.4%), glucagon with the Coat-A-Count Double Antibody Glucagon kits (Siemens Medical Solutions Diagnostics; 3.2-6.5% CV), and T_3 and T_4 with the Coat-A-Count Total T_3 kit (Siemens Medical Solutions Diagnostics; 3.8-8.9% CV) and Coat-A-Count Total T_4 kit (Siemens Medical Solutions Diagnostics; 2.7-3.8% CV), respectively. The homeostatic model assessment (HOMA) to assess insulin resistance was calculated as the insulin concentration (uU/mL) multiplied by the blood glucose concentration (mmol/L) divided by a constant of 22.5 (Mathews et al. 1985).

3.2.4.3 Liver Analyses

The total lipid content of liver samples was measured following the Folch et al. (1957) method using 2:1 chloroform: methanol ratio. Liver DM was measured by freeze drying liver samples in duplicate. To determine relative size of the liver in relation to the body mass, the hepatosomatic index (HSI) was calculated as the % liver weight over body weight and the ratio of liver weight to BMI (L: BMI).

The formalin preserved liver samples were processed for histological evaluation and stained with hematoxylin and eosin at the histology laboratory of the Veterinary Services of the Nova Scotia Department of Agriculture and Fisheries, Quality Evaluation Branch (Truro, N.S.). In brief, the automated method uses the Tissue Tek® VIP™ (Sakura; Torrance, CA, USA) to dehydrate the liver samples and infuse with wax. Following this the samples were embedded using the Tissue Tek® Tec™ (Sakura; Torrance, CA, USA) where cassettes of the liver samples are made. The cassettes were then sliced on a microtome to 5 μm in thickness (Microm HM 335E, Microtome Laborgerate GmbH, Gemany), and placed on a slide. The slides undergo an automated staining process using the Tissue-Tek ® DRS™ 2000 (Sakura; Torrance, CA, USA). The liver sections were evaluated for degree and type of steatosis using a method modified from Kleiner et al. (2005). Each liver sample was examined on three separate occasions and assigned scores corresponding to the degree of macrovesicular and microvesicular steatosis. The scores were: 0 (<5%), 1 (5-33%), 2 (33-66%) and 3 (>66%). In addition, the location of the steatosis was evaluated: 0 (central or zone 3), 1 (periportal or zone 1), 2 (azonal), and 3 (panacinar). In order for a liver evaluation for a sample to be considered, the same scores must have been achieved a minimum of two out of the three times.

3.2.5 Statistical Analysis

A split-plot factorial design was used in the analysis of the feeding trial. The whole plot treatment was sex and the subplot treatments were the diet and feeding intensity treatment combinations with 6 replicates within each. The dam's dam was used as a blocking factor and was nested within sex. The growth data, and monthly blood glucose levels were analyzed to examine the effects of feeding intensity, dietary fat source and sex on the measured response variables using Proc MIXED in SAS® v.9 (SAS Institute Inc., Cary, NC) with the repeated measures option and random statement (subject = mink ID).

For the fasted mink, a split-plot factorial design was used to determine body weight and BCS changes during relocation to the quarantine shed and the fasting period.

The same experimental design was used from the feeding trial, with the exception of the replicates being reduced to 3 in each diet and feeding intensity combination.

For the final sampling data, a split-split-plot factorial design was used, with fasting treatments as the whole plot, sex as the sub-plot and the diet and feeding intensity treatment combinations as the sub-sub-plots, with 3 replicates within each. The haematology, clinical chemistry, endocrinology and liver responses were analyzed using Proc MIXED to examine the effects of feeding intensity, diet, fasting, and sex on the response variables.

A multiple means comparison test (PDiff) was used to identify where differences existed when significant effects were found at $P < 0.05$. In cases where a significant 3-way interaction that included fasting as an effect or a 4-way interaction, comparisons were made within NF and F, and between individual NF and F treatments only. A regression model was developed for the prediction of liver lipid content from liver DM using Proc REG in SAS.

3.3 Results

3.3.1 Diet Nutrient Composition and Metabolizable Energy Distribution

The diet nutrient composition, energy distribution, and fatty acid composition of treatment diets is shown in Table 3.2. The fatty acid profiles of the diets differed with the n-3 PUFA diets having the highest levels of saturated fatty acids, MUFA and n-3 PUFA, EPA and DHA and lowest proportion of n-6 PUFAs resulting in a low n-6: n-3 PUFA ratio in the diet. The n-6 PUFA diet had high levels of total n-3 and n-6 PUFAs, making it the highest total PUFA containing diet. The high n-6 content of the n-6 PUFA diet, however gave it the highest n-6: n-3 PUFA ratio. The n-9 MUFA had the highest levels of MUFA and lowest PUFA levels, and was intermediate in the n-6: n-3 PUFA ratio compared to the other diets. The differences in the fatty acid profiles of the diets are a direct reflection of the fatty acid content of the oils (Table 3.3).

Table 3.2. Nutrient composition, metabolizable energy (ME) content and distribution, and fatty acid profiles of the n-3 PUFA, n-6 PUFA and n-9 MUFA experimental diets.

		Experimental Diet		
		n-3 PUFA	n-6 PUFA	n-9 MUFA
Composition (as fed)	Samples, n	3	3	3
	% DM	39.08 ±0.74	39.77 ±0.91	39.49 ±0.46
	% CP	15.10 ±0.30	15.63 ±0.51	14.83 ±0.31
	% CF	6.72 ±0.43	7.66 ±0.91	6.52 ±0.49
	% CHO	12.50 ±0.84	13.48 ±1.19	13.86 ±0.48
	% Ash	4.04 ±0.19	3.84 ±0.19	3.75 ±0.32
ME Distribution	ME kcal/kg	1520 ±23.9	1649 ±72.1	1532 ±28.6
	% Protein	38.01 ±0.22	36.29 ±0.51	37.06 ±1.19
	% Fat	37.75 ±1.82	39.48 ±3.02	36.31 ± 2.04
	% Carbohydrate	24.25 ±1.98	24.23 ±2.78	26.63 ±1.31
Fatty Acid Profile (mole %)	14:0	5.45 ± 0.14^{A}	0.25 ± 0.14^{B}	0.18 ± 0.14^{B}
	16:0	14.63 ± 0.26^{A}	13.38 ± 0.26^{B}	9.26 ± 0.26^{C}
	18:0	3.93 ± 0.31^{B}	5.69 ± 0.31^{A}	4.27 ± 0.31^{AB}
	Total SFA	25.71 ± 0.46^{A}	20.38 ± 0.41^{B}	14.90 ± 0.46^{C}
	16:1 n-9	0.19 ± 0.004^{A}	0.13 ± 0.004^{B}	0.13 ± 0.004^{B}
	16:1 n-7	7.05 ± 0.07^{A}	0.54 ± 0.07^{B}	0.58 ± 0.07^{B}
	18:1 n-9	10.64 ± 0.26^{C}	17.38 ± 0.26^{B}	48.27 ± 0.26^{A}
	18:1 n-7	2.03 ± 0.06^{B}	1.47 ± 0.06^{C}	2.89 ± 0.06^{A}
	Total MUFA	53.17 ± 0.29^{A}	20.99 ± 0.29^{B}	53.85 ± 0.29^{A}
	18:2 n-6 (LA)	8.86 ± 0.08^{C}	45.98 ± 0.08^{A}	21.18 ± 0.02^{B}
	20:4 n-6 (AA)	1.47 ±0.13	1.23 ±0.13	1.08 ±0.13
	22:4 n-6	0.16 ±0.02	0.12 ±0.02	0.13 ±0.02
	22:5 n-6	0.11 ± 0.01^{A}	0.06 ± 0.01^{B}	0.04 ± 0.01^{B}
	Total n-6 PUFA	10.83 ± 0.55^{C}	47.56 ± 0.55^{A}	22.61 ± 0.55^{B}
	18:3 n-3 (ALA)	0.75 ± 0.14^{C}	8.12 ± 0.14^{A}	6.65 ± 0.14^{B}
	20:5 n-3 (EPA)	4.05 ± 0.07^{A}	0.82 ± 0.07^{B}	0.54 ± 0.07^{C}
	22:5 n-3	0.70 ± 0.02^{A}	0.24 ± 0.02^{B}	0.19 ± 0.02^{B}
	22:6 n-3 (DHA)	3.40 ± 0.18^{A}	1.52 ± 0.18^{B}	1.03 ± 0.18^{B}
	Total n-3 PUFA	10.29 ± 0.29^{A}	10.97 ± 0.29^{A}	8.57 ± 0.29^{B}
	UFA: SFA	2.94 ± 0.11^{C}	3.92 ± 0.11^{B}	5.71 ± 0.11^{A}
	Total PUFA	21.12 ± 0.61^{C}	58.62 ± 0.61^{A}	31.25 ± 0.61^{B}
	n-6: n-3 PUFA	1.05 ± 0.03^{C}	4.35 ± 0.03^{A}	2.63 ± 0.03^{B}

$^{A-C}$ Values within response rows differ ($P < 0.05$).

Table 3.3. Fatty acid profile of the oils used in the n-3 PUFA, n-6 PUFA and n-9 MUFA diets.

	Experimental Diet		
mole %	n-3 PUFA	n-6 PUFA	n-9 MUFA
14:0	8.56	0.10	0.07
16:0	11.97	10.57	4.75
18:0	0.96	3.51	1.93
Total SFA	22.95	14.96	7.83
16:1 n-9	0.05	0.02	0.04
16:1 n-7	10.04	0.11	0.25
18:1 n-9	6.73	16.70	58.41
18:1 n-7	2.01	1.15	3.13
Total MUFA	63.29	18.54	63.34
18:2 n-6 (LA)	0.80	55.31	19.67
20:4 n-6 (AA)	0.21	0.004	0.002
22:4 n-6	0.02	0.001	0.02
22:5 n-6	0.07	0.002	0.001
Total n-6 PUFA	1.27	55.39	19.77
18:3 n-3 (ALA)	0.39	10.96	8.72
20:5 n-3 (EPA)	0.23	0.04	0.02
22:5 n-3	0.71	0.002	0.13
22:6 n-3 (DHA)	3.63	0.01	0.13
Total n-3 PUFA	12.16	11.06	9.02
UFA: SFA	3.36	5.68	11.79
Total PUFA	13.76	66.50	28.84
n-6: n-3 PUFA	0.10	5.00	2.17

3.3.2 Feeding Trial

A significant interaction occurred over time with feeding intensity and sex for body weight and feeding intensity for body condition score (Table 3.4). At the start of the trial, there was a difference in the body weights between the sexes, but not between animals assigned to the various dietary regimes (Figure 3.2). Both the male and female mink lost weight when fed at the lower feeding intensity throughout October and November, while only the males had significant weight gain on the high feeding intensity, with the weight gains plateauing after October. Changes in the BCS score of the animals were only observed in November with a significantly lower BCS for the 80%

RDA group from previous scorings, and a higher BCS for the 120% RDA from previous scorings (Table 3.5).

The feed intake of the mink differed between the sexes and time for feeding intensity and oil separately. The males consumed more feed than the females (347 ±6 kcal/day and 84.1 ±1.5 g DM/day vs. 248 ±6 kcal/day and 60.0 ±1.5 g DM/day). Feed intake remained constant for the animals on the lower feeding intensity (Table 3.6). In the high feeding intensity the feed intake increased throughout the trial, but never reached the intended 120% RDA. Diet affected the DM intake per day, with the mink fed the n-3 PUFA diet increasing consumption from October to November (Table 3.7). The consumption of the other two diets did not change over time. During November, the n-6 PUFA diet had a higher intake than the n-9 MUFA diet.

The blood glucose levels of the mink differed significantly over time during the feeding trial. Blood glucose levels measured in September, at the start of the trial, were significantly higher than in the other months (September: 4.6± 0.1, October: 3.8± 0.1 and November 3.7 ± 0.1 mmol/L).

Table 3.4. P-values of the main effects and interactions for mink body weights, BCS, blood glucose and feed intake during the feeding trial from September to November. Highest order significant main effects and interactions are bolded.

Effect	Body Weights	BCS	Feed Intake (kcal/day)	Feed Intake (DM g/day)	% RDA intake	Blood Glucose
Sex	<0.001	0.273	**<0.001**	**<0.001**	0.462	0.757
Diet	0.305	0.391	0.002	0.639	0.677	0.510
Sex*Diet	0.586	0.179	0.246	0.351	0.737	0.817
FI	<0.001	0.001	<0.001	<0.001	<0.001	0.293
Sex*FI	0.267	0.601	0.062	0.063	0.478	0.945
Diet*FI	0.995	0.970	0.835	0.735	0.656	0.836
Sex*Diet*FI	0.863	0.913	0.710	0.678	0.718	0.315
Time	<0.001	0.272	<0.001	0.570	<0.001	**<0.001**
Sex*Time	0.001	0.521	0.513	0.901	0.583	0.489
Diet*Time	0.236	0.242	**0.001**	0.715	0.384	0.272
Sex*Diet*Time	0.170	0.386	0.885	0.948	0.259	0.437
FI*Time	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.109
Sex*FI*Time	**<0.001**	0.862	0.850	0.970	0.611	0.787
Diet*FI*Time	0.254	0.446	0.296	0.496	0.414	0.365
Sex*Diet*FI*Time	0.550	0.985	0.536	0.517	0.239	0.697

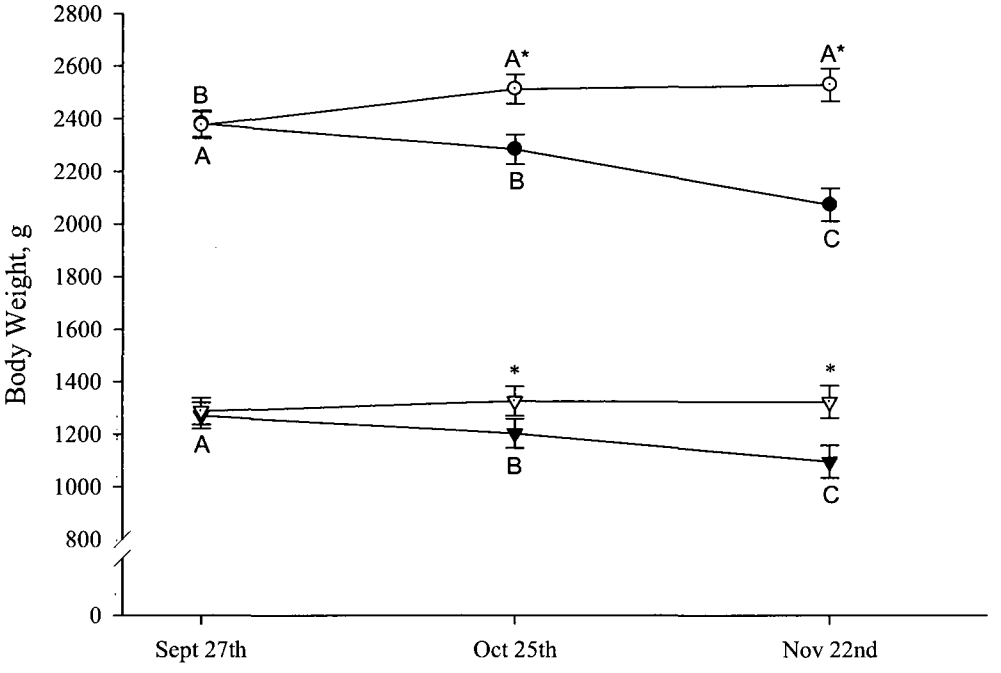

Figure 3.2. Body weight of the male and female mink fed at 80% and 120% RDA throughout the feeding trial. Male mink are shown in circles, and female mink in triangles. Closed symbols represent the 80% RDA and open symbols represent the 120% RDA. Sex*FI*Time P =<0.001. $^{A-C}$ Values differ within sex and feeding intensity. * Values differ between feeding intensity within sex. Lsmeans ± SEM are presented.

Table 3.5. Effect of feeding intensity on BCS of mink throughout the feeding trial.

BCS	80% RDA	120% RDA
Sept 27th	3.0 ± 0.01^{B}	3.0 ± 0.01^{B}
Oct 25th	3.1 ± 0.01^{B}	3.1 ± 0.01^{B}
Nov 22nd	2.9 ± 0.01^{C}	3.3 ± 0.01^{A}

$^{A-C}$ Values differ within response (P <0.05).
Lsmeans ± SEM are presented.

Table 3.6. Feed intake of the mink fed at 80% and 120% RDA during the feeding trial.

	80% RDA			120% RDA		
	DM g/day	kcal/day	% RDA	DM g/day	kcal/day	% RDA
Oct	70.2 ±2.2^{Ba}	274 ±8^{Ab}	80 ±2^{Ab}	74.8 ±2.3^{Ba}	292 ±9^{Ba}	89 ±2^{Ba}
Nov	61.1 ±1.6^{Bb}	266 ±7^{Ab}	80 ±1^{Ab}	82.0 ±1.6^{Aa}	357 ±7^{Aa}	103 ±1^{Aa}

$^{A-B}$ Values differ within response columns (P <0.05).
$^{a-b}$ Values differ within response rows (P <0.05).
Lsmeans ± SEM are presented.

Table 3.7. Feed intake (DM g/day) of the mink fed the experimental diets during the feeding trial.

Feed intake, DM g/day	n-3 PUFA	n-6 PUFA	n-9 MUFA
Oct	285.1 ±10.7^{Ba}	285.7±10.7^{a}	278.3 ±10.7^{a}
Nov	349.4 ±8.7^{Aa}	307.3 ±8.7^{b}	277.3 ±8.7^{c}

$^{A-B}$ Values differ within response columns (P <0.05).
$^{a-c}$ Values differ within response rows (P <0.05).
Lsmeans ± SEM are presented.

3.3.3 Effect of the Fasting Treatment on Final Body Condition

All mink completed the fasting treatment without apparent detrimental health effects and remained alert during the 5-day food deprivation period. The tips of the tails of four mink, all in different diet and feeding intensity treatment combinations, showed minor chewing after one day of fasting, but showed no progression throughout the rest of the fast.

The F mink lost weight over time and differences were observed in body weights between feeding intensities and sex (Table 3.8). A continuation of the size difference between the male and female was observed throughout fasting, with the males being larger than the females (Table 3.9). In both sexes, the fasting treatment resulted in a decrease in body weight in addition to the weight loss during the time from being relocated to the quarantine shed and the 24 hour fasting mark. The body weights were also dependent on the feeding intensity, with higher weights in the mink fed at 120% RDA (Table 3.10). During the fasting period, both feeding intensities showed weight loss during the transition to the quarantine to 24 hours fasting mark to final weighing. The weight loss was also reflected in the BCS of the mink fed at 80% RDA, but this was not observed in the mink fed at 120% RDA.

P-values for the final body weight, BCS, and BMI of the mink are shown in Table 3.11. The final body weights of the mink fed at 80% RDA were lower than those fed at 120% RDA (Figure 3.3). A fasting, diet and sex interaction was observed, where the males fed n-3 PUFA at 120% RDA were found to be the heaviest mink in the NF group and lightest in the F group. No differences were observed between the n-6 PUFA and n-9 MUFA fed male mink within the NF and F male groups. No significant differences were observed in the body weight of the females. The final BCS of the mink was influenced by fasting with lower scores in the fasted mink (3.1 ±0.1 vs. 2.7 ±0.1). In addition the mink fed at 80% RDA were also found to have a lower BCS than the mink fed at 120% RDA (2.5 ±0.1 vs. 3.3±0.1). Changes associated with fasting and feeding intensity were also reflected in BMI of the mink. The NF mink had a higher BMI than the F mink (19.1 ±0.4 vs. 17.3 ±0.4) and the mink fed at 80% RDA had a lower mean BMI than the mink fed at 120% RDA (16.1 ±0.4 vs. 20.4 ±0.4). A sex difference was also noted for BMI with the males having a higher BMI than the females (19.2 ±0.4 vs. 17.3 ±0.4).

Table 3.8. P-values of the main effects and interactions of fasting on body weight and BCS. Highest order significant main effects and interactions are bolded.

	Body Weight	BCS
Sex	0.001	0.280
Diet	0.912	0.252
Sex*Diet	0.432	0.597
FI	<0.001	<0.001
Sex*FI	0.018	0.678
Diet*FI	0.879	0.779
Sex*Diet*FI	0.903	0.944
Time	<0.001	0.024
Sex*Time	**<0.001**	0.761
Diet*Time	0.424	0.875
Sex*Diet*Time	0.706	0.894
FI*Time	**0.002**	**0.014**
Sex*FI*Time	0.795	0.125
Diet*FI*Time	0.823	0.875
Sex*Diet*FI*Time	0.233	0.376

Table 3.9. Effect of fasting and sex on body weight.

	Body Weight, g	
	Male	Female
Nov 29th	2284 ±75[A]	1183 ±75[D]
24 hrs Fasting	2153 ±75[B]	1108 ±75[E]
Final	1902 ±75[C]	949 ±75[F]

[A-F] Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 3.10. Effect of fasting and feeding intensity on body weight and BCS during the 5-day fasting period.

	Body Weight, g		BCS	
	80% RDA	120% RDA	80% RDA	120% RDA
Nov 29th	1497 ±64[D]	1971 ±64[A]	2.9 ±0.2[B]	3.1 ±0.2[A]
24 hrs Fasting	1411 ±64[E]	1851 ±64[B]	2.6 ±0.2[C]	3.4 ±0.2[A]
Final	1211 ±64[F]	1640 ±64[C]	2.4 ±0.2[D]	3.1 ±0.2[A]

[A-F] Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 3.11. P-values of the main effects and interactions of the final body weight, BCS and BMI. Highest order significant main effects and interactions are bolded.

Effect	Body Weight	BCS	BMI
Fast	<0.001	**0.004**	**0.003**
Sex	0.001	0.163	**0.034**
Fast*Sex	0.217	0.664	0.616
Diet	0.123	0.717	0.176
Fast*Diet	0.271	0.717	0.742
Sex*Diet	0.284	0.953	0.907
Fast*Sex*Diet	**0.009**	0.542	0.061
FI	<0.001	**<0.001**	**<0.001**
Fast*FI	0.776	1.000	0.463
Sex*FI	**0.004**	0.664	0.297
Fast*Sex*FI	0.785	0.087	0.558
Diet*FI	0.719	0.375	0.804
Fast*Diet*FI	0.957	0.653	0.738
Sex*Diet*FI	0.524	0.717	0.328
Fast*Sex*Diet*FI	0.902	0.717	0.973

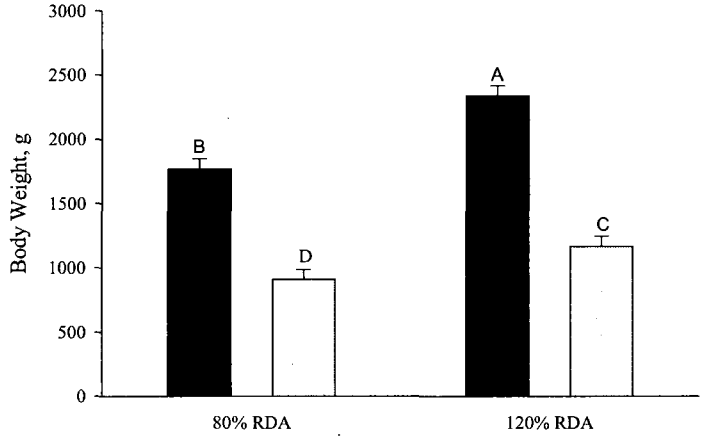

Figure 3.3. Final body weights of the mink fed at 80% and 120% RDA. Male mink are shown in black and female mink in grey. Sex*FI P = 0.004. $^{A-D}$ Values differ (P <0.05). Lsmeans ± SEM are presented.

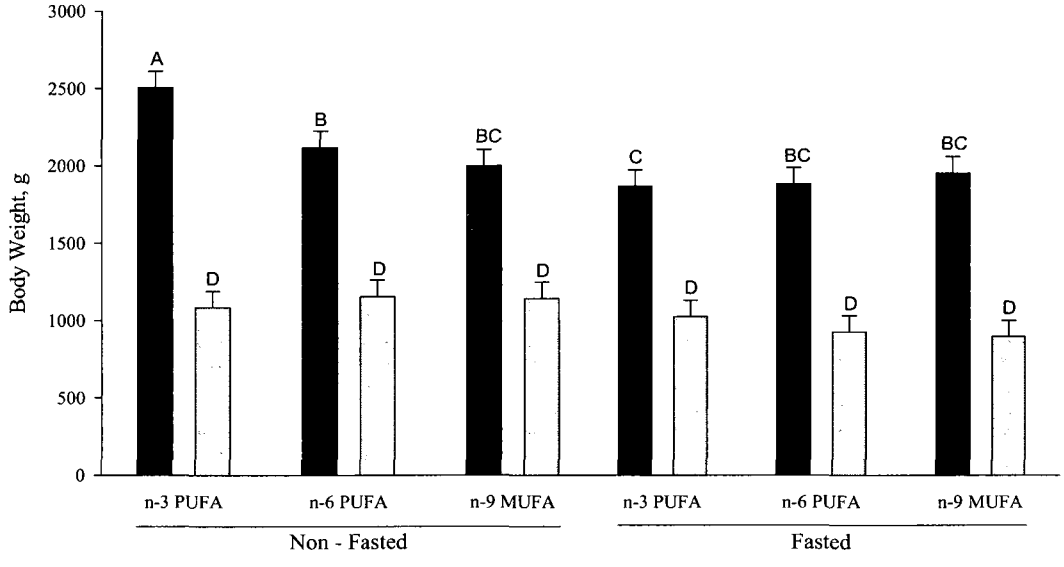

Figure 3.4. Final body weights of the non-fasted and fasted mink fed the experimental diets. Male mink are shown in black and female mink in grey.Fast*Sex*Diet P = 0.009. $^{A-D}$ Values differ (P <0.05). Lsmeans ± SEM are presented.

3.3.4 Haematology

Significant interactions were detected in the red blood cell (RBC) count with the fasting, sex, and oil interaction and the fasting, sex, and feeding intensity interaction (Table 3.12.a). In both interactions, the males tended to have higher RBC counts than the females, but this was not true in every case. For the fasting, sex and oil interaction, in the NF group the female mink fed the n-3 PUFA diet had a lower RBC count than their male counterparts, which was not observed in the other diets (Table 3.13). Fasting resulted in a decrease in the RBC count of the female mink fed the n-6 PUFA diet and an increase in the female mink fed the n-9 MUFA diet. Examining the F mink, only the n-9 MUFA mink did not show a difference in the RBC count between sexes. With regards to the fasting, sex and feeding intensity effect on RBC count, no difference was observed between the F and NF mink (Table 3.14). In the NF mink, feeding at 80% RDA resulted in lower RBC count in females compared to males, which was not observed in NF mink fed at 120% RDA. However in the F mink, feeding at 120% RDA resulted in a decrease in the RBC count in the females compared to the males. Fasting led to an increase in the haemoglobin (HGB) levels of the female mink fed at 80% RDA and the male mink fed at 120% RDA, who also had the highest HGB levels within the F mink. The F mink also had increased haematocrit (HCT), mean corpuscular volume (MCV), and mean corpuscular haemoglobin values (MCH) (Table 3.15). Diet had a significant effect on the MCH, with the n-6 PUFA diet having higher levels than the n-3 PUFA diet (Table 3.16), while the higher feeding intensity increased the MCV as well from 31.4 ±0.1 um^3 in the 80% RDA group to 31.7 ±0.1 um^3 in the 120% RDA group. A significant interaction with the RBC distribution width (RDW) was observed, with the lowest value observed in the n-6 PUFA mink fed at 80% RDA within the NF (Table 3.17). In the F mink, the n-9 MUFA mink fed at 80% RDA had a lower RDW value than those fed the n-9 MUFA diet at 120% RDA and n-3 PUFA diet at 80% RDA. Fasting resulted in an increase in the RDW in the mink fed the n-3 and n-6 PUFA diets at 80% RDA.

Platelet (PLT) number and mean platelet volume (MPV) of the mink was affected by fasting and diet (Table 3.12.a). Fasting decreased the PLT number, but showed an increase in the MPV (Table 3.15). The lowest PLT counts were observed in the mink fed

the n-3 PUFA diet, followed by the n-9 MUFA diet and n-6 PUFA diet (Table 3.16), while the n-3 PUFA group had the highest MPV compared to the other two diets.

A fasting, sex, diet and feeding intensity interaction was found with the white blood cell (WBC) count for the mink (Table 3.12.b). In the NF mink, the highest WBC count was observed in the male mink fed the n-9 MUFA diet at 120% RDA, which differed from its 80% RDA counterpart (Table 3.18). The female mink fed the n-3 PUFA diet at 80% also had a lower WBC count compared to the female mink fed the same diet at 120% RDA in the NF group. In the fasted mink the lowest WBC count was found in the female mink fed the n-6 PUFA diet at 80% RDA, which differed from its counterparts fed at 120% RDA. The proportion of lymphocytes (LYM) decreased in the F mink while the proportion of the granulocytes (GRA) increased (Table 3.15). The monocytes (MON) decreased as a result of fasting in mink fed at the 80% RDA level, but not at the 120% RDA level. The F mink fed at the 80% RDA level also had lower proportion of MON than the mink fed at 120% RDA.

A sex, diet and RDA interaction was also observed for the % LYM, MON, and GRA. The % LYM was the highest in the male mink fed the n-3 PUFA diet at 80% RDA, and the lowest in the female mink fed then 6-PUFA diet at 120% RDA (Table 3.19). The % MON were the highest in the males fed the n-6 PUFA diet at 80% RDA, and the lowest in the female mink fed the n-3 and n-6 PUFA diets at 120% RDA. The % MON tended to decrease with increasing feeding intensity and was generally lower in the female mink. Changes in the % MON as a result of feeding intensity were also dependent on the fast, with lower proportions in the NF mink, but higher in the F with the 120% RDA (Table 3.21). The % GRA were the highest in the female mink fed the n-6 PUFA diet at the 120% RDA level and the lowest in the male mink fed the n-3 PUFA diet at the 80% RDA level. For both males and females fed the n-6 PUFA diet, increasing the RDA to 120% increased the % GRA.

Feeding intensity and diet had an effect on the LYM count. LYM count was higher in the mink fed at 120% RDA (1.2 ±0.1) compared to the 80% RDA group (0.8 ±0.1), while, the mink fed the n-3 PUFA diet had higher counts than both the n-6 PUFA and n-9 MUFA diets (Table 3.16). With regards to the MON count, a significant interaction with diet and feeding intensity was found. Increasing feeding intensity

increased the MON count in the n-3 PUFA diet, but decreased the count in the n-6 PUFA diet, and no effect was observed in the n-9 MUFA diet (Table 3.20). There were no effects on GRA number (2.5 ±0.1 $10^3/mm^3$).

Table 3.12.a P-values of the main effects and interactions for haematological responses. Highest order significant main effects and interactions are bolded.

Effect	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV
Fast	0.368	0.001	**<0.001**	**0.001**	**0.019**	**0.027**	<0.001	**<0.001**	**0.002**
Sex	0.062	0.194	0.423	**0.034**	0.097	0.177	0.299	0.064	0.427
Fast*Sex	0.545	0.095	0.405	0.824	0.433	0.206	0.156	0.238	0.320
Diet	0.993	0.172	0.825	0.901	0.297	**0.039**	0.196	**<0.001**	**<0.001**
Fast*Diet	0.271	0.276	0.227	0.859	0.970	0.623	0.908	0.476	0.636
Sex*Diet	0.182	0.612	0.866	0.263	0.559	0.302	0.360	0.752	0.426
Fast*Sex*Diet	**0.021**	0.144	0.116	0.487	0.367	0.591	0.155	0.943	0.429
FI	0.860	0.306	0.967	0.755	0.291	**0.049**	0.012	0.366	0.131
Fast*FI	0.956	0.788	0.619	0.624	0.879	0.181	0.838	0.487	0.861
Sex*FI	0.877	0.145	0.441	0.268	0.131	0.169	0.336	0.239	0.951
Fast*Sex*FI	**0.039**	**0.019**	0.076	0.688	0.945	0.376	0.055	0.519	0.311
Diet*FI	0.834	0.822	0.812	0.615	0.942	0.383	0.018	0.639	0.200
Fast*Diet*FI	0.317	0.132	0.142	0.799	0.806	0.928	**0.049**	0.759	0.963
Sex*Diet*FI	0.842	0.108	0.157	0.218	0.259	0.992	0.959	0.586	0.335
Fast*Sex*Diet*FI	0.569	0.655	0.913	0.845	0.933	0.761	0.844	0.425	0.715

RBC = red blood cell count; HGB = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red blood cell distribution; PLT = platelet count; MPV = mean platelet volume.

Table 3.12.b. P-values of the main effects and interactions for haematological responses. Highest order significant main effects and interactions are bolded.

Effect	WBC	LYM %	MON %	GRA %	LYM #	MON #	GRA #
Fast	0.071	**<0.001**	<0.001	**<0.001**	0.057	0.002	0.967
Sex	0.244	0.213	0.048	0.139	0.421	0.365	0.185
Fast*Sex	0.924	0.717	0.102	0.863	0.871	0.204	0.881
Diet	0.024	0.005	0.472	0.013	**0.016**	**0.039**	0.732
Fast*Diet	0.066	0.150	0.357	0.133	0.080	0.743	0.097
Sex*Diet	0.659	0.933	0.334	0.900	0.133	0.598	0.068
Fast*Sex*Diet	0.496	0.524	0.742	0.527	0.837	0.454	0.532
FI	<0.001	0.073	0.524	0.075	**0.037**	0.459	0.225
Fast*FI	0.749	0.068	**0.040**	0.052	0.968	0.097	0.602
Sex*FI	0.774	0.738	0.991	0.738	0.968	0.314	0.707
Fast*Sex*FI	0.399	0.674	0.036	0.533	0.186	0.839	0.058
Diet*FI	0.995	0.428	0.229	0.409	0.664	**0.006**	0.368
Fast*Diet*FI	0.363	0.673	0.826	0.663	0.584	0.567	0.794
Sex*Diet*FI	0.054	**0.030**	**0.030**	**0.022**	0.673	0.088	0.433
Fast*Sex*Diet*FI	**0.045**	0.155	0.300	0.139	0.697	0.065	0.130

WBC = white blood cell count; LYM % = lymphocyte %; MON % = monocyte %; GRA % = granulocyte %; LYM # = lymphocyte count; MON # = monocyte count; GRA # = granulocyte count.

Table 3.13. Effect of diet, sex and fasting on red blood cell (RBC) count.

RBC, 10^6/mm^3		Non-Fasted	Fasted
n-3 PUFA	Male	9.94 ±0.20^{A}	10.00 ±0.20^{A}
	Female	9.24 ±0.20^{B}	9.38 ±0.20^{BC}
n-6 PUFA	Male	9.73 ±0.20^{AB}	10.08 ±0.20^{A}
	Female	9.69 ±0.20^{AB*}	9.12 ±0.20^{C}
n-9 MUFA	Male	9.72 ±0.20^{AB}	9.78 ±0.20^{AB}
	Female	9.28 ±0.20^{B*}	9.81 ±0.20^{AB}

$^{A-C}$ Values differ within in response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P <0.05).
Lsmean ± SEM are presented.

Table 3.14. Effect of feeding intensity, sex, and fasting on red blood cell (RBC) count and haemoglobin (HGB).

		RBC, 10^6/mm^3		HGB, g/dL	
		Non-Fasted	Fasted	Non-Fasted	Fasted
80%	Male	9.92 ±0.18^{A}	9.86 ±0.18^{AB}	18.8 ±0.3	19.3 ±0.3^{B}
RDA	Female	9.30 ±0.18^{B}	9.54 ±0.18^{BC}	18.5 ±0.3*	19.3 ±0.3^{B}
120%	Male	9.67 ±0.18^{AB}	10.04 ±0.18^{A}	18.8 ±0.3*	20.2 ±0.3^{A}
RDA	Female	9.51 ±0.18^{AB}	9.33 ±0.18^{C}	18.8 ±0.3	18.8 ±0.3^{B}

$^{A-C}$ Values differ within response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P <0.05).
Lsmean ± SEM are presented.

Table 3.15. Effect of fasting on haematological responses. Responses shown are haemoatocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), mean platelet volume (MPV), lymphocyte (LYM) and granulocytet (GRA).

	Non-Fasted	Fasted
HCT, %	59.1 ±0.6^{B}	61.8 ±0.6^{A}
MCV, um^3	61.6 ±0.5^{B}	63.9 ±0.5^{A}
MCH, pg	19.6 ±0.2^{B}	20.0 ±0.2^{A}
MCHC, g/dL	31.7 ±0.1^{A}	31.4 ±0.1^{B}
PLT, 10^3/mm^3	633 ±15^{A}	446 ±15^{B}
MPV, um^3	10.5 ±0.3^{A}	9.9 ±0.3^{B}
LYM, %	32.9 ±1.3^{A}	25.8 ±1.2^{B}
GRA, %	61.6 ±1.3^{B}	69.7 ±1.2^{A}

^{A,B} Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.16. Effect of diet on mean corpuscular volume (MCHC), platelets (PLT), mean platelet volume (MPV), monocytes (MON), and lymphocytes (LYM).

	n-3 PUFA	n-6 PUFA	n-9 MUFA
MCHC, g/dL	31.4 ±0.1^{B}	31.8 ±0.1^{A}	31.5 ±0.1^{AB}
PLT, 10^3/mm^3	410 ±19^{C}	641 ±19^{A}	568 ±19^{B}
MPV, um^3	11.3 ±0.3^{A}	9.5 ±0.3^{B}	9.9 ±0.3^{B}
LYM, 10^3/mm^3	1.3 ±0.1^{A}	0.9 ±0.1^{B}	0.9 ±0.1^{B}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.17. Effect of fasting, diet and feeding intensity on red blood distribution width (RDW) and monocytes (MON).

		RDW, %		MON, 10^s/mm^3	
		Non-Fasted	Fasted	Non-Fasted	Fasted
n-3 PUFA	80% RDA	13.5 ±0.2^{A*}	14.1 ±0.2^{AB}	0.3 ±0.04^{A}	0.2 ±0.04^{A}
	120% RDA	13.4 ±0.2^{A}	13.9 ±0.2^{ABC}	0.1 ±0.04^{B}	0.1 ±0.04^{A}
n-6 PUFA	80% RDA	12.9 ±0.2^{B*}	13.7 ±0.2^{BC}	0.1 ±0.04^{B}	0.03 ±0.04^{B}
	120% RDA	13.7 ±0.2^{A}	13.9 ±0.2^{ABC}	0.2 ±0.04^{AB}	0.1 ±0.04^{AB}
n-9 MUFA	80% RDA	13.5 ±0.2^{A}	13.6 ±0.2^{C}	0.2 ±0.04^{AB*}	0.05 ±0.04^{B}
	120% RDA	13.6 ±0.2^{A*}	14.3 ±0.2^{A}	0.2 ±0.04^{AB}	0.1 ±0.04^{AB}

$^{A-C}$ Values differ within response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P <0.05).
Lsmean ± SEM are presented.

Table 3.18. Effect of fasting, diet, feeding intensity and sex on white blood cell count (WBC) count.

WBC, $10^3/mm^3$			Non-Fasted	Fasted
n-3 PUFA	80% RDA	Male	4.2 ± 0.8^{BC}	5.2 ± 0.8^{AB}
		Female	3.7 ± 0.8^{C}	5.0 ± 0.8^{AB}
	120% RDA	Male	6.2 ± 0.8^{AB}	6.7 ± 0.8^{A}
		Female	6.3 ± 0.8^{AB}	5.6 ± 0.8^{AB}
n-6 PUFA	80% RDA	Male	4.9 ± 0.8^{AB}	3.3 ± 0.8^{BCD}
		Female	4.0 ± 0.8^{BC}	2.2 ± 0.8^{D}
	120% RDA	Male	4.8 ± 0.8^{BC}	4.9 ± 0.8^{ABC}
		Female	6.3 ± 0.8^{BC}	5.0 ± 0.8^{AB}
n-9 MUFA	80% RDA	Male	3.3 ± 0.8^{C}	3.3 ± 0.8^{BCD}
		Female	4.6 ± 0.8^{BC}	2.5 ± 0.8^{CD}
	120% RDA	Male	$7.9 \pm 0.8^{A*}$	4.3 ± 0.8^{ABCD}
		Female	3.7 ± 0.8^{C}	4.3 ± 0.8^{ABCD}

$^{A-D}$ Values differ within response columns ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.19. Effect of feeding intensity, diet and sex on lymphocytes (LYM), monocytes (MON), and granulocytes (GRA).

		80% RDA			120% RDA		
%		n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
LYM	Male	35.8 ±3.4^{A}	22.2 ±3.0^{C}	28.2 ±3.0^{ABC}	35.0 ±3.0^{A}	33.2 ±3.0^{A}	29.5 ±3.0^{ABC}
	Female	31.5 ±3.0^{AB}	27.5 ±3.0^{ABC}	21.0 ±3.0^{C}	32.5 ±3.0^{AB}	24.4 ±3.0^{BC}	31.1 ±3.0^{AB}
MON	Male	5.9 ±0.4^{AB}	4.8 ±0.4^{BC}	5.5 ±0.4^{ABC}	5.1 ±0.4^{BC}	6.2 ±0.4^{A}	5.2 ±0.4^{ABC}
	Female	4.1 ±0.4^{C}	4.9 ±0.4^{BC}	4.7 ±0.4^{C}	4.3 ±0.4^{C}	4.7 ±0.4^{C}	5.0 ±0.4^{BC}
GRA	Male	58.4 ±3.5^{C}	73.0 ±3.2^{AB}	66.4 ±3.2^{ABC}	59.9 ±3.2^{C}	60.5 ±3.2^{C}	65.3 ±3.2^{BC}
	Female	64.3 ±3.2^{BC}	67.6 ±3.2^{ABC}	74.4 ±3.2^{A}	63.2 ±3.2^{BC}	70.9 ±3.2^{AB}	63.9 ±3.2^{C}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.20. Effect of diet and feeding intensity on monocyte (MON) count.

MON, $10^3/mm^3$	n-3 PUFA	n-6 PUFA	n-9 MUFA
80% RDA	0.22 ±0.03^{A}	0.07 ±0.03^{C}	0.11 ±0.03^{BC}
120% RDA	0.14 ±0.03^{BC}	0.17 ±0.03^{AB}	0.14 ±0.03^{BC}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.21. Effect of fasting and feeding intensity on % monocytes (MON).

MON, %	Non-Fasted	Fasted
80% RDA	5.7 ±0.2^{A}	4.2 ±0.2^{C}
120% RDA	5.4 ±0.2^{AB}	4.8 ±0.2^{B}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

3.3.5 Clinical Chemistry

The p-values for the main effects and interactions of the clinical chemistry responses are shown in Table 3.22. Fasting and feeding intensity had a significant effect on blood glucose levels at sampling, with the NF mink having higher levels than the F mink (Table 3.23). Within the F mink, the animals fed at 120% RDA had higher levels than those fed at 80% RDA.

Fasting significantly increased plasma alanine transaminase (ALT) levels (Table 3.24). An interaction between fasting and feeding intensity was observed for plasma alkaline phosphatase (AKPH) levels where the F mink fed at 80% RDA had the highest values, followed by the NF mink at both feeding intensity. The lowest AKPH levels were seen in the fasted mink fed at 120% RDA.

Total protein levels within the blood were influenced by diet, fasting and sex, and fasting and feeding intensity interactions. Within the dietary treatments, the highest levels were recorded for the mink fed the n-9 MUFA diet and no differences between either the n-3 or n-6 PUFA diets (Table 3.25). Higher protein levels were observed in the NF females, compared to the fasted females, whereas no differences were present between the males (Table 3.26). The NF mink fed at 120% feeding intensity had higher protein levels in the plasma compared to the F mink fed at the same feeding intensity, while no differences between the 80% RDA and fasting were detected (Table 3.23). Differences were also observed within the two components that make up total plasma protein. Albumin was found to be affected by diet, with the mink fed the n-3 PUFA diet having lower levels compared to the n-6 PUFA and n-9 MUFA diets (Table 3.25). Globulin was affected by the fasting and feeding intensity interaction, with the F mink fed at 120% RDA having lower globulin levels than the NF mink or F mink fed at 80% RDA. The albumin: globulin ratio was also increased as a result of fasting (Table 3.24).

Plasma urea values responded to the fasting and feeding intensity, with the highest levels being observed in the NF mink fed at 120% RDA and the lowest in the F mink fed at 120% RDA (Table 3.23). No effect of fasting was observed on the mink fed at 80% RDA. Significant differences in plasma uric acid were observed due to the fasting, sex, diet and feeding intensity interaction. No difference was observed within the

NF mink, however, within the F mink, the females fed the n-3 PUFA diet at 120% RDA had higher uric acid levels than the females fed the n-6 PUFA diet at 80% RDA or the n-9 MUFA diet at 120% RDA (Table 3.27). Fasting resulted in a decrease in uric acid levels for the male mink fed the n-3 PUFA diet at 120% RDA and the female mink fed the n-6 PUFA diet at 120% RDA. No differences were observed for plasma NH_3 levels (645 ±1 mmol/L).

Plasma creatinine levels in the NF mink were found to be the highest in the female n-6 PUFA fed at 120% which differed from the female mink fed the n-9 MUFA diet at both feeding intensities (Table 3.27). In the F animals, differences were found in the female mink with those fed the n-3 PUFA diet at 80% RDA having higher levels than the females fed any of the diets at 120% RDA. Creatine kinase levels increased as a result of fasting from 167 ±25 U/L in the NF mink to 297 ±25 U/L in the F mink. A feeding intensity effect on total antioxidant score was found with higher levels in the mink fed at 120% RDA (1.51 ±0.02 mmol/L) compared to the mink fed at 80% RDA (1.46 ±0.02 mmol/L).

LDL was found to respond to fasting and feeding intensity. In the NF animals, mink fed at 120% RDA had higher LDL levels than the mink fed at 80% RDA (Table 3.23). However, this effect was lost in the F mink, but who had lower LDL levels than the NF mink. Fasting also led to a decrease in the HDL levels (3.64 ±0.08 vs. 3.32 ±0.08). Furthermore, feeding intensity had an effect on HDL levels, with a decreased HDL levels when feeding the mink at 120% RDA compared to 80% RDA (3.643 ±0.08 vs. 3.29 ±0.08). Both LDL and HDL showed a significant sex and diet interaction. For LDL, the n-9 MUFA diet males had higher levels than the females while no sex differences were observed in the other diets (Table 3.28). With regards to HDL, the females had lower values only when fed the n-3 or n-6 PUFA diets compared to the males. In addition, in the female mink the highest HDL levels were seen in the n-6 PUFA diets, while in the male mink the highest levels were observed in the n-9 MUFA diets and the lowest in the n-3 PUFA diets. Differences in HDL levels were also detected between diets and feeding intensities, with the mink fed the n-6 PUFA diet at 80% having higher HDL levels than all other groups (Table 3.29).

A significant interaction response to the fasting, diet and feeding was observed with plasma NEFA and TAG levels. NEFA levels tended to increase as a result of fasting, with the exception of the n-3 PUFA mink fed at 120% RDA. The highest NEFA levels were found in the fasted mink fed the n-6 PUFA diet at 120% RDA and n-9 PUFA diet at 80% RDA (Table 3.30). Within the non-fasted mink, diet and feeding intensity did not result in differences in NEFA levels. TAG levels also tended to increase as a result of fasting in the diet and feeding intensity, with the exception of the n-6 PUFA diet fed at 120% which showed no significant difference between the F and NF mink. A sex effect was also observed for TAG with the males having lower levels than the females (0.94 ±0.04 vs. 1.13 ±0.04).

Table 3.22a. P-values of the main effects and interactions for the clinical chemistry responses. Highest order significant main effects and interactions are bolded.

Effect	Glucose	ALT	AKPH	TProt	ALB	Glob	A:G	Urea
Fast	<0.001	**<0.001**	0.569	0.155	0.098	0.009	**0.002**	0.002
Sex	0.147	0.754	0.620	0.685	0.392	0.166	0.087	0.561
Fast*Sex	0.485	0.280	0.978	**0.023**	0.075	0.266	0.678	0.379
Diet	0.375	0.223	0.759	**0.019**	**0.006**	0.311	0.140	0.092
Fast*Diet	0.762	0.940	0.784	0.927	0.595	0.718	0.493	0.215
Sex*Diet	0.566	0.850	0.895	0.501	0.764	0.670	0.745	0.966
Fast*Sex*Diet	0.291	0.946	0.232	0.078	0.205	0.545	0.809	0.767
FI	0.186	0.909	0.043	0.787	0.125	0.166	0.099	0.392
Fast*FI	**0.050**	0.260	**0.047**	**0.015**	0.518	**0.036**	0.300	**<0.001**
Sex*FI	0.648	0.464	0.594	0.928	0.897	0.852	0.996	0.793
Fast*Sex*FI	0.169	0.281	0.978	1.000	0.605	0.709	0.523	0.757
Diet*FI	0.566	0.519	0.969	0.464	0.548	0.511	0.420	0.285
Fast*Diet*FI	0.940	0.056	0.427	0.184	0.657	0.321	0.552	0.865
Sex*Diet*FI	0.101	0.229	0.878	0.495	0.442	0.123	0.229	0.752
Fast*Sex*Diet*FI	0.221	0.377	0.666	0.858	0.595	0.691	0.460	0.514

ALT = alanine transaminase; AKPH = alkaline phosphatase; TPro = total plasma protein; ALB = albumin; Glob = globulin; A:G = albumin: globulin.

Table 3.22b. P-values of the main effects and interactions for the clinical chemistry responses. Highest order significant main effects and interactions are bolded.

Effect	UA	NH_3	Crea	CK	LDL	HDL	TAG	NEFA	TAS
Fast	0.045	0.172	0.003	**<0.001**	<0.001	**0.004**	<0.001	<0.001	0.586
Sex	0.807	0.159	0.172	0.696	0.520	0.022	**0.039**	0.312	0.770
Fast*Sex	0.973	0.327	0.407	0.429	0.439	0.482	0.338	0.702	0.516
Diet	0.518	0.160	0.791	0.546	0.754	0.004	0.053	0.299	0.616
Fast*Diet	0.657	0.234	0.490	0.257	0.606	0.648	0.172	0.526	0.067
Sex*Diet	0.159	0.104	0.465	0.848	**0.042**	**0.035**	0.390	0.831	0.482
Fast*Sex*Diet	0.233	0.805	0.036	0.522	0.373	0.364	0.231	0.490	0.187
FI	0.810	0.102	0.957	0.228	0.002	0.001	0.173	0.578	**0.024**
Fast*FI	0.673	0.275	0.974	0.090	**0.026**	0.229	0.279	0.404	0.361
Sex*FI	0.844	0.933	0.432	0.854	0.171	0.682	0.954	0.944	0.821
Fast*Sex*FI	0.619	0.190	0.113	0.109	0.425	0.173	0.945	0.676	0.700
Diet*FI	0.923	0.458	0.766	0.577	0.074	**0.002**	0.513	0.015	0.396
Fast*Diet*FI	0.850	0.623	0.178	0.820	0.371	0.803	**0.042**	**0.016**	0.299
Sex*Diet*FI	0.174	0.310	0.266	0.152	0.643	0.247	0.489	0.454	0.839
Fast*Sex*Diet*FI	**0.014**	0.200	**0.043**	0.998	0.561	0.458	0.136	0.320	0.059

UA = uric acid; NH_3 = ammonia; NEFA = non-esterified fatty acids; LDL = low density lipoprotein; HDL = high density lipoprotein; TAG = triacylglyceride TAS= total antioxidant status.

Table 3.23. Effect of fasting and feeding intensity on plasma glucose, urea, alkaline phosphatase (AKPH), total protein, globulin and LDL.

	Non-Fasted		Fasted	
	80 % RDA	120% RDA	80 % RDA	120% RDA
Glucose, mmol/L	8.3 ±0.3^{A}	8.5 ±0.3^{A}	6.5 ±0.3^{C}	7.6 ±0.3^{B}
Urea, mmol/L	4.1 ±0.3^{B}	5.0 ±0.3^{A}	4.4 ±0.3^{AB}	3.1 ±0. 3^{C}
AKPH, U/L	22 ±3^{B}	21 ±3^{B}	29 ±3^{A}	17 ±3^{C}
Total Protein, g/L	57 ±1^{AB}	58 ±1^{A}	57 ±1^{AB}	56 ±1^{B}
Globulin, g/L	29 ±1^{A}	29 ±1^{A}	28 ±1^{A}	26 ±1^{B}
LDL, mmol/L	0.58 ±0.03^{A}	0.41 ±0.03^{B}	0.28 ±0.03^{C}	0.26 ±0.03^{C}

^{A,B,C} Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.24. Effect of fasting on alanine transaminase (ALT), albumin: globulin (A:G), and HDL.

	Non-Fasted	Fasted
ALT, U/L	139 ±22^{B}	299 ±22^{A}
A:G	1.00 ±0.02^{B}	1.09 ±0.02^{A}
HDL, mmol/L	3.29 ±0.08^{B}	3.64 ±0.08^{A}

^{A,B} Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.25. Effect of diet on total plasma protein and albumin.

	n-3 PUFA	n-6 PUFA	n-9 MUFA
Total Protein, g/L	56 ±1^{B}	57 ±1^{B}	58 ±1^{A}
Albumin, g/L	28 ±0.4^{B}	29 ±0.4^{A}	30 ±0.4^{A}

^{A,B} Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.26. Effect of fasting and sex on total plasma protein.

	Male	Female
Non-Fasted	57 ±1^{AB}	58 ±1^{A}
Fasted	58 ±1^{AB}	56 ±1^{B}

^{A,B} Values are differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.27. Effect of diet, feeding intensity, sex, and fasting on plasma uric acid and creatinine levels.

			Uric acid, µmol/L		Creatinine, µmol/L	
			Non-Fasted	Fasted	Non-Fasted	Fasted
n-3 PUFA	80% RDA	Male	74.45 ±13.96	82.10 ±13.96^{AB}	178.8 ±79.4^{AB}	372.0 ±79.4^{AB}
		Female	105.60 ±13.96	75.47 ±13.96^{AB}	153.0 ±79.4^{AB*}	458.2 ±79.4^{A}
	120% RDA	Male	99.33 ±13.96*	55.32 ±13.96^{B}	133.7 ±79.4^{AB}	324.8 ±79.4^{AB}
		Female	82.87 ±13.96	108.37 ±13.96^{A}	134.2 ±79.4^{AB}	228.7 ±79.4^{B}
n-6 PUFA	80% RDA	Male	101.58 ±13.96	92.00 ±13.96^{AB}	197.5 ±79.4^{AB}	371.2 ±79.4^{AB}
		Female	79.50 ±13.96	68.30 ±13.96^{B}	143.3 ±79.4^{AB}	262.7 ±79.4^{AB}
	120% RDA	Male	88.23 ±13.96	92.00 ±13.96^{AB}	138.7 ±79.4^{AB}	250.5 ±79.4^{AB}
		Female	113.63 ±13.96*	60.40 ±13.96^{B}	347.5 ±79.4^{A}	207.3 ±79.4^{B}
n-9 MUFA	80% RDA	Male	76.60 ±13.96	65.03 ±13.96^{B}	155.2 ±79.4^{AB}	264.2 ±79.4^{AB}
		Female	86.90 ±13.96	91.53 ±13.96^{AB}	123.2 ±79.4^{B}	333.7 ±79.4^{AB}
	120% RDA	Male	90.08 ±13.96	80.48 ±13.96^{AB}	169.8 ±79.4^{AB}	323.4 ±79.4^{AB}
		Female	74.95 ±13.96	68.57 ±13.96^{B}	124.3 ±79.4^{B}	164.3 ±79.4^{B}

^{A,B,C} Values within response columns differ ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.28. Effect of sex and diet on plasma LDL and HDL levels.

	Male			Female		
	n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
LDL, mmol/L	0.35 ±0.04^{AB}	0.34 ±0.04^{AB}	0.44 ±0.04^{A}	0.44 ±0.04^{AB}	0.39 ±0.04^{AB}	0.34 ±0.04^{B}
HDL, mmol/L	2.97 ±0.13^{D}	3.27 ±0.13^{CD}	3.45 ±0.13^{BC}	3.51 ±0.13^{BC}	4.01 ±0.13^{A}	3.58 ±0.13^{B}

^{A,B} Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.29. Effect of feeding intensity and diet on plasma HDL levels.

HDL, mmol/L	n-3 PUFA	n-6 PUFA	n-9 MUFA
80% RDA	3.35 ±0.12^{B}	4.05 ±0.12^{A}	3.52 ±0.12^{B}
120% RDA	3.13 ±0.12^{B}	3.22 ±0.12^{B}	3.51 ±0.12^{B}

^{A,B} Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.30. Non-esterified fatty acid (NEFA) and triacylglycerol (TAG) levels of the fasted and non-fasted mink fed experimental diets at 80% and 120% RDA.

		NEFA mmol/L		TAG, mmol/L	
		Non-Fasted	Fasted	Non-Fasted	Fasted
n-3 PUFA	80% RDA	0.50 ±0.14*	1.32 ±0.14^{ABC}	0.94 ±0.09^{AB*}	1.31 ±0.09^{A}
	120% RDA	0.58 ±0.14	0.95 ±0.14^{C}	0.89 ±0.0^{AB*}	1.34 ±0.09^{A}
n-6 PUFA	80% RDA	0.44 ±0.14*	0.96 ±0.14^{C}	0.72 ±0.09^{B*}	1.15 ±0.09^{AB}
	120% RDA	0.46 ±0.14*	1.52 ±0.14^{AB}	1.06 ±0.09^{A}	1.02 ±0.09^{B}
n-9 MUFA	80% RDA	0.60 ±0.14*	1.63 ±0.14^{A}	0.76 ±0.09^{B*}	1.11 ±0.09$^{AB\backslash}$
	120% RDA	0.57 ±0.14*	1.10 ±0.14^{ABC}	0.85 ±0.09^{AB*}	1.25 ±0.09^{AB}

$^{A-C}$ Values differ within responses ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmean ± SEM are presented.

3.3.6 Endocrinology

The p-values of the main effects and interactions for endocrinology responses are shown in Table 3.31. Insulin was significantly affected by fasting, sex, and feeding intensity. The males had lower insulin levels than the females (4.32 ±0.35 μU/mL vs. 5.98 ±0.35 μU/mL), while the mink fed at 120% RDA had higher insulin levels compared to the mink fed at 80% RDA (5.67 ±0.36 μU/mL vs. 4.63 ±0.36 μU/mL). In addition fasting decreased insulin levels compared to the non-fasted mink (4.34 ±0.36 μU/mL vs. 5.95 ±0.36 μU/mL). HOMA also showed a significant difference with fasting resulting in a decrease in the ratio (NF: 2.20 ±0.13 vs. F: 1.33 ±0.13). Glucagon did not show any significant main effects or interactions with a mean of 35.29 ±1.21 pg/mL.

Plasma leptin showed difference with regards to feeding intensity and fasting, where lower leptin levels were observed in the F mink and within the NF mink the highest concentrations were observed at the 120% RDA (Table 3.32). Cortisol levels increased with feeding at 120% RDA (80% RDA: 37.04 ±7.16 nmol/L vs. 120% RDA: 62.87 ±7.16 nmol/L).

The thyroid hormones responded differently to sex, diet, feeding intensity, and fasting treatments. The T_3 levels were the lowest in the male mink fed at 80% RDA, and the highest in the female mink fed the n-3 PUFA diet at 120% RDA (Table 3.33). Differences were observed between the sexes in the n-3 PUFA and n-9 MUFA diets fed at 120% with higher levels in the female mink. T_3 levels also increased as a result of fasting in the male mink fed the n-3 and n-6 PUFA diets and the female mink fed the n-9 MUFA diet (Table 3.34). T_4 levels increased with the higher feeding intensity for the n-3 PUFA diet, which was not observed in the other diets, and with the 120% RDA group, the n-6 PUFA mink had lower T_4 levels than the mink fed the n-3 PUFA diet. Feeding intensity and the fasting and diet interaction had a significant effect on the T_3: T_4 ratio. Increasing the feeding intensity resulted in an increase in the T_3: T_4 ratio (80% RDA: 0.07 ±0.004 vs. 120% RDA: 0.09 ±0.004). With regards to fasting, diet affected the T_3: T_4 response with an increase as a result of fasting in both the n-3 PUFA and n-9 MUFA diets but not the n-6 PUFA diet. Within the NF mink, those fed the n-6 PUFA diet had a higher T_3: T_4 ratio than the animals fed the n-3 PUFA and n-9 MUFA diet (Table 3.35).

Table 3.31. P-values of the main effects and interactions for the endocrinology responses. Highest order significant main effects and interactions are bolded.

Effect	Leptin	Cortisol	Glucagon	T_3	T_4	Insulin	HOMA	T_3:T_4
Fast	<0.001	0.108	0.056	<0.001	**<0.001**	**0.003**	**<0.001**	0.060
Sex	0.055	0.275	0.436	0.045	0.746	**0.030**	0.098	0.117
Fast*Sex	0.430	0.552	0.834	0.806	0.369	0.196	0.215	0.157
Diet	0.111	0.605	0.753	0.821	0.678	0.703	0.354	0.590
Fast*Diet	0.625	0.389	0.351	0.009	0.077	0.300	0.729	**0.034**
Sex*Diet	0.420	0.245	0.760	0.477	0.814	0.551	0.667	0.991
Fast*Sex*Diet	0.265	0.707	0.677	**0.029**	0.604	0.576	0.356	0.506
FI	<0.001	**0.013**	0.088	<0.001	0.996	**0.044**	0.138	**0.001**
Fast*FI	**0.001**	0.856	0.248	0.847	0.108	0.243	0.051	0.130
Sex*FI	0.669	0.348	0.850	0.289	0.384	0.159	0.301	0.468
Fast*Sex*FI	0.244	0.991	0.544	0.387	0.143	0.513	0.928	0.131
Diet*FI	0.333	0.376	0.842	0.318	**0.021**	0.603	0.734	0.240
Fast*Diet*FI	0.951	0.209	0.826	0.399	0.141	0.352	0.439	0.314
Sex*Diet*FI	0.801	0.955	0.168	**0.009**	0.233	0.554	0.411	0.916
Fast*Sex*Diet*FI	0.266	0.276	0.328	0.334	0.653	0.232	0.739	0.970

HOMA = the homeostatic model assessment

Table 3.32. Plasma leptin levels of fasted and non-fasted mink fed at 80% RDA and 120% RDA.

Leptin, ng/mL	80 % RDA	120% RDA
Non-Fasted	1.91 ± 0.19^{B}	3.41 ± 0.19^{A}
Fasted	1.25 ± 0.19^{C}	1.36 ± 0.19^{C}

$^{A-B}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.33. Effect of feeding intensity and diet on plasma T_3 and T_4 levels.

		80% RDA			120% RDA		
		n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
T_3, n*M*	Male	0.65 ± 0.06^{E}	0.65 ± 0.06^{E}	0.69 ± 0.06^{E}	0.82 ± 0.06^{CDE}	0.91 ± 0.06^{BC}	0.84 ± 0.06^{CD}
	Female	0.73 ± 0.06^{DE}	0.85 ± 0.06^{CD}	0.78 ± 0.06^{CDE}	1.10 ± 0.06^{A}	0.94 ± 0.06^{BC}	1.07 ± 0.07^{AB}
T_4, n*M*		10.16 ± 0.80^{B}	11.74 ± 0.80^{AB}	11.79 ± 0.85^{AB}	13.12 ± 0.99^{A}	10.00 ± 0.85^{B}	10.58 ± 0.86^{AB}

$^{A-E}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.34. Effect of diet, sex and fasting on plasma T_3.

T_3, n*M*		Non-Fasted	Fasted
n-3 PUFA	Male	$0.81 \pm 0.05^{C*}$	0.65 ± 0.05^{B}
	Female	$0.98 \pm 0.05^{AB*}$	$0.85\ 0.06^{B}$
n-6 PUFA	Male	$0.86 \pm 0.05^{BC*}$	0.70 ± 0.05^{B}
	Female	$1.07 \pm 0.05^{A*}$	0.71 ± 0.06^{B}
n-9 MUFA	Male	$0.83 \pm 0.05^{C*}$	0.70 ± 0.05^{B}
	Female	0.92 ± 0.05^{BC}	0.93 ± 0.07^{A}

$^{A-C}$ Values differ within response columns ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.35. Effect of fasting and diet on T_3:T_4 ratio.

T_3:T_4	n-3 PUFA	n-6 PUFA	n-9 MUFA
Non-Fasted	0.063 ± 0.007^{C}	0.087 ± 0.007^{A}	0.068 ± 0.006^{BC}
Fasted	0.086 ± 0.006^{AB}	0.077 ± 0.007^{ABC}	0.089 ± 0.008^{A}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

3.3.7 Liver Evaluation

The p-values for the main effects and interactions for the liver responses are shown in Table 3.36. Liver weight in the mink was affected by sex, with heavier livers found in the males than the females (Table 3.37, Table 3.38). No differences in liver weights were observed in the female mink with regards to fasting and diet. However, in the male mink the heaviest livers were found in the NF n-3 PUFA fed group, which differed from all other groups except the F n-9 MUFA fed males. The higher feeding intensity led to increased liver weight in both male and female mink. The effect of feeding intensity on liver weight was also observed in the n-3 and n-6 PUFA fed mink which had heavier livers, however this was not observed in the n-9 MUFA fed mink (Table 3.39).

The HSI showed significant differences due to sex, fasting, and diet and feeding intensity. The female mink had a higher HSI than the male mink (female: 2.76 ±0.06 vs. male: 2.38 ±0.06). Fasting increased HSI from 2.37 ±0.08 in the NF mink to 2.77 ±0.06 in the F mink. The mink fed the n-3 PUFA diet at 80% had the highest HSI, which was not significantly different than the n-6 PUFA diet at 120% RDA and the n-9 MUFA diet at both RDA.

The liver weight: BMI ratio (L: BMI) could be another way to express the relationship between liver to weight and body size. Unlike HSI, The female mink had lower L: BMI ratio compared to the male mink (male: 2.23 ±0.03 vs. female: 1.64 ±0.03). The L: BMI also showed a difference to the fasting and feeding intensity, with an increase in the ratio with fasting in the mink fed at 120% RDA, while the ratio was not affected in the mink fed at 80% RDA (Table 3.40). In the NF group, the mink fed at 80% RDA a higher L: BMI ratio than the 120% RDA group, with the opposite being true in the F group. The n-6 PUFA diet resulted in an increased L: BMI when the mink were fed

at the 120% RDA levels compared to the 80% RDA level, which was not observed in either of the n-3 or n-6 PUFA diets.

Significant differences in liver lipid levels were observed in the fasting and feeding intensity, and the diet and feeding intensity interactions. It was found that fasting resulted in increased liver lipid levels in the mink, with the increase being the most severe in the mink fed at 120% RDA (Figure 3.5). The highest liver lipid levels were found in the mink fed the n-6 PUFA and n-9 MUFA diets at the 120% RDA level. The lowest were found in the mink fed the n-6 PUFA diet at 80% RDA, which did not differ from the n-3 PUFA diet fed at either the 80% or 120% RDA level (Figure 3.6). Changes in the liver lipid levels (%) were also reflected in the total amount of lipid present in the livers. A sex effect was also observed for total amount of liver lipid, with the male mink having higher total liver lipid than the females (5.81 ±0.36 g vs. 3.79 ±0.36 g). Non-fasted mink with a BCS of 2 or 3 were found to have a liver lipid level of 6.0 ±0.3 %. Mink with histologically normal livers, with no macrovesicular and microvesicular steatosis, had liver lipid levels of 6.6 ±0.6 %.

The liver DM levels showed a difference due to fasting, with increasing % DM as a result of fasting and were similar to the results observed for liver lipid %. Within the fasted mink, the DM levels were the highest in the n-6 PUFA mink fed at 120% RDA, and the lowest in the n-6 PUFA mink fed at 80% (Table 3.41). A regression equation was generated for predicting liver lipid levels from % liver DM with the equation: lipid % = 1.02DM % - 27.2 ($P < 0.001$; $R^2 = 89.7\%$) (Figure 3.7).

Histological evaluation of hepatic lipidosis showed no significant differences between macrovesicular steatosis scores (0.72 ±0.09). For microvesicular steatosis an interaction for sex, diet and feeding intensity was observed, where within the n-9 MUFA mink fed at 120% RDA the female mink had a higher microvesicular steatosis score than the males (Table 3.42). As well the female mink fed the n-9 MUFA diet at 120% RDA had a higher microvesicular score than all the other female mink. The lipid accumulation of the liver sections showed steatosis in the periportal regions and progressing towards the central vein with increasing severity of the lipid accumulation. When macrovesicular steatosis was present, it was located 64% (24/37) of the time in the periportal region, and was present panacinarly 35% (13/37) of the time. For microvesicular steatosis, it was

observed to occur 50% (17/34) in the periportal region, 47% (16/34) with a panacinar distribution, and 3% following an azonal distribution (1/34).

Table 3.36. P-values of the main effects and interactions for the liver responses. Highest order significant main effects and interactions are bolded.

Effect	Liver Lipid %	Liver Weight/ BMI	HSI	Liver Weight	DM	Total lipid liver content	Microvesicular Steatosis	Macrovesicular Steatosis
Fast	<0.001	0.002	**<0.001**	0.950	<0.001	<0.001	0.308	0.156
Sex	0.562	**<0.001**	**0.013**	0.001	0.252	**0.016**	1.000	1.000
Fast*Sex	0.643	0.577	0.261	0.787	0.669	0.080	0.205	0.774
Diet	0.327	0.955	0.575	0.179	0.440	0.300	0.171	0.291
Fast*Diet	0.979	0.751	0.965	0.406	0.783	0.715	0.546	0.676
Sex*Diet	0.677	0.819	0.685	0.716	0.393	0.279	0.862	0.830
Fast*Sex*Diet	0.641	0.675	0.862	**0.023**	0.510	0.262	0.731	0.529
FI	0.004	0.880	0.185	<0.001	0.001	<0.001	0.078	0.254
Fast*FI	**0.020**	**0.003**	0.444	0.071	0.111	**0.007**	0.129	0.156
Sex*FI	0.187	0.499	0.394	**0.005**	0.528	0.878	0.308	0.567
Fast*Sex*FI	0.119	0.058	0.669	0.352	0.119	0.464	0.078	0.774
Diet*FI	**0.031**	**0.041**	**0.020**	**0.033**	0.053	**0.048**	0.602	0.369
Fast*Diet*FI	0.081	0.625	0.629	0.448	**0.003**	0.222	0.708	0.205
Sex*Diet*FI	0.852	0.257	0.466	0.650	0.693	0.727	**0.011**	0.598
Fast*Sex*Diet*FI	0.955	0.255	0.159	0.406	0.147	0.909	0.891	0.529

HIS = hepatosomatic index, DM = dry matter.

Table 3.37. Effect of sex, diet and fasting on liver weight.

Liver weight, g		Non-Fasted	Fasted
n-3 PUFA	Male	54.74 ±2.44^{A*}	47.00 ±2.44^{A}
	Female	27.62 ±2.44^{C}	30.42 ±2.44^{B}
n-6 PUFA	Male	45.56 ±2.44^{B}	48.30 ±2.44^{A}
	Female	27.40 ±2.44^{C}	27.30 ±2.44^{B}
n-9 MUFA	Male	44.77 ±2.44^{B}	49.51 ±2.44^{A}
	Female	28.70 ±2.44^{C}	26.79 ±2.44^{B}

$^{A-C}$ Values differ within response columns ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.38. Effect of sex and feeding intensity on liver weight and hepatosomatic index (HSI).

	Male		Female	
	80% RDA	120% RDA	80% RDA	120% RDA
Liver weight, g	41.87 ±1.67^{B}	55.09 ±1.67^{A}	25.16 ±1.67^{D}	30.92 ±1.67^{C}
HSI	2.40 ±0.08^{B}	2.36 ±0.08^{B}	2.84 ±0.08^{A}	2.68 ±0.08^{A}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmean ± SEM are presented.

Table 3.39. Effect of diet and feeding intensity on liver weight, hepatosomatic index (HSI), liver weight over BMI, and total liver lipid.

	80% RDA			120% RDA		
	n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
Liver Weight, g	37.00 ±1.73^{B}	30.35 ±1.73^{C}	33.19 ±1.73^{B}	42.89 ±1.73^{A}	44.43 ±1.73^{A}	41.69 ±1.73^{ABC}
HSI	2.74 ±0.09^{A}	2.42 ±0.09^{C}	2.70 ±0.09^{AB}	2.43 ±0.09^{BC}	2.61 ±0.09^{ABC}	2.52 ±0.09^{ABC}
Liver weight/ BMI	2.16 ±0.06^{AB}	1.98 ±0.06^{B}	2.10 ±0.06^{AB}	2.03 ±0.06^{AB}	2.17 ±0.06^{A}	2.06 ±0.06^{AB}
Total Liver Lipid, g	4.4 ±0.6^{B}	2.6 ±0.59^{C}	4.4 ±0.9^{B}	5.0 ±0.6^{AB}	6.2 ±0.6^{A}	6.2 ±0.6^{A}

$^{A-C}$ Values differ within responses ($P < 0.05$)
Lsmean ± SEM are presented.

Table 3.40. Effect of fasting and feeding intensity on total liver lipid content and liver weight over BMI.

	Non-Fasted		Fasted	
	80% RDA	120% RDA	80% RDA	120% RDA
Total Liver Lipid, g	2.1 ±0.5^{C}	2.7 ±0.4^{C}	5.5 ±0.5^{B}	8.9 ±0.5^{A}
Liver Weight/ BMI	2.08 ±0.05^{B}	1.92 ±0.05^{C}	2.09 ±0.05^{B}	2.26 ±0.05^{A}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

Table 3.41. Effect of diet and feeding intensity and fasting on liver DM.

Liver DM, %		Non-Fasted	Fasted
n-3 PUFA	80% RDA	31.90 ±1.58*	44.56 ±1.58^{B}
	120% RDA	35.40 ±1.58*	44.70 ±1.58^{B}
n-6 PUFA	80% RDA	32.39 ±1.58*	37.76 ±1.58^{C}
	120% RDA	32.84 ±1.58*	50.40 ±1.58^{A}
n-9 MUFA	80% RDA	32.76 ±1.58*	45.23 ±1.58^{B}
	120% RDA	34.31 ±1.58*	46.86 ±1.58^{AB}

$^{A-C}$ Values differ within response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P <0.05).
Lsmean ± SEM are presented.

Table 3.42. Effect of sex, diet and feeding intensity on microvesicular steatosis score.

Microvesicular steatosis score	80% RDA			120% RDA		
	n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
Males	0.17 ±0.39^{B}	0.67 ±0.39^{B}	1.17 ±0.39^{AB}	0.83 ±0.39^{B}	1.17 ±0.39^{AB}	0.50 ±0.39^{B}
Females	0.17 ±0.39^{B}	1.00 ±0.39^{B}	0.17 ±0.39^{B}	0.67 ±0.39^{B}	0.67 ±0.39^{B}	1.83 ±0.39^{A}

^{A,B} Values differ within responses (P <0.05).
Lsmean ± SEM are presented.

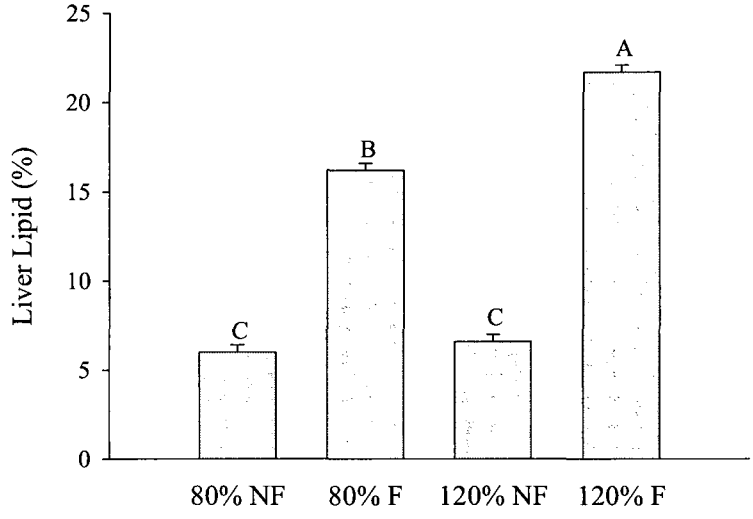

Figure 3.5. Liver lipid content of the fasted and non-fasted mink fed at 80% and 120% RDA. Fast*FI P = 0.020. $^{A-C}$ Values differ (P <0.05). Lsmean ± SEM are presented.

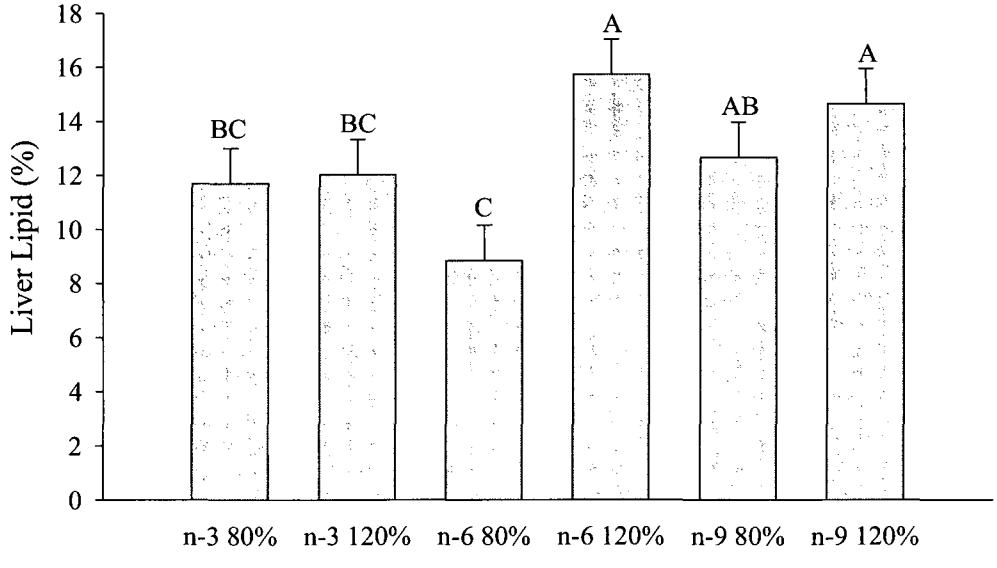

Figure 3.6. Liver lipid content of mink fed experimental diets at 80% and 120% RDA. Diet*FI P = 0.031. $^{A-C}$ Values differ (P <0.05). Lsmean ± SEM are presented.

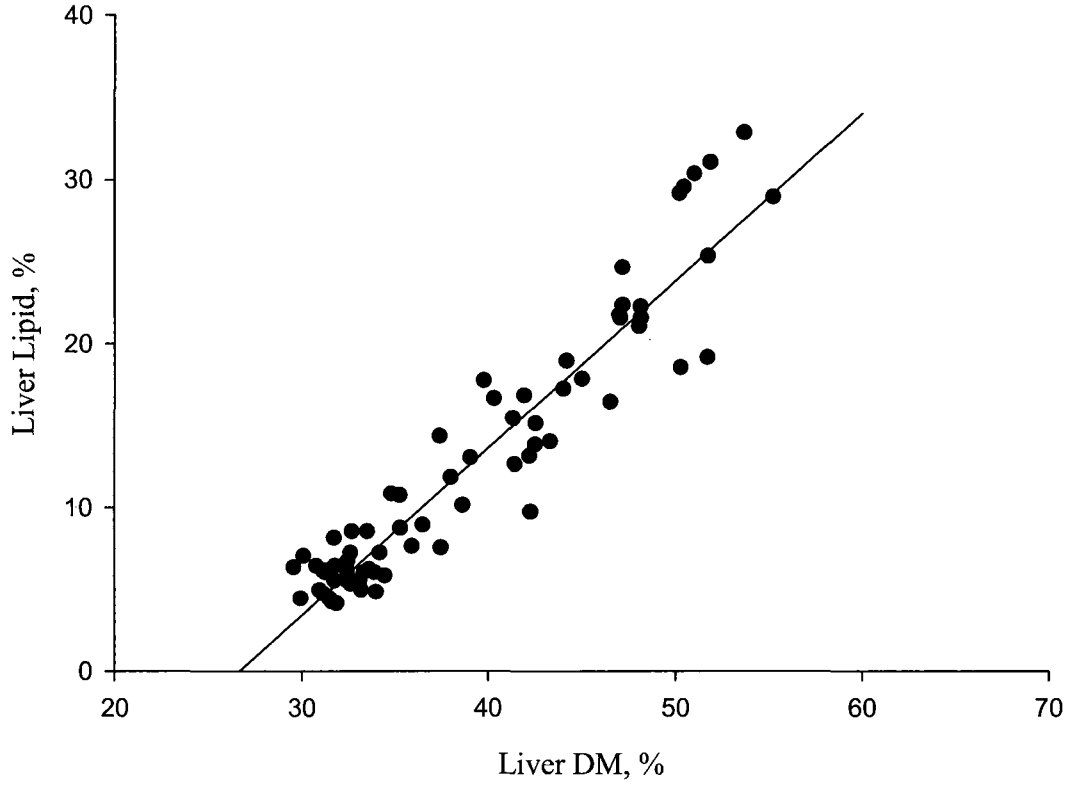

Figure 3.7. Relationship between the liver dry matter (DM) content and the liver lipid content. Regression equation (Liver lipid, % = 1.02Liver DM -27.2; R^2= 89.7, p-value < 0.001).

3.4 Discussion

The development of fatty liver and related diseases in mink, cats, and humans is thought to be influenced by dietary history either through inadequate diet composition or obesity (Hunter and Barker 1996; Rouvinen-Watt 2003; Adams et al. 2005; Allard et al. 2008; Armstrong and Blanchard 2009). Dietary modifications or long term gradual weight loss have been shown to be effective methods to improve liver health and decrease lipid content (Dixon et al. 2004; Alwayn et al. 2005). This study examined the effects of prior feeding history on the development of fasting-induced fatty liver in order

to determine the effects of fatty acid nutrition and feeding intensity on the overall health of the mink and particularly the health of the liver.

3.4.1 Body Weight and Condition

Feeding the experimental diets at 80% RDA decreased body weight and condition throughout the feeding trial portion of the study resulting in lean mink. There was not as dramatic an increase in the body condition of the animals fed above the recommended RDA. Feeding at 120% RDA did increase body weight in male mink, but no significant weight gain was observed in female mink. Regardless of sex, a subtle increase in BCS was observed during the feeding trial with the increased feeding intensity. Differences in autumnal fattening in mink are known to be differ by sex. Female mink reach maximum body weight between November and December, earlier than males, which occurs during December to January (Korhonen and Niemelä 1997, 1998). The time frame of this study could have resulted in the female mink reaching maximum body weight during the mid-point of the feeding trial, while the males could potentially have reached their maximum body weight during the final two weeks of the study. Differences in the energy requirements between males and females may explain the differences in weight gain in mink fed at 120% RDA. Being smaller, female mink have a higher surface area-to-volume ratio than males and this would result in increased heat loss in relation to body weight (Korhonen and Niemelä 1998; Thom et al. 2004). This would be intensified during the onset of winter weather, which occurred during the latter part of the study. Having smaller stomachs, female mink could be limited in feed intake as well (Korhonen and Niemelä 1998). Thus, females would have a lower energy surplus for fat deposition in comparison to males, and as such may reach their maximum weight before males.

It should also be noted that the feed intake of mink fed at 120% RDA was actually closer to the estimated energy requirement or 100% RDA. Mink housed individually during the growing-furring period ate and weighed less than mink housed in male-female pairs (Korhonen and Niemelä 1997). Having the mink housed individually in this study, in order to control their feed consumption, may have limited their potential weight gain compared to if they had been housed in pairs as standard practice. A diet effect was noted

in the DM intake per day, but this could be a result of subtle difference in body weight, as it was not reflected in the estimated RDA which was adjusted to the individual mink's body weight. This is supported by the final body weight of NF male mink fed the n-3 PUFA diet being significantly larger than the other groups.

For the F mink, both sexes lost weight and body condition during the period from moving into the quarantine shed to the 24 hour fasting mark, and after 5 days of fasting. The mink fed at 80% RDA remained lighter than the mink fed at 120% RDA throughout the fasting period, with comparable weight losses between the feeding intensities. Although the weight losses may be similar, decreases in the BCS as a result of fasting were only observed in the mink fed at 80% RDA. However, when compared to the NF mink, all F mink had lowered BCS.

3.4.2 Liver Health

In the diagnosis of fatty liver, a threshold of 5% liver TAGs is used in humans and cats (Pazak et al. 1998; Brunt et al. 1999). This level is likely applicable to mink, however the use of TAG in defining fatty liver limits the interpretation of this study and others investigating the development of fatty liver in mink (Clausen and Sandbøl 2005, 2006) since the liver lipid level corresponding to 5% TAG has not been determined. It would be beneficial to develop a regression model for the purposes of being able to determine the critical liver lipid threshold. Examining the results of other studies, that measured both hepatic total lipid and TAG content, suggests that the threshold for liver lipid content for fatty liver in mink is between 9 to 12% (Käkelä et al. 2001; Rouvinen-Watt et al. 2010). This relates well to normal liver lipid content of mink which can range from 2 to 9.4% (Kannan et al. 2002; Clausen and Sandbøl 2005; Mitchell and Rouvinen-Watt 2008). In the current study the mink with histologically normal livers had a liver lipid content of 6.6% and the non-fasted mink in ideal body condition had a liver lipid content of 6.0%.

The role of fasting in the development of fatty liver in mink had been shown previously (Bjornvad et al. 2004; Clausen and Sandbøl 2005; Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). In this study, dietary history was found to influence the

development of fatty liver in the mink. Both the F mink fed at 80% and 120% showed drastically elevated liver lipid contents. The role of feeding intensity and obesity in mink have been proposed as causative factors, as fatty liver is commonly found in obese mink (Hunter 1996; Koskinen and Lassén 2006) and higher liver lipid levels have been observed in overweight male mink (Clausen and Sandbøl 2006).

An effect of diet and feeding intensity also showed that the mink fed the n-6 PUFA or n-9 MUFA diets at 120% RDA had the highest liver lipid levels. This is in disagreement with the results of a previous study by Käkelä et al. (2001), where mink fed capelin oil had the highest liver lipid levels, compared to a variety of fat sources including soybean oil. Although none of the mink in that study had liver TAG above 5%. Studies have shown that the supplementation of the n-3 PUFAs, particularly the LCPUFAs EPA and DHA, helps in the treatment of NAFLD observed through decreased hepatic steatosis, decreased plasma transaminases and normalization of ultrasonographic evidence of fatty liver (Hatzitolios et al. 2004; Capanni et al. 2006; Zhu et al. 2008).

Differences in liver lipid content were also reflected in liver DM among the dietary and feeding intensity treatments in the F mink. However, the interaction of feeding intensity and fasting on liver lipid level was not observed in DM. A regression model has been used by Clausen and Sandbøl (2004) for the estimation of liver lipid from DM. This study does support the use of a regression model in the estimation of liver lipid content. Yet, in light of the discrepancy in interaction effects, caution may be needed for detecting subtle differences in liver lipid levels between treatments in studies such as this.

Liver weight reflected the known sexual dimorphism seen in mink (Thom et al. 2004). Mink fed at 80% RDA had smaller livers, and this was true within the sexes and fasting treatments. Fasting the mink did result in an increase in liver weight, presumably caused by the accumulation of lipids. The total liver lipid content reflected the relative liver lipid levels, and the subtle differences among treatment groups could in turn reflect differences in liver weights related to body size.

HSI is a ratio that is used to relate the size of the liver to the weight of the body. In this study HSI increased in the fasted animals, indicating that lipid accumulation increased the size of the liver in relation to body size. A study by Rouvinen-Watt et al. (2010) found that HSI increased with increasing lipid accumulation in the liver with

fasting. However, limitations to the use of HSI are noted in this study. Mink fed the n-3 PUFA diet at 80% RDA had the highest HSI ratio, which was not different from the mink fed the n-6 PUFA diet at 120% RDA and the n-9 MUFA diets at both RDAs, the groups with the highest liver lipid levels. The difference in results between the liver lipid content and HSI could be a result of large differences in body fat reserves, which would also affect the HSI ratio. Higher HSI ratios in mink with lean body conditions compared to overweight have been reported before, showing opposite trends to liver fat content (Clausen and Sandbøl 2006). Therefore, the use of HSI in evaluating liver health should be considered carefully, and this index should not be used as the sole indicator of liver health in mink.

Histological evaluation of the livers showed significant differences in the degree of microvesicular steatosis, but not macrovesicular steatosis. The score for microvescular steatosis was found to be the highest for female mink fed the n-9 MUFA diet at 120% RDA at 1.83. The mink fed the n-9 MUFA diet at 120% RDA did have high liver lipid levels. However, the pattern for higher or lower liver lipid levels and the corresponding changes in microvesicular steatosis score was not observed for all treatments. Both macrovesicular and microvesicular steatosis were observed more often in severely fatty livers in this study. The addition of a combined score could reflect the changes observed in liver lipid levels. Limitations in the histological evaluation may also be due to the disadvantages of nonparametric statistics in the analysis of factorial designs. Currently there are few options for the analysis of ordinal data, such as the grading system used here, in factorial designs; none of which are ideal (Shah and Madden 2004). Complex experimental designs, such as the one used in this study, would benefit from the use of scoring systems that combine many features in the generation of the score, as this may not require the use of nonparametric statistics in the analysis. The full method of histological evaluation developed by the Nonalcoholic Steatohepatitis Clinical Research Network generates a score out of 26 by assessing the degree of steatosis, fibrosis, inflammation and liver cell injury (Kleiner et al. 2005). Using this method would allow for a broader examination of the liver, and may allow for further comparison to human NAFLD as demonstrated by Nieminen et al. (2009) for polecats.

Cases of less severe steatosis were localized in the periportal region of the liver lobule. With increasing lipid accumulation it appears as if the steatosis proceeds towards the centrilobular area. This could be a key indication of the cause of the development of steatosis in fasting-induced fatty liver. It has been shown that, under normal metabolic conditions, fatty acid oxidation occurs in the periportal regions of the liver lobule (Guzman et al. 1995; Jungermann and Kietsmann 2000; Braeuning et al. 2006). The increased delivery of fatty acids to the liver may be taken up by the periportal hepatocytes. If the fatty acid uptake by these hepatocytes exceeds the ability of the cell to oxidize or secrete the excess, fatty acids may accumulate in the hepatocytes. There is some flexibility in the liver, and fatty acid oxidation can expand and become panacinar, but this does not appear to be true for fatty acid synthesis (Guzman et al. 1995). Changes to the location of the steatosis within the liver may reflect an increase in the liver's efforts to keep up with the lipolysis occurring. Periportal steatosis has also been observed in ferrets with diabetes and rapid weight loss (Benoit-Biancamano et al. 2005).

Clinical chemistry indicators of liver dysfunction were observed with elevated levels of ALT found with fasting and these levels exceeded the normal reference range for mink (Hunter 1996). Increases in plasma ALT can indicate hepatocyte damage, and is used as a plasma indicator for NAFLD in humans (Brunt 2004), and have been observed in other fasting studies in mink (Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). Differences with AKPH were also observed but the levels were well within the normal range of mink suggesting that there were no abnormalities within the bile ducts (Hunter 1996; Giannini et al. 2005).

3.4.3 Haematology

Haematological variables for red blood cells were within normal range for the most part. Exceptions to this were increased HCT, MCV and MCH which were higher than the reference ranges regardless of treatment (Hunter 1996; Berestov and Brandt 1989), but did compare to values obtained by Muise (2008) who used mink of similar genetic background and management practices. Mustonen et al. (2005a) found that fasting resulted in increased MCV after 5 days, and similar to this study all values were

above reference range regardless of treatment. Without a decrease in RBC count, determining the biological relevance of the increase MCV and MCH is difficult. WBC and individual cell counts all fell within normal ranges (Hunter 1996; Berestov and Brandt 1989). Diet did influence platelet profiles in the mink. Increasing dietary n-3 PUFA intake is known to decrease platelet count (Chang and Huang 1998) and has been documented in mink before (Damgaard et al. 2000). These data are similar to those observed in cats with hepatic lipidosis, where alterations to the blood cell count do not have any prognostic value since cats typically do not show alterations in haematological profiles (Armstrong and Blanchard 2009). Furthermore, in healthy obese cats fed diets high in saturated fats or n-3 PUFAs undetectable differences in immune system were observed when compared to lean cats fed the same diets (Jaso-Friedmann et al. 2008). Thus, although fasting, feeding intensity and diet may influence the development of fatty liver in the mink, like cats, they did not show strong haematological evidence of ill health in this study.

3.4.3 Carbohydrate Metabolism

The mink maintained normoglycemia throughout the feeding trial, the blood glucose concentrations remaining below 7 mmol/L mark considered to be the threshold for hyperglycemia in mink (Hynes and Rouvinen-Watt 2007). The higher blood glucose levels measured in September, at the start of the trial, may reflect handling stress from relocation of the experimental animals. This differs from previous studies in mink where high feeding intensities led to the development of hyperglycemia (Rouvinen-Watt et al. 2004). The development of hyperglycemia during fasting could not be deciphered as handling stress and the use of ketamine during anesthesia prior to blood sampling can increase blood glucose levels (Hsu and Hembrough 1982). Thus, the actual blood glucose levels at sampling were not determined. It has been shown that mink can maintain glucose levels during fasting (Mustonen et al. 2005a) with similar results observed in polecats (Mustonen et al. 2009). This differs from mink nursing sickness and FHL. Mink with nursing sickness are hyperglycemic (Wamberg et al. 1992), while in FHL transient hyperglycemia can often be observed (Biourge et al. 1990). Hyperglycemia can also be

seen during experimentally induced rapid weight loss in obese cats (Biourge et al. 1997). It is possible that the limited length of the fasting period used in this study, like others, does not have the same metabolic consequences as nursing or prolonged durations of obesity. Nursing is metabolically demanding, and development of nursing sickness typically occurs during the period of highest milk production (Clausen et al. 1992). While in cats chronic obesity causes the development of impaired glucose metabolism (Biourge et al. 1997). Thus, the duration of obesity or other metabolic stressors may be a factor in terms of the development of metabolic dysfunctions and associated pathological consequences, such as hyperglycaemia. Short term fasting, such as the duration used in this study, may not be a strong enough stressor to cause this metabolic disruption. This is supported by a 7-week severe weight loss period in obese cats, where glucose tolerance worsened after weight loss (Biourge et al. 1997). This may reflect the cumulative effect of metabolic stressors such as obesity and rapid weight loss.

3.4.5 Lipid Metabolism

Both plasma NEFA and TAG levels increased with fasting. For NEFA there were no differences in the NF mink with regards to diet or feeding intensity. In the F mink, NEFA levels tended to be the highest in the mink with the highest liver lipid levels, and the lowest in the mink with the lowest liver lipid levels. The exceptions to this were the mink fed the n-3 PUFA diet at 80% RDA. Previous studies have shown that NEFA levels in mink increase after 7 days of fasting (Mustonen et al. 2005a). The development of hepatic lipidosis in cats is associated with increased plasma NEFAs. Increased feeding intensity, leading to increase in body condition, may increase the quantity of NEFAs released into circulation (Jensen et al. 1989; Hoenig et al. 2007; Armstrong and Blanchard 2009). Increased NEFAs observed in this study would be expected to result in increased delivery of fatty acids to the liver which can cause lipid accumulation to occur and could be amplified if the animal was obese.

It is interesting to note that the highest plasma TAG levels in the NF mink and the lowest in the F mink were observed in the mink fed the n-6 PUFA diet at 120% RDA. A high n-6: n-3 PUFA ratio is thought to decrease liver secretion of triglycerides (El-Badry

et al. 2007). Thus, a decrease in liver secretion of lipids would explain the increased lipid content in mink fed the n-6 PUFA diet at 120% RDA, while no significant increase in TAG levels with fasting was observed in other groups in this study. These data are in agreement with recent findings in another fasting study in mink (Rouvinen-Watt et al. 2010).

Differences in the cholesterol metabolism were found in both LDL and HDL in this study. LDL was found to be lower in mink fed at 120% RDA compared to mink fed at 80% RDA, and regardless of feeding intensity lower levels were found with fasting. These results are not in agreement with Mustonen et al. (2005a) who found no effect of fasting on LDL levels in mink and Ibrahim et al. (2000), who found an increase in plasma LDL levels after diet-induced obesity in cats. No clear explanations are apparent for these differences. HDL levels were the highest in the F mink, which is a difference not observed previously in mink (Mustonen et al. 2005a). Increased circulating HDL could be the result of decreased clearance by the liver (Ibrahim et al. 2000). Decreased clearance could be the result of decreased receptors in the liver for HDL such as cholesteryl ester transfer protein (Zhou et al. 2006) or decreased production of signalling molecules in the liver such as apolipoprotein E. Impaired or absent apolipoprotein E expression in humans is associated with the development of hyperlipidemia, since apolipoprotein E is required for the clearance of lipids from the blood (Davignon et al. 1999).

In cats with hepatic lipidosis, VLDL is not altered; as such it was suggested that other mechanisms dominate the development of lipid accumulation in the liver (Pazak et al. 1998; Ibrahim et al. 2000). Armstrong and Blanchard (2009) proposed that it was likely increased lipolysis resulting in increased circulating NEFAs and TAGs that leads to the development of HL in cats, rather than a decrease in secretion of lipid from the liver. From this study and others in mink, the increased lipolysis of adipose tissue during food deprivation appears to be the leading cause and source of the lipid accumulation in the liver during negative energy balance. In addition, decreased or insufficient secretion of lipids from the liver may play a critical supporting role further exacerbating the lipid accumulation. Metabolic consequences that may impair lipid secretion will be discussed later in more detail.

3.4.6 Protein Metabolism

Plasma NH_3 levels showed no difference with any of the treatments, similar to the results found by Rouvinen-Watt et al. (2010). This result is different from that of Mustonen et al. (2005b) who found an increase in NH_3 with 5 days of fasting in mink, and Mustonen et al. (2009) who found a decrease with fasting in polecats. A decrease in plasma urea levels was observed with fasting in the mink fed at 120% RDA. Fasting has been shown in previous studies to result in a decrease in plasma urea levels in mink and polecats (Tauson et al. 1997; Mustonen et al. 2005b, 2009). Uric acid levels were found to decrease in two cases in the current study: in male mink fed the n-3 PUFA diet at 120% RDA and female mink fed the n-6 PUFA diet at 120% RDA. In previous studies uric acids levels have been shown to decrease as a result of fasting (Tauson et al. 1997), and in other cases have been shown to increase after 5 days of fasting, but no effect was not observed after 7 days of fasting (Mustonen et al. 2005b). Putting obese cat on severe caloric restriction resulted in no changes to the indicators of protein metabolism with regards to the effect of dietary protein or fat source (Sazbo et al. 2000). Creatinine levels showed a fasting effect with female mink fed the n-3 PUFA diet at 80% RDA and is likely the result of high creatinine levels found in the F mink in this group. Other fasting studies have shown that creatinine levels decrease with short term fasting (Tauson et al. 1997), or show no change with varying durations of fasting (Mustonen et al. 2005a). Elevated creatinine levels could indicate decreased kidney filtration rate (Fettman and Rebar 2004). Additionally, lean body mass makes a small contribution to plasma creatinine levels (Swaminathan et al. 2000), however there were no differences in lean body mass observed between diets in the female mink (Table 4.3). As such, the cause for the differences in plasma creatinine levels remains unclear. The findings of this study support that kidney function was not drastically altered. Thus, feeding intensity, dietary fatty acids, or fasting did not cause severe alterations in the ability of the liver to detoxify NH_3 or the ability of the kidney to excrete urea, uric acid, and creatinine from the body.

Total protein levels, albumin and globulin levels were within normal range for mink (Hunter 1996). Protein is known to be important as an energetic fuel source for

mink and other strict carnivores, with approximately 39% of the body's heat production attributed to protein oxidation (Tauson et al. 1997). During food deprivation mink are able to maintain normoglycemia, due to the utilization of amino acids for gluconeogenesis (Mustonen et al. 2005a). Decreases in the protein concentration of the quadriceps muscle in mink could indicate that protein catabolism is occurring, providing the energetic fuel source the mink is metabolically accustomed to using (Mustonen et al. 2005b). It is indeed likely that the mink utilized muscle protein as an energy source during food deprivation. Concomitantly, creatine kinase levels did increase in this study as a result of fasting. Anorexia in cats is associated with increased serum creatine kinase activities, and is thought to be the result of muscle catabolism to supply amino acids for gluconeogenesis (Fascetti et al. 1997).

3.4.7 Endocrinology

In previous fasting experiments, decreases in insulin levels have occurred with increases in plasma NEFAs (Mustonen et al. 2005a, 2005c), demonstrating insulin's role in sparing glucose and increasing fat mobilization in response to food deprivation. This is also likely the case for the differences in insulin levels observed between the two feeding intensities in the current study. HOMA is an index used to assess insulin resistance (McAuley et al. 2001). In this study, in accordance with similar studies in mink (Mustonen et al. 2005a), a decrease in HOMA was observed. As mentioned before, the glucose measurements during the final blood sampling do not entirely reflect the effects of fasting and dietary treatments. Since this measurement would be used in the calculation of the HOMA index, this too would be affected by stress of restraint and anaesthesia. Glucagon levels showed no differences in this study. Previous studies in mink showed an increase after 5 days of fasting but not after 7 (Mustonen et al. 2005c). A similar fasting study in polecats yielded a decrease in glucagon (Mustonen et al. 2009).

In the NF mink, leptin levels were increased with the higher feeding intensity. Fasting resulted in a decrease in leptin levels compare to the NF, with no effect of feeding intensity. Decreasing leptin levels, previously observed in fasted mink (Mustonen et al. 2005c; Rouvinen-Watt et al. 2010), serve to remove the satiety signal and increase foraging behavior. Increasing body weight, achieved from the increased feeding intensity,

has been shown to correlate with higher leptin levels (Tauson and Forsberg 2002). It is interesting to note, that Mustonen et al. (2005c) did report a correlation between intra-abdominal fat and leptin levels in fasted animals. It is likely that fasting has a stronger influence on leptin levels than body mass, as leptin also serves as a trigger for the neuroendocrine response to fasting (Ahima et al. 1996). This response was evident in the current study with no difference with regards to feeding intensity in the F animals.

The higher feeding intensity also led to increased cortisol levels in the mink. Levels in the 120% RDA group exceeded values previously reported in mink (Mustonen et al. 2005c). In humans, it has been shown that increasing activity of the hypothalamic-pituitary-adrenal axis, which would lead to increase in cortisol levels, promotes selective visceral weight gain, and weight regain (Purnell et al. 2009). This may have implications for mink management, where over feeding during the fall period may set the mink up for continuous weight management problems during the following periods of production.

T_3 levels generally decreased as a result of fasting, a result observed previously by Mustonen et al. (2005c, 2009) and Rouvinen-Watt et al. (2010). Since insulin is required for the conversion of T_4 to T_3, the decrease in T_3 levels during fasting could be a consequence of decreased insulin levels and serves to conserve energy (Rouvinen-Watt et al. 2010). Within the NF mink the females tended to have higher T_3 levels compared to males. The T_4 levels in this study were found to vary with feeding intensity and diet. Previous studies have also shown no effect of fasting on T_4 levels in mink and polecats (Mustonen et al. 2005c, 2009). It was suggested that since T_4 is a prohormone for T_3, it may not reflect changes in nutritional status in the same manner as T_3 (Mustonen et al. 2005c). Although T_4 may not be a suitable indicator of immediate nutritional status, Ferguson et al. (2007) proposed that obesity may result in a relative state of thyroid hormone resistance in cats, which could be identified through elevated T_4, with or without elevated levels of thyroid stimulating hormone. The differences between Ferguson et al. (2007) and fasting studies may reflect prolonged consequences of obesity on hormone function in a similar manner as was observed in insulin. In both studies on mink and polecats, fasting was either found to decrease the T_3: T_4 ratio (Mustonen et al. 2005c, 2009) or resulted in no difference (Mustonen et al. 2005c). This was not observed

in this study, except in mink fed the n-6 PUFA diet. Thus, it is somewhat unclear how the T_3: T_4 ratio is affected by fasting.

3.7.8 Mechanisms in the Development of Fatty Liver

It has been well documented in humans, cats and certain mustelids that negative energy balance caused by food deprivation or severe caloric restriction results in the development of hepatic lipidosis (Szabo et al. 2000; Bjornvad et al. 2004; Mustonen et al. 2005a, 2009; Wang et al. 2006; Rouvinen-Watt et al. 2010). This development is observed with increases in plasma NEFAs caused by increased lipolysis during fasting. Other suggested mechanisms may be involved in the accumulation of lipids in the liver such as decreased excretion of lipids through VLDLs, impaired β-oxidation or increased fatty acid synthesis (Adams et al. 2005).

In this study, the highest NEFA levels were also observed in the mink with the highest liver lipid content. This was true with the exception of the n-3 PUFA mink, who had the lower lipid content in the over fed mink, but still maintained elevated NEFAs. Fatty liver may be the consequence of excessive mobilization of fatty acids, but fatty acids may also play metabolic roles in preventing excessive accumulation as well. Lipolysis caused by anorexia or severe and rapid weight loss has been suggested as the principal mechanism in the development of hepatic lipidosis in cats (Armstrong and Blanchard 2009).

Fatty liver is often observed in obese mink (Hunter and Barker 1996). This experiment illustrated increased severity in the degree of hepatic lipidosis with the high feeding intensity. This may be due to increased supply of NEFAs to the liver, as obesity is associated with increase plasma levels of NEFAs (Jensen et al. 1989). Increased feeding intensity in mink has also been suggested to cause the development of decreased insulin sensitivity (Rouvinen-Watt et al. 2004). This study cannot confirm this, but insulin resistance does play a role in NAFLD (Day 2002). Insulin resistance decreases the ability to regulate mobilization of TAGs in adipose tissue, as insulin decreases the activity of HSL in releasing fatty acids from adipose tissue (Bradbury et al. 2006). Lower insulin levels could result in elevated plasma NEFAs since the uptake of NEFAs by adipocytes is performed by LPL, which is also insulin dependent (Sjaastad et al. 2003).

This is in contrast to fatty acid uptake by the liver which changes in relation to plasma NEFA concentrations, and reflects the liver's role in maintaining a steady state in the body (Bradbury et al. 2006). Fasting leads to decreased insulin levels and in a way has a similar effect as insulin resistance on adipose tissue leading to increased lipolysis and plasma NEFA and consequential NEFA uptake by the liver. The uptake of NEFAs by the liver may be increased with increasing adiposity. Visceral fat accumulates in the mink with increasing body condition during autumnal fattening (Rouvinen and Kiiskinen 1989) and is highly mobilized during fasting (Mustonen et al. 2005a). Increasing body fat in human leads to an increase in the intra-abdominal fat, some of which when mobilized would enter the liver through the portal vein without potential buffering and uptake by muscle tissue (Day 2002). It is likely that this occurs in the mink as well, and will be discussed in Chapter 4.

While the liver's uptake of fatty acids from the blood is influenced by plasma NEFAs, within the liver mechanisms to decrease the accumulation may aid in delaying or reducing the severity of fatty liver. PPAR-α has been proposed to play a prominent role in the pathogenesis of NAFLD by increasing energy utilization (Reddy and Rao 2006). n-3 PUFAs activate PPAR-α leading to increase in β-oxidation (Krey et al. 1997; Levy et al. 2004) and function as a lipid sensor in the liver by responding to influxes of fatty acids with increased transcription of genes for mitochondrial and peroxisomal β-oxidation (Rao and Reddy 2004). Increasing n-3 LCPUFAs has been shown to restore hepatic PPAR-α expression and improve liver condition in rats with fatty liver (Svegliati-Baroni et al. 2006). The decrease in the severity of hepatic lipidosis in the mink fed the n-3 PUFA diet in the current study compared to mink fed the n-6 PUFA diet may be due to effects of PPAR-α through the upregulation of fatty acid oxidation systems. PPAR-α has been shown to mediate the adaptive response in mice during fasting (Kersten et al. 1999). The potential role for increased fatty acid oxidation in decreasing lipid accumulation in the mink may also be found in the location of the lipids within the liver lobule. Lipid accumulation in the periportal area suggests that increased fatty acid uptake is not being matched with sufficient oxidation (Braeuning et al. 2006). In the current study, the histological evaluations did reveal periportal accumulation, the proposed area for fatty

acid oxidation, suggesting that increased PPAR-α could help prevent hepatic lipid accumulation during periods of increase NEFA delivery.

Decreased secretion of lipids from the liver could be attributed to lipid accumulation in NAFLD (Adams et al. 2005) whereas n-3 PUFAs have a role in increased secretion (Lindén et al. 2002). While an increase in VLDL secretion can be observed in some cases of FHL, in others no changes are observed, although insufficient assembly and secretion likely does play a supporting role in liver lipid accumulation (Armstrong and Blanchard 2009). The parallels in the causes of development of fasting-induced fatty liver in mink and FHL suggest that this could also be the case for mink as well. This study supports the idea that increased delivery to the liver rather than decreased secretion from the liver is likely the principal mechanism for the accumulation of lipids during fasting. Furthermore, during periods of fasting decreased insulin levels inhibit LPL activity reducing lipid uptake by adipocytes (Bradbury et al. 2006). If the lipids have no destination, increasing VLDL production would not aid in the long run, as the lipid contents could potentially return back to the liver resulting in a perpetual vicious cycle (Bradbury et al. 2006).

While the experimental model of fasting-induced fatty liver in mink may not result in the clinical development of insulin resistance observed in many patients with NAFLD (Bugianesi et al. 2006) or in female mink with nursing sickness (Rouvinen-Watt 2003), it does, however, illustrate a realistic factor in the development of hepatic lipidosis and mechanisms that alter the degree of lipid accumulation in the liver. The fasting method used in this study, as well as many others, likely represents the first hit in the 2-hit hypothesis proposed for human NAFLD. This stage is benign in humans, just as fatty liver in mink is often asymptomatic (Hunter and Barker 1996). The mink remained "healthy" despite the drastic increases in liver lipids. Yet, mink do succumb to fatty liver. This first hit may not be sufficient enough to cause severe liver damage, but it does predispose the liver for the second hit caused by oxidative stress (Malinska et al. 2010). Oxidative stress is likely a key factor in the second hit, and could contribute to the development of insulin resistance (Day 2002). This study did not show alterations to plasma TAS as a result of fasting, suggesting that fasting for five days likely did not result in an increase in ROS.

It has been proposed that mink succumb to FLS as a result of the metabolic consequences of competing pathways between protein and lipid metabolism (Rouvinen-Watt et al. 2010). Catabolising protein for energy, seen in the current study, would result in ammonia waste that would need to be detoxified in the urea cycle and would also require a source of arginine (Elliott and Elliott 2005). Thus, failure of the urea cycle from insufficient arginine could lead to the development of hyperammonia which can lead to coma and death (Cagnon and Braissant 2007). Insufficient arginine also results in increased production of orotic acid, a hepato-toxin which can inhibit secretion of lipids from the liver (Cornelius and Jacobs 1989). Fasting does result in a depletion of plasma arginine (Mustonen et al. 2005b, 2009), suggesting the development of a deficiency, while increased competition for arginine with lipid metabolism may also be observed, as arginine is also required for the production of VLDL (Maugeais et al. 2000). Thus, fasting may result in a deficiency of arginine and may compromise the secretion of lipids from the liver through VLDL or detoxification of ammonia (Rouvinen-Watt et al. 2010).

3.5 Conclusion

During the feeding trial portion of the trial, the mink fed at 80% RDA lost body weight, and only the males gained weight when fed at 120% RDA. All the mink maintained normoglycaemia through the feeding trial. The development of fatty liver in the mink was shown to be strongly influenced by fasting. Increasing the feeding intensity increased the severity of the development of fatty liver with fasting, while feeding diets high in n-3 PUFA helped in reducing the severity in the mink fed above the RDA. Increased plasma NEFAs suggest that the mobilization of fat reserves occurs during fasting, leading to increased uptake by the liver and subsequent lipid accumulation. The higher feeding intensity increased the NEFAs, suggesting an excessive mobilization of the fat reserves during fasting. However, feeding the n-3 PUFA diet may promote increased levels of PPAR-α, increasing fatty acid oxidation in the liver and helping to lower the severity of hepatic lipid accumulation. Despite the development of fatty liver, the mink were otherwise in good health as indicated by the normal haematology and clinical chemistry values. Evidence of increased muscle catabolism as a result of fasting

was noted. The results of this study suggest that avoiding overconditioning in mink during the fall and increasing dietary intake of n-3 PUFAs may help prevent the development of fatty liver during periods of food deprivation.

CHAPTER 4. Effect of Feeding Intensity and Dietary Fat source on the Deposition and Mobilization of Fat and Fatty Acids in the Mink (*Neovison vison*).

4.1. Introduction

During the fall, mink prepare for the winter by depositing body fat which provides an energy reserve during times of food scarcity as well as additional insulation (Rouvinen and Kiiskinen 1989). However, in captivity the energetic needs of the mink differ from those in the wild. As a consequence of a sedentary lifestyle and a calorically dense, high-fat and high carbohydrate diet, farmed mink are frequently found to be obese at the beginning of winter (Koskinen and Lassén 2006; Mustonen et al. 2009). Increased adiposity in mink can have negative health consequences, including an increased risk in the development of fatty liver syndrome (Hunter and Barker 1996).

Fatty liver syndrome in mink is typically found in obese animals during stressful periods in which an animal may go off feed for a period of a few days (Hunter and Barker 1996). Food deprivation or fasting in mink and polecats results in the mobilization of fat, particularly from the abdominal cavity and leads to the development of fatty liver in these animals (Mustonen et al. 2005a, 2009; Rouvinen-Watt et al. 2010). Intra-abdominal fat is of particular concern as the fatty acids mobilized from this depot enter into portal circulation and are delivered to the liver and can lead to hepatic lipid accumulation (Smith and Schenk 2000).

Dietary fatty acids have also been implicated in the development of fatty liver, with low levels of n-3 PUFA or a high n-6: n-3 PUFA ratio leading to the development of fatty liver in humans (Araya et al. 2004). A derangement to liver lipid metabolism caused by an imbalance in n-3 and n-6 PUFAs may lead to conditions in the liver that promote lipid accumulation (El-Badry et al. 2007). During fasting, preferential mobilization of n-3 PUFAs from the fat depots and a decrease of n-3 PUFAs from the liver in mink and polecats occurs (Nieminen et al. 2009; Rouvinen-Watt et al. 2010). This suggests that increasing n-3 PUFAs may help promote and maintain normal liver metabolism in mink during fasting.

Altering the dietary history of the mink during the fall by preventing the development of obesity may help in the prevention of the development of fatty liver. In

addition, increasing intake of n-3 PUFA may help maintain an optimal balance of n-3 and n-6 PUFAs. Dietary fatty acids are strongly reflected in the fatty acid profile of the fat depots in mink (Rouvinen and Kiiskinen 1989, Iverson et al. 2004). Thus feeding diets high in n-3 PUFAs would increase the body's reserves of n-3 PUFAs. This could benefit the mink during periods of fasting by preventing the suggested deficiency in n-3 PUFAs.

The objective of this study is to determine how feeding intensity and the relative dietary fatty acid composition can affect the deposition and mobilization of fat and fatty acids in the mink, and the subsequent alterations in the liver lipids. It was hypothesized that feeding at 120% RDA would increase visceral body fat, which would be mobilized during periods of fasting. Dietary fatty acid composition would be reflected in the fatty acid profile of the fat depots and liver, and fasting would result in a decrease of n-3 PUFAs in the mesenteric fat and liver.

4.2 Material and Methods

4.2.1 Animals

Seventy-two (72) standard dark mink, half male and half female, were allocated to one of three diets and two feeding intensities starting in September 2007. The diets included one of three fat sources: herring oil (n-3 PUFA), soya oil (n-6 PUFA) and canola oil (n-9 MUFA). The oils were chosen to give the diets distinct fatty acid profiles with regards to n-3 and n-6 PUFAs, with herring oil being rich in n-3 PUFAs, soya oil rich in n-6 PUFAs, and canola oil being intermediate in both n-3 and n-6 PUFAs and rich in n-9 MUFAs. The two feeding intensities were 80% RDA and 120% RDA. After feeding the mink for 10 weeks, half the mink in each diet and feeding intensity combination were subjected to a 5-day fast and the other half an overnight fast as a control. Following this, the mink were euthanized for blood and tissue sampling. Further details of the trial, diet formulation and composition, and tissue sampling are described in Section 3.2. Liver lipid content was analyzed following the Folch et al. (1957) method.

4.2.2 Quantitative Dissections

Each mink carcass was quantitatively dissected for the following body fat depots to the nearest 0.1 g: subcutaneous, intermuscular, omental, mesenteric, perirenal, and diaphragmatic. To measure the subcutaneous fat each mink pelt was weighed before and after scraping, and the difference was used to calculate the amount of subcutaneous fat that remained on the pelt. The heart, stomach, pancreas, intestine, spleen, kidneys, reproductive tract, and testes were weighed to the nearest 0.1g, while ovaries, thyroid and adrenal glands were weighed to the nearest 0.001 g.

Total body fat was calculated as the sum of all the fat depots. Total intra-abdominal fat was calculated as the sum of the omental, mesenteric, retroperitoneal and diaphragmatic fat for each mink. Total visceral fat, as the sum of the omental and mesenteric depots, was also calculated. Lean body mass (LBM) was calculated as the total body fat subtracted from the body weight. The relative fat depot weights were expressed both as % body weight and % LBM. The % body fat was calculated for the fat depots as the % weight of the depot of the total body fat reserves.

4.2.3 Fatty Acid Analysis

Fatty acid analysis was performed on the mink plasma, liver, and mesenteric fat. Fatty acid analysis, gas chromatography and identification methodology are described in Section 3.2.4.1. Plasma samples were evaporated under nitrogen to near dryness before methylation solvents were added. Mole percentages are reported for the SFAs, MUFAs, total n-6 PUFAs, total n-3 PUFAs, LA, AA, ALA, EPA, and DHA. Product: Precursor (Pro: Pre) ratios were calculated for the n-3 PUFAs as the combined mole % of EPA and 22:6 -3 over ALA (Nieminen et al. 2009). For the n-6 PUFA, the Pro: Pre ratio was calculated as the ratio of AA to LA.

4.2.4 Statistical Analysis

Data from the dissections and fatty acid profiles were analyzed in a split-split-plot factorial design using Proc MIXED in SAS® v.9 (SAS Institute Inc., Cary, NC). The fasting treatments were used as the whole plot, sex as the sub-plot and the subplot

treatments as the sub-sub-plots, with 3 replicates within each. A blocking factor of dam's dam was used and was nested within sex. A multiple mean comparison test (PDiff) was used to identify where differences existed when significant effects were found at $P < 0.05$. Where 3-way interactions that included fasting as effect or 4-way interactions were found to be significant, comparisons were made within NF and F mink, and between NF and F treatments only. Data are presented as lsmeans ± SEM.

4.3 Results

4.3.1 Liver Lipid Content

Liver lipid content was found to be significantly affected by the interactions between diet and feeding intensity and between fasting and feeding intensity. The mink fed the n-6 PUFA and n-9 MUFA diets at 120% RDA had the highest liver lipid content (Table 4.1). The lowest % liver lipid was observed in the mink fed the n-6 PUFA fed at 80% RDA, which did not differ significantly from the n-3 PUFA diet fed at either feeding intensity. Fasting significantly increased the % liver lipid, with the lipid accumulation being the most severe in the mink fed at 120% RDA (Table 4.2). For a more detailed description of liver health see Chapter 3.

Table 4.1. Effect of feeding intensity and diet on liver lipid content. P-value 0.031.

Liver lipid, %	n-3 PUFA	n-6 PUFA	n-9 MUFA
80% RDA	11.7 ± 1.3^{BC}	8.9 ± 1.3^{C}	12.0 ± 1.3^{AB}
120% RDA	12.0 ± 1.3^{BC}	15.7 ± 1.3^{A}	12.7 ± 1.3^{A}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.2. Effect of fasting and feeding intensity on liver lipid content. P-value 0.020.

Liver lipid, %	80% RDA	120% RDA
Non-Fasted	6.0 ± 1.1^{C}	6.6 ± 1.1^{C}
Fasted	16.2 ± 1.1^{B}	21.7 ± 1.1^{A}

$^{A-C}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

4.3.2 Organ and Fat Depot Weights

4.3.2.1 Absolute Weights

Significant differences were found between the absolute weights of the main internal organs, body fat depots and other dissected tissues (Table 4.3). As described in Chapter 3, the final body weights of the mink fed at 80% RDA were less than those fed at 120% RDA (Table 4.4). Higher body weights were found in the males fed the n-3 PUFA diet at 120% RDA in the NF mink, and these showed a decrease with fasting (Table 4.5). The males fed the n-6 or n-9 MUFA did not differ within or between fasting treatments. The final body weights of the females did not differ within diets or between fasting regimes, and remained significantly less than the male mink. The lean body mass of the mink was larger in the males than the females and increased for both sexes when the mink were fed at 120% RDA. Fasting decreased the lean body mass of the mink (Table 4.6). The estimated total body fat content was found to be the lowest in female mink, and highest in the male mink fed the n-3 PUFA diet. Fasting resulted in a significant total body fat loss only in the male mink fed the n-3 PUFA diet. The 120% RDA feeding intensity resulted in increased total body fat content for both the males and females.

The subcutaneous fat depots were larger in the males than the females. The higher feeding intensity increased the mass of the subcutaneous fat reserves. The largest subcutaneous depots were found in the male mink fed the n-3 PUFA diet, which were also the only group to show a decrease in weight with fasting. Intermuscular fat also was notably elevated in the mink fed at 120% RDA (Table 4.7). The males tended to have more intermuscular fat than the females, and only the male mink fed the n-3 or n-6 PUFA diets showed a decrease in the intermuscular fat depot mass in response to fasting.

The intra-abdominal fat reserves, consisting of omental, mesenteric, diaphragmatic, perirenal fat depots, were the largest in the mink fed at 120% RDA. In the NF mink, males fed the n-3 PUFA diet had the most intra-abdominal fat while, the lowest amounts were found in the female mink. Fasting decreased the intra-abdominal depot for the males fed the n-3 and n-6 PUFA diets. The fasted males tended to have more visceral fat (omental and mesenteric fat) than the females with the exception of n-3 PUFA mink, where no significant difference was found between the sexes. The total visceral fat depot

was the largest in the males, and increased for both sexes with increasing feeding intensity. The increase in these visceral fat reserves was particularly high for males fed the n-3 PUFA diet. Only the males fed the n-3 and n-6 PUFA diet had a significant decrease in the visceral depots with fasting. The omental weight also increased with increasing feeding intensity. It was the largest in the males, with the biggest reserve in NF males fed the n-3 PUFA diet, which was also the only group to show a decrease with fasting. A similar pattern was also observed for the mesenteric fat depot weights with the exception of a general decrease in mesenteric weight with fasting. The perirenal fat depot weight increased with increasing feeding intensity and decreased with fasting. The perirenal mass was the highest in mink fed the n-3 PUFA diet (Table 4.8). Diaphragmatic fat depot was larger in the male mink, and decreased in both sexes due to fasting.

The male hearts were larger in mass than the female hearts and the weight increased with feeding at 120% RDA regardless of sex (Table 4.9). Kidneys also increased in mass with increasing feeding intensity, and were heavier in the males than the females. Kidney weight only decreased in the male mink fed the n-3 PUFA diet. Spleens increased in mass when the mink were fed at 120% RDA. The female mink tended to have slightly smaller spleens than the males, but this was only observed in the F mink. The males had larger stomachs and intestines than the females. Stomach weight increased with the higher feeding intensity. Stomachs were the largest in NF mink fed the n-6 PUFA diet and the smallest in F mink fed the n-6 PUFA diet, and decreased in weight for mink fed the n-6 and n-9 MUFA diet with fasting (Table 4.10). Both the male and female intestines decreased in mass with fasting and were larger in the males. The female pancreases were smaller than those of the males, and decreased in weight with fasting for both sexes. The mink fed the n-9 MUFA diet had smaller pancreases than those fed the n-3 or n-6 PUFA diets. Both male and female reproductive tracts increased in weight with increased feeding intensity. Male reproductive tracts and testes were larger in fasted males. Ovary weight did not respond to any treatment and had a mean of 0.128 ±0.011g. Adrenal glands weighed more in the fasted animals and as well as males, particularly those fed at 120% RDA, while the thyroid glands were heavier in males and decreased in mass as a result of feeding at 80% RDA or fasting.

Table 4.3.a P-values of the main effects and interactions for mink body weight and the weights of fat depots and organs. Highest order significant main effects and interactions are bolded.

Effect	Body Weight	Subcutaneous Fat	Intermuscular Fat	Intra-abdominal Fat	Visceral Fat	Total Body Fat
Fast	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Sex	0.001	0.003	0.001	0.010	0.006	0.003
Fast*Sex	0.217	0.483	**0.028**	0.102	0.069	0.299
Diet	0.123	0.093	0.223	0.013	0.017	0.054
Fast*Diet	0.271	0.251	0.142	0.232	0.249	0.202
Sex*Diet	0.284	0.284	0.270	0.437	0.383	0.267
Fast*Sex*Diet	**0.009**	**0.008**	**0.007**	**0.010**	**0.008**	**0.005**
FI	<0.001	<0.001	**<0.001**	**<0.001**	<0.001	<0.001
Fast*FI	0.776	0.682	0.989	0.656	0.553	0.678
Sex*FI	**0.004**	**0.028**	0.122	0.071	**0.031**	**0.027**
Fast*Sex*FI	0.785	0.960	0.774	0.772	0.699	0.946
Diet*FI	0.719	0.765	0.883	0.580	0.520	0.817
Fast*Diet*FI	0.957	0.521	0.391	0.737	0.725	0.505
Sex*Diet*FI	0.524	0.157	0.669	0.951	0.887	0.293
Fast*Sex*Diet*FI	0.902	0.759	0.692	0.924	0.979	0.812

Table 4.3.b P-values of the main effects and interactions for mink body weight and the weights of fat depots and organs. Highest order significant main effects and interactions are bolded.

Effect	Body Length	Omental	Mesenteric	Perirenal	Diaphagmatic	Lean Body Mass
Fast	0.002	0.008	<0.001	**<0.001**	0.139	**<0.001**
Sex	**<0.001**	0.005	0.004	0.055	0.003	<0.001
Fast*Sex	0.350	0.709	0.044	0.398	0.462	0.212
Diet	0.413	0.106	0.006	**0.038**	0.123	0.458
Fast*Diet	0.829	0.382	0.327	0.423	0.544	0.414
Sex*Diet	0.196	0.842	0.147	0.961	0.503	0.440
Fast*Sex*Diet	0.413	**0.010**	**0.010**	0.053	0.465	0.066
FI	0.130	**<0.001**	<0.001	**<0.001**	<0.001	<0.001
Fast*FI	**0.038**	0.276	0.421	0.709	0.159	0.271
Sex*FI	0.961	0.149	**0.043**	0.929	**0.008**	**0.001**
Fast*Sex*FI	0.186	0.439	0.749	0.601	0.447	0.518
Diet*FI	0.880	0.830	0.513	0.469	0.505	0.654
Fast*Diet*FI	0.737	0.480	0.569	0.511	0.289	0.214
Sex*Diet*FI	0.339	0.549	0.852	0.776	0.475	0.650
Fast*Sex*Diet*FI	0.702	0.495	0.960	0.503	0.240	0.422

Table 4.3.c P-values of the main effects and interactions for mink body weight and the weights of fat depots and organs. Highest order significant main effects and interactions are bolded.

Effect	Kidney	Intestine	Heart	Spleen	Stomach	Pancreas
Fast	0.039	<0.001	0.371	0.170	<0.001	**<0.001**
Sex	<0.001	<0.001	**0.001**	0.017	**<0.001**	**0.001**
Fast*Sex	0.617	**0.015**	0.117	0.123	0.498	0.233
Diet	0.007	0.141	0.920	0.178	0.812	**0.047**
Fast*Diet	0.023	0.629	0.960	0.335	**0.008**	0.815
Sex*Diet	0.057	0.711	0.515	0.404	0.303	0.755
Fast*Sex*Diet	**0.032**	0.554	0.374	**0.009**	0.193	0.429
FI	**<0.001**	**0.008**	**0.024**	**<0.001**	**0.019**	0.472
Fast*FI	0.491	0.790	0.684	0.787	0.558	0.394
Sex*FI	0.067	0.949	0.183	0.707	0.291	0.597
Fast*Sex*FI	0.328	0.745	0.662	0.217	0.968	0.415
Diet*FI	0.367	0.839	0.720	0.522	0.883	0.355
Fast*Diet*FI	0.667	0.411	0.099	0.559	0.885	0.137
Sex*Diet*FI	0.849	0.226	0.496	0.428	0.798	0.676
Fast*Sex*Diet*FI	0.440	0.253	0.871	0.841	0.970	0.699

Table 4.3.d P-values of the main effects and interactions for mink body weight and the weights of fat depots and organs. Highest order significant main effects and interactions are bolded.

Effect	Female Repro	Male Repro	Testes	Ovaries	Adrenal	Thyroid
Fast	0.176	**0.023**	**0.023**	0.754	**<0.001**	**0.005**
Sex	-	-	-	-	0.001	**0.020**
Fast*Sex	-	-	-	-	0.139	0.622
Diet	0.314	0.897	0.890	0.393	0.243	0.595
Fast*Diet	0.500	0.284	0.569	0.974	0.181	0.814
Sex*Diet	-	-	-	-	0.081	0.725
Fast*Sex*Diet	-	-	-	-	0.064	0.064
FI	**0.011**	**0.032**	0.083	0.465	**0.016**	**0.004**
Fast*FI	0.346	0.948	0.651	0.577	0.946	0.878
Sex*FI	-	-	-	-	**0.004**	0.052
Fast*Sex*FI	-	-	-	-	0.200	0.181
Diet*FI	0.720	0.774	0.860	0.640	0.546	0.783
Fast*Diet*FI	0.993	0.745	0.788	0.467	0.318	0.724
Sex*Diet*FI	-	-	-	-	0.485	0.076
Fast*Sex*Diet*FI	-	-	-	-	0.915	0.951

Table 4.4. Effect of feeding intensity and sex on mink body weight, lean body mass and the weights of the fat depots, and adrenal glands.

	80% RDA		120% RDA	
Component (g)	Male	Female	Male	Female
Body Weight	1770 ±78^{B}	909 ±78^{C}	2338 ±78^{A}	1168 ±78^{C}
Lean Body Mass	1302.7 ±44.0^{B}	686.0 ±44.0^{D}	1569.9 ±44.0^{A}	784.9 ±44.0^{C}
Total Body Fat	467.0 ±39.8^{B}	222.7 ±39.8^{C}	768.5 ±39.8^{A}	383.2 ±39.8^{B}
Subcutaneous Fat	375.6 ±33.2^{B}	174.1 ±33.2^{C}	614.1 ±33.2^{A}	300.1 ±33.2^{B}
Visceral	40.7 ±4.8^{B}	20.5 ±4.8^{C}	74.7 ±4.8^{A}	36.7 ±4.8^{B}
Mesenteric Fat	24.9 ±2.8^{B}	12.4 ±2.8^{C}	46.9 ±2.8^{A}	24.1 ±2.8^{B}
Diaphragmatic Fat	2.3 ±0.2^{B}	1.4 ±0.2^{C}	4.1 ±0.2^{A}	2.0 ±0.2^{B}
Adrenal Gland	0.132 ±0.005^{B}	0.085 ±0.005^{C}	0.153 ±0.005^{A}	0.082 ±0.005^{C}

$^{A-D}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.5. Effect of fasting, sex and diet on mink body weight, and the weights of fat depots, and organs.

Component (g)		Male			Female		
		n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
Body Weight	Non-Fasted	2504 ±106^{A*}	2117 ±106^{B}	1999 ±106^{B}	1085 ±106^{C}	1155 ±106^{C}	1143 ±106^{C}
	Fasted	1867 ±106^{A}	1884 ±106^{A}	1954 ±106^{A}	1025 ±106^{B}	928 ±106^{B}	895 ±106^{B}
Total Body Fat	Non-Fasted	888.6 ±58.9^{A*}	646.3 ±58.9^{B}	557.9 ±58.9^{B}	328.3 ±58.9^{D}	368.2 ±58.9^{CD}	355.3 ±58.9^{CD*}
	Fasted	526.3 ±58.9^{A}	498.6 ±58.9^{A}	588.8 ±58.9^{A}	314.9 ±58.9^{B}	232.9 ±58.9^{BC}	218.3 ±58.9^{C}
Subcutaneous Fat	Non-Fasted	694.8 ±48.2^{A*}	502.5 ±48.2^{B}	450.8 ±48.2^{B}	254.3 ±48.2^{C}	289.2 ±48.2^{C}	278.7 ±48.2^{C}
	Fasted	428.7 ±48.2^{A}	411.8 ±48.2^{A}	480.9 ±48.2^{A}	242.9 ±48.2^{B}	182.7 ±48.2^{B}	174.8 ±48.2^{B}
Intermuscular Fat	Non-Fasted	58.4 ±3.9^{A*}	49.4 ±3.9^{A*}	36.2 ±3.9^{B}	19.5 ±3.9^{C}	25.3 ±3.9^{BC}	23.5 ±3.9^{C}
	Fasted	32.0 ±3.9^{A}	28.9 ±3.9^{A}	36.5 ±3.9^{A}	20.3 ±3.9^{B}	17.0 ±3.9^{B}	14.8 ±3.9^{B}
Intra-abdominal	Non-Fasted	133.0 ±10.7^{A*}	92.5 ±10.7^{B*}	69.5 ±10.7^{BC}	53.4 ±10.7^{C}	53.1 ±10.7^{C}	51.8 ±10.7^{C}
	Fasted	64.3 ±10.7^{A}	56.7 ±10.7^{A}	69.8 ±10.7^{A}	50.9 ±10.7^{AB}	32.6 ±10.7^{B}	28.3 ±10.7^{B}
Visceral	Non-Fasted	93.0 ±7.4^{A*}	67.5 ±7.4^{B*}	49.0 ±7.4^{BC}	32.6 ±7.4^{C}	34.1 ±7.4^{C}	33.1 ±7.4^{C}
	Fasted	46.5 ±7.4^{A}	40.3 ±7.4^{AB}	50.0 ±7.4^{A}	33.1 ±7.4^{ABC}	21.4 ±7.4^{BC}	17.2 ±7.4^{C}
Omental Fat	Non-Fasted	27.7 ±2.6^{A*}	19.9 ±2.6^{B}	18.6 ±2.3^{B}	12.0 ±2.3^{C}	12.0 ±2.3^{C}	11.6 ±2.3^{C}
	Fasted	16.2 ±2.3^{A}	16.7 ±2.3^{A}	21.1 ±2.3^{A}	12.6 ±2.3^{B}	6.9 ±2.3^{B}	6.7 ±2.3^{B}
Mesenteric Fat	Non-Fasted	60.1 ±4.5^{A*}	42.2 ±4.5^{B*}	30.3 ±4.5^{BC}	20.6 ±4.5^{C}	22.2 ±4.5^{C}	21.5 ±4.5^{C}
	Fasted	30.3 ±4.5^{A}	23.5 ±4.5^{AB}	28.9 ±4.5^{A}	20.5 ±4.5^{ABC}	14.5 ±4.5^{C}	10.5 ±4.5^{C}
Kidney	Non-Fasted	8.9 ±0.4^{A*}	7.7 ±0.4^{B}	7.2 ±0.4^{B}	3.6 ±0.4^{C}	3.9 ±0.4^{C}	3.9 ±0.4^{C}
	Fasted	7.0 ±0.4^{B}	8.8 ±0.4^{A}	6.2 ±0.4^{B}	3.5 ±0.4^{C}	3.6 ±0.4^{C}	3.0 ±0.4^{C}
Spleen	Non-Fasted	7.4 ±0.8	6.3 ±0.8	6.1 ±0.8*	4.1 ±0.8	5.6 ±0.8	6.8 ±0.8*
	Fasted	6.6 ±0.8^{AB}	4.9 ±0.8^{BC}	8.5 ±0.8^{A}	5.0 ±0.8^{BC}	3.7 ±0.8^{C}	3.6 ±0.8^{C}

$^{A-D}$ Values differ within response rows ($P < 0.05$).

* Values differ between non-fasted and fasted mink ($P < 0.05$).

Lsmeans ± SEM are presented.

Table 4.6. Effect of fasting on mink lean body mass, and the weights of the perirenal fat and organs.

Component (g)	Non-Fasted	Fasted
Lean Body Mass	1143 ±31^{A}	1029±31^{B}
Perirenal Fat	20.3 ±1.1^{A}	13.4±1.1^{B}
Pancreas	3.72 ±0.10^{A}	3.07±0.10^{B}
Adrenal	0.120 ±0.004^{B}	0.105±0.004^{A}
Thyroid	0.319 ±0.019^{A}	0.247 ±0.019^{B}
Male Reproductive Organs	2.8 ±0.2^{B}	3.6 ±0.2^{A}
Testes	5.7 ±0.4^{B}	6.9 ±0.4^{A}

$^{A-B}$Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.7. Effect of feeding intensity on the weights of fat depots and organs in mink.

Component (g)	80% RDA	120% RDA
Intramuscular Fat	24.0 ±1.7^{B}	36.3 ±1.7^{A}
Intra-abdominal Fat	45.2 ±4.9^{B}	80.8 ±4.9^{A}
Omental Fat	11.9 ±1.1^{B}	18.4 ±1.1^{A}
Perirenal Fat	12.8 ±1.1^{B}	20.9 ±1.1^{A}
Kidney	5.1 ±0.2^{B}	6.1 ±0.2^{A}
Intestine	30.4 ±0.7^{B}	33.2 ±0.7^{A}
Heart	8.8 ±0.3^{B}	9.7 ±0.3^{A}
Spleen	4.6 ±0.3^{B}	6.8 ±0.3^{A}
Stomach	7.8 ±0.2^{B}	8.3 ±0.2^{A}
Female Reproductive Organs	1.18 ± 0.09^{B}	1.54 ± 0.09^{A}
Male Reproductive Organs	5.69 ±0.36^{B}	6.84 ±0.36^{A}
Thyroid	0.246 ±0.019^{B}	0.321 ±0.019^{A}

$^{A-B}$Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.8. Effect of diet on the weights of the perirenal fat depot and the pancreas in mink.

Component (g)	n-3 PUFA	n-6 PUFA	n-9 MUFA
Perirenal Fat	19.7 ±1.3^{A}	15.7 ±1.3^{B}	15.2 ±1.3^{B}
Pancreas	3.5 ±0.1^{A}	3.5 ±0.1^{A}	3.1 ±0.1^{B}

$^{A-B}$Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.9. Effect of sex on mink body length and organ weights.

Component (g)	Male	Female
Body Length, cm	47.4 ±0.4^{A}	39.1 ±0.4^{B}
Heart, g	11.8 ±0.4^{A}	6.6 ±0.4^{B}
Stomach, g	9.9 ±0.2^{A}	6.2 ±0.2^{B}
Pancreas, g	4.1 ±0.1^{A}	2.7 ±0.1^{B}
Thyroid, g	0.336 ±0.020^{A}	0.231 ±0.020^{B}

$^{A-B}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.10. Effect of fasting and diet on mink stomach weight.

Stomach (g)	n-3 PUFA	n-6 PUFA	n-9 MUFA
Non-Fasted	8.1 ±0.3^{BC}	9.1 ±0.3^{A}	8.4 ±0.3^{AB}
Fasted	8.1 ±0.3^{BC}	7.1 ±0.3^{D}	7.4 ±0.3^{CD}

$^{A-D}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.11. Effect of fasting and sex on intestinal weight in mink.

Intestine (g)	Male	Female
Non-Fasted	45.3 ±1.0^{A}	35.3 ±1.0^{B}
Fasted	25.6 ±1.0^{C}	20.9 ±1.0^{D}

$^{A-D}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

4.3.2.2 Lean Body Mass and Fat Depot Weights in Relation to Body Weight

Significant differences were found in the relative weights of the fat depots and lean body mass (expressed as % body weight) (Table 4.12). The relative lean body mass decreased in the mink fed at 120% RDA (Table 4.13), and was the lowest in the NF males fed the n-3 PUFA diet (Table 4.14). Fasting increased the relative lean body mass of the males fed the n-3 PUFA diet, and the females fed either the n-6 PUFA or the n-9 MUFA diets while there were no differences in % lean body mass in the F mink. An opposite trend was found for percent body fat, as it was decreasing with the lower RDA or in response to fasting (Table 4.15).

The proportion of subcutaneous in relation to body weight decreased with fasting and also tended to decrease with feeding at 80% RDA with the exceptions of the males fed the n-3 PUFA diet at 80% RDA and the females fed the n-9 MUFA diet at 120% RDA (Tables 4.16). The intermuscular fat depots decreased in proportion to body weight with fasting and feeding at 80% RDA.

Increasing the feeding intensity resulted in an increased proportion of intra-abdominal fat. The relative weight of this fat depot did not differ between the sexes fed the same diets in the NF mink. The males fed the n-3 PUFA diet had a higher proportion than the n-6 PUFA or n-9 MUFA fed mink, and this was observed for the females in the F group. Fasting resulted in a decrease in the percent of intra-abdominal fat for the males fed the n-3 or n-6 PUFA diet and the females fed the n-9 MUFA diet. All fat depots within the intra-abdominal compartment increased in relation to body weight with feeding at 120% RDA. The relative weights of the omental and perirenal fat depots decreased due to fasting. The percentage of mesenteric fat in relation to body weight decreased only in the males fed the n-3 or n-6 PUFA diet and the females fed the n-9 MUFA diet with fasting. Within the NF mink the lowest proportion of mesenteric fat in relation to body fat was observed in males fed the n-9 MUFA diet, and highest in males fed the n-3 PUFA diet. These changes were also reflected in the combined relative weights of these visceral fat depots. Diet influenced the proportion of perirenal fat, with the highest percentages in mink fed the n-3 PUFA diet (n-3 PUFA: 1.3 ±0.1; n-6 PUFA: 1.0 ±0.1; n-9 MUFA: 1.0 ±0.1).

Table 4.12.a. P-values of the main effects and interactions for relative fat depot weights. Highest order significant main effects and interactions are bolded.

Effect	Body Fat	Intra-abdominal Fat	Visceral Fat	Mesenteric Fat	Omental Fat	Perirenal Fat
Fast	**0.005**	<0.001	0.001	<0.001	**0.029**	<0.001
Sex	0.503	0.165	0.929	0.790	0.624	**0.006**
Fast*Sex	0.695	0.682	0.509	0.403	0.777	0.966
Diet	0.138	0.008	0.013	0.005	0.156	**0.008**
Fast*Diet	0.467	0.587	0.495	0.590	0.505	0.661
Sex*Diet	0.614	0.970	0.968	0.620	0.681	0.764
Fast*Sex*Diet	0.048	**0.033**	**0.014**	**0.011**	0.080	0.152
FI	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**
Fast*FI	0.994	0.750	0.936	0.791	0.621	0.547
Sex*FI	0.584	0.747	0.990	0.723	0.672	0.245
Fast*Sex*FI	0.851	0.798	0.775	0.951	0.615	0.881
Diet*FI	0.950	0.592	0.474	0.508	0.519	0.709
Fast*Diet*FI	0.098	0.333	0.238	0.106	0.675	0.644
Sex*Diet*FI	0.123	0.975	0.876	0.831	0.877	0.837
Fast*Sex*Diet*FI	0.708	0.935	0.998	0.853	0.706	0.657

Table 4.12.b. P-values of the main effects and interactions for the relative weights of the fat depots and lean body mass. Highest order significant main effects and interactions are bolded.

Effect	Diaphragmatic Fat	Subcutaneous Fat	Intermuscular Fat	Lean Body Mass
Fast	0.291	**0.026**	**0.001**	0.005
Sex	0.673	0.344	0.896	0.500
Fast*Sex	0.368	0.488	0.224	0.693
Diet	0.068	0.262	0.548	0.138
Fast*Diet	0.352	0.564	0.168	0.471
Sex*Diet	0.564	0.537	0.632	0.619
Fast*Sex*Diet	0.667	0.092	0.071	**0.047**
FI	**0.001**	<0.001	**0.003**	**<0.001**
Fast*FI	0.188	0.903	0.586	0.995
Sex*FI	0.138	0.595	0.577	0.578
Fast*Sex*FI	0.756	0.833	0.660	0.860
Diet*FI	0.819	0.957	0.629	0.955
Fast*Diet*FI	0.173	0.111	0.128	0.099
Sex*Diet*FI	0.936	**0.046**	0.361	0.123
Fast*Sex*Diet*FI	0.518	0.591	0.576	0.709

Table 4.13. Effect of feeding intensity on the relative weights of the fat depots and lean body mass.

(% Body Weight)	80% RDA	120% RDA
Body Fat	24.3 ±0.9^{B}	32.3 ±0.9^{A}
Intra-abdominal Fat	3.3 ±0.2^{B}	4.6 ±0.2^{A}
Visceral Fat	2.2 ±0.1^{B}	3.1 ±0.1^{A}
Mesenteric Fat	1.3 ±0.1^{B}	2.0 ±0.1^{A}
Omental Fat	0.8 ±0.1^{B}	1.1 ±0.1^{A}
Perirenal Fat	1.0 ±0.1^{B}	1.3 ±0.1^{A}
Diaphragmatic Fat	0.1 ±0.0^{B}	0.2 ±0.0^{A}
Intermuscular Fat	1.7 ±0.1^{B}	2.1 ±0.1^{A}
Lean body mass	75.7 ±0.9^{A}	67.7 ±0.9^{B}

$^{A-B}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.14. Effect of fasting, sex and diet on the relative weight of the mink fat depots and lean body mass.

(% Body Weight)		Male			Female		
		n-3 PUFA	n-6 PUFA	n-9 MUFA	n-3 PUFA	n-6 PUFA	n-9 MUFA
Intra-abdominal Fat	Non-Fasted	$5.2 \pm 0.4^{A*}$	$4.2 \pm 0.4^{B*}$	3.4 ± 0.4^{B}	4.8 ± 0.4^{A}	4.5 ± 0.4^{AB}	$4.5 \pm 0.4^{AB*}$
	Fasted	3.3 ± 0.4^{B}	2.7 ± 0.4^{B}	3.5 ± 0.4^{B}	4.8 ± 0.4^{A}	3.3 ± 0.4^{B}	3.0 ± 0.4^{B}
Visceral Fat	Non-Fasted	$3.6 \pm 0.3^{A*}$	$3.0 \pm 0.2^{AB*}$	2.4 ± 0.3^{B}	2.9 ± 0.2^{AB}	2.9 ± 0.2^{AB}	$2.9 \pm 0.2^{AB*}$
	Fasted	2.4 ± 0.2^{AB}	1.9 ± 0.3^{B}	2.5 ± 0.2^{AB}	3.1 ± 0.3^{A}	2.2 ± 0.3^{B}	1.9 ± 0.3^{B}
Mesenteric Fat	Non-Fasted	$2.3 \pm 0.2^{A*}$	$1.9 \pm 0.2^{AB*}$	1.5 ± 0.2^{B}	1.8 ± 0.2^{AB}	1.9 ± 0.2^{AB}	$1.8 \pm 0.2^{AB*}$
	Fasted	1.6 ± 0.2^{AB}	1.1 ± 0.2^{B}	1.4 ± 0.2^{AB}	1.9 ± 0.2^{A}	1.5 ± 0.2^{AB}	1.1 ± 0.2^{B}
Lean body mass	Non-Fasted	$64.9 \pm 2.3^{B*}$	70.3 ± 2.3^{AB}	73.2 ± 2.3^{A}	71.2 ± 2.3^{AB}	$68.8 \pm 2.3^{AB*}$	$69.9 \pm 2.3^{AB*}$
	Fasted	72.1 ± 2.3	75.4 ± 2.3	70.9 ± 2.3	70.9 ± 2.3	75.7 ± 2.3	76.3 ± 2.3

$^{A-B}$ Values differ within responses ($P < 0.05$).

* Values differ between non-fasted and fasted mink ($P < 0.05$).

Lsmeans ± SEM are presented.

Table 4.15. Effect of fasting on the relative weight of the mink fat depots.

(% Body Weight)	Non-Fasted	Fasted
Body Fat	30.2 ± 0.9^{A}	26.4 ± 0.9^{B}
Subcutaneous Fat	23.7 ± 0.1^{A}	21.2 ± 0.1^{B}
Intermuscular Fat	2.1 ± 0.1^{A}	1.7 ± 0.1^{B}
Omental Fat	1.1 ± 0.1^{A}	0.9 ± 0.1^{B}
Perirenal Fat	1.3 ± 0.1^{A}	1.0 ± 0.1^{B}

$^{A-B}$ Values differ within responses ($P < 0.05$).

Lsmeans ± SEM are presented.

Table 4.16. Effect of sex, diet and feeding intensity on the relative weight of subcutaneous fat.

Subcutaneous Fat (% Body Weight)	n-3 PUFA		n-6 PUFA		n-9 MUFA	
	80% RDA	120% RDA	80% RDA	120% RDA	80% RDA	120% RDA
Male	24.4 ±1.9^{AB}	25.9 ±1.9^{A}	18.0 ±1.9^{D}	25.4 ±1.9^{A}	18.4 ±1.9^{CD}	26.8 ±1.9^{A}
Female	17.1 ±1.9^{D}	27.5 ±1.9^{A}	18.9 ±1.9^{CD}	24.7 ±1.9^{A}	18.9 ±1.9^{BCD}	23.5 ±1.9^{ABC}

$^{A-D}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

4.3.2.3 Fat Depot Weights in Relation to Total Body Fat

Significant differences in the relative weights of the fat depots compared to total body fat are shown in Table 4.17. The proportion of subcutaneous fat increased with fasting (Table 4.18). Subcutaneous fat percentage was the highest in the males fed the n-3 PUFA diet at 80% RDA and the lowest in the female mink fed the n-3 PUFA diet at 80% RDA (Table 4.19). The relative weight of the intermuscular fat depot was the highest in males fed the n-6 PUFA diet at 80% RDA, and the lowest in females fed the n-3 PUFA diet at 120% RDA. A sex difference was observed for the amount of mesenteric fat in relation to total body fat, with the females having a higher percentage (14.8 ±0.5 % vs. 12.7 ±0.5 %). Fasting decreased the proportion of intra-abdominal fat in the mink, and this was also reflected in decreases in the visceral, mesenteric and perirenal fat depots in relation to total body fat. The proportion of mesenteric fat increased with increasing feeding intensity from 80% RDA to 120% RDA (5.4 ±0.2 % vs. 6.0 ±0.2 %). Diet influenced the proportion of mesenteric fat, which was the highest in the mink fed the n-3 PUFA diet, and was also reflected in the proportion of visceral fat (Table 4.20). Perirenal fat depot weight in relation to total body fat was the highest in the females fed the n-3 PUFA diet at 80% RDA and lowest in males fed the n-3 PUFA diet at 80% RDA. The weight of the diaphragmatic fat was lower in males fed at 80% RDA compared to females in relation to total body fat reserves (Table 4.21).

Table 4.17. P-values of the main effects and interactions for fat depot weights in relation to total body fat. Highest order significant main effects and interactions are bolded.

Effect	Subcutaneous Fat	Intermuscular Fat	Intra-abdominal Fat	Visceral Fat	Omental Fat	Mesenteric Fat	Perirenal Fat	Diaphragmatic Fat
Fast	**0.004**	0.226	**0.003**	**0.008**	0.174	**<0.001**	**0.001**	0.632
Sex	0.091	0.341	**0.037**	0.523	0.980	0.542	0.002	0.208
Fast*Sex	0.122	0.118	0.311	0.209	0.560	0.306	0.542	0.542
Diet	0.303	0.136	**0.036**	0.088	0.532	**0.008**	0.014	0.141
Fast*Diet	0.850	0.511	0.946	0.608	0.876	0.221	0.633	0.481
Sex*Diet	0.507	0.795	0.418	0.660	0.267	0.909	0.152	0.596
Fast*Sex*Diet	0.380	0.274	0.367	0.137	0.737	0.061	0.892	0.392
FI	0.967	0.026	0.250	0.117	0.867	**0.024**	0.773	0.888
Fast*FI	0.176	0.647	0.196	0.375	0.266	0.879	0.093	0.064
Sex*FI	0.688	0.627	0.542	0.458	0.340	0.377	0.974	**0.027**
Fast*Sex*FI	0.575	0.644	0.962	0.922	0.895	0.388	0.958	0.868
Diet*FI	0.627	0.337	0.301	0.173	0.311	0.272	0.380	0.685
Fast*Diet*FI	0.711	0.573	0.714	0.773	0.915	0.359	0.361	0.541
Sex*Diet*FI	**0.044**	**0.006**	0.189	0.426	0.663	0.167	**0.046**	0.510
Fast*Sex*Diet*FI	0.378	0.198	0.659	0.739	0.283	0.972	0.299	0.477

Table 4.18. Effect of fasting on the relative weights of the mink fat depots.

(% Body Fat)	Non-Fasted	Fasted
Subcutaneous Fat	77.9 ± 0.7^{B}	80.4 ± 0.7^{A}
Intra-abdominal Fat	14.7 ± 0.5^{A}	12.8 ± 0.5^{B}
Visceral Fat	9.8 ± 0.3^{A}	8.6 ± 0.3^{B}
Mesenteric Fat	6.2 ± 0.2^{A}	5.2 ± 0.2^{B}
Perirenal Fat	4.4 ± 0.2^{A}	3.6 ± 0.2^{B}

$^{A-B}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.19. Effect of sex, diet and feeding intensity on the relative weights of the fat depots.

		n-3 PUFA		n-6 PUFA		n-9 MUFA	
(% Body Fat)		80% RDA	120% RDA	80% RDA	120% RDA	80% RDA	120% RDA
Subcutaneous Fat	Male	81.7 ± 1.5^{A}	78.6 ± 1.5^{BC}	80.0 ± 1.5^{AB}	80.5 ± 1.5^{AB}	79.6 ± 1.5^{AB}	81.6 ± 1.5^{AB}
	Female	74.7 ± 1.5^{C}	78.2 ± 1.5^{BC}	79.8 ± 1.5^{AB}	77.4 ± 1.5^{BC}	79.0 ± 1.5^{AB}	78.9 ± 1.5^{AB}
Intermuscular Fat	Male	6.1 ± 0.5^{CD}	6.5 ± 0.5^{BCD}	8.0 ± 0.5^{A}	6.1 ± 0.5^{CD}	6.2 ± 0.5^{CD}	6.2 ± 0.5^{CD}
	Female	8.0 ± 0.5^{AB}	5.5 ± 0.5^{D}	7.2 ± 0.5^{ABC}	7.3 ± 0.5^{ABC}	6.9 ± 0.5^{ABCD}	6.8 ± 0.5^{ABCD}
Perirenal Fat	Male	3.0 ± 0.4^{F}	3.6 ± 0.4^{DEF}	3.0 ± 0.4^{F}	3.1 ± 0.4^{F}	3.3 ± 0.4^{EF}	2.8 ± 0.4^{F}
	Female	6.1 ± 0.4^{A}	5.1 ± 0.4^{AB}	3.9 ± 0.4^{CDEF}	4.9 ± 0.4^{BC}	4.4 ± 0.4^{BCDE}	4.7 ± 0.4^{BCD}

$^{A-F}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.20. Effect of diet on the relative weights of the mink visceral and mesenteric fat depots.

(% Body Fat)	n-3 PUFA	n-6 PUFA	n-9 MUFA
Intra-abdominal Fat	14.9 ±0.6^{A}	13.2 ±0.6^{B}	13.2 ±0.6^{B}
Mesenteric Fat	6.2 ±0.2^{A}	5.5 ±0.2^{B}	5.4 ±0.2^{B}

$^{A-B}$Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.21. Effect of feeding intensity and sex on the relative weights of the mink diaphragmatic fat.

Diaphragmatic Fat (% Body Fat)	Male	Female
80% RDA	0.49 ±0.04^{B}	0.62 ±0.04^{A}
120% RDA	0.57 ±0.04^{AB}	0.54 ±0.04^{AB}

$^{A-B}$Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

4.3.3 Fatty Acid Profiles

4.3.3.1 Plasma Fatty Acid Profile

The fatty acid profile of the plasma was affected by the treatment main effects and the interactions (Table 4.22). The mole percent of total SFA increased with feeding the mink at 120% RDA (Table 4.23). Fasting was found to increase total SFA (Table 4.24). Total plasma MUFAs were affected by fasting, sex and feeding intensity, and diet. The MUFA proportion increased due to fasting (Table 4.25). Within the NF mink, the females fed at 80% RDA had a higher MUFA proportion than the females fed at 120% RDA. The fasted males fed at 80% RDA had higher MUFA levels than those fed at 120% RDA. Diet also influenced the MUFA percentage in the plasma, with the lowest proportions observed in the n-6 PUFA group, and no difference between the n-3 PUFA and n-9 MUFA diets (Table 4.26).

The plasma n-6: n-3 PUFA ratio was the lowest in the mink fed the n-3 PUFA diet and the highest in the mink fed the n-6 PUFA diet (Table 4.27 and 4.28). Fasting decreased the ratio in the mink fed the n-6 PUFA and n-9 MUFA diets and removed the difference between these two diets. In addition, the females had a higher n-6: n-3 PUFA ratio than the males when fed the n-6 PUFA diet.

The proportion of n-6 PUFAs in the plasma also responded to diet, with increased proportions in the mink fed the n-6 PUFA diets and the lowest in the mink fed the n-3 PUFA diet. Total n-6 PUFAs decreased with fasting and within the NF mink fed at 80%

RDA, the males had a higher proportion than the females. Differences were noted within the n-6 PUFA fatty acid class. The proportion of LA in plasma was altered by diet and feeding intensity, with the proportion being higher in the mink fed at 80% RDA (Table 4.29). Within both feeding intensities the proportions followed the same rank order, being the highest in the n-6 PUFA group and the lowest in the n-3 PUFA group. Fasting was not found to decrease the proportion of plasma LA although it did for total n-6 PUFAs. An increase was noted in the males fed at 80% RDA, who also had a higher proportion than all the other NF mink. Differences in the proportion of AA were present due to diet with the highest levels in the mink fed the n-9 MUFA diet. Fasting or feeding at 80% RDA resulted in a decrease in the plasma AA percentage.

Total n-3 PUFAs were the highest in the mink fed the n-3 PUFA diet. The n-3 PUFA diet also responded to fasting with a decreased proportion of total n-3 PUFAs, while an increase occurred with fasting for both n-6 PUFA and n-9 MUFA diets. The proportion of ALA were the lowest in the mink fed the n-3 PUFA diet, and only the mink fed the n-6 and n-9 MUFA diets showed decreases in the plasma ALA proportion in response to fasting (Table 4.30). The plasma percentage of EPA was the highest in the mink fed the n-3 PUFA diet and for the NF group lowest in the mink fed the n-9 MUFA diet (Table 4.31). With regards to diet, fasting decreased the proportion of EPA in plasma for mink fed the n-3 PUFA diet only. Feeding at 80% RDA resulted in a higher proportion of EPA, with fasting reducing the proportion for both feeding intensities and eliminating the difference (Table 4.32). The percent of DHA in plasma was the highest in the mink fed the n-3 PUFA diet and lowest in those fed the n-6 PUFA diet, and decreased with fasting.

Table 4.22.a. P-values of the main effects and interactions for the plasma fatty acid profile. Highest order significant main effects and interactions are bolded.

Plasma Effect	SFA	MUFA	UFA: SFA	n-6: n-3 PUFA	LA	AA	n-6 PUFA	n-6 PUFA Pro: Pre
Fast	**0.013**	<0.001	0.106	<0.001	0.027	**<0.001**	<0.001	**0.001**
Sex	0.640	0.912	0.424	0.146	0.719	0.874	0.441	0.396
Fast*Sex	0.704	0.329	0.965	0.102	0.250	0.779	0.117	0.866
Diet	0.060	**<0.001**	0.349	<0.001	<0.001	**0.017**	**<0.001**	**<0.001**
Fast*Diet	0.518	0.095	0.403	**<0.001**	0.274	0.682	0.427	0.454
Sex*Diet	0.155	0.805	0.679	**0.003**	**0.016**	0.592	0.165	0.100
Fast*Sex*Diet	0.666	0.242	0.327	0.715	0.216	0.128	0.253	0.054
FI	**<0.001**	0.001	**0.001**	0.543	<0.001	**<0.001**	0.902	**<0.001**
Fast*FI	0.606	0.375	0.247	0.949	0.342	0.451	0.246	0.348
Sex*FI	0.430	0.569	0.762	0.552	0.072	0.112	0.934	0.175
Fast*Sex*FI	0.805	**0.005**	0.590	0.801	**0.019**	0.897	**0.028**	0.746
Diet*FI	0.528	0.064	0.376	0.385	**0.001**	0.227	0.077	0.293
Fast*Diet*FI	0.371	0.704	0.407	0.716	0.577	0.404	0.926	0.330
Sex*Diet*FI	0.993	0.318	0.609	0.558	0.538	0.077	0.176	0.490
Fast*Sex*Diet*FI	0.735	0.688	0.653	0.573	0.106	0.211	0.806	0.167

Table 4.22.b. P-values of the main effects and interactions for the plasma fatty acid profile. Highest order significant main effects and interactions are bolded.

Plasma Effect	ALA	EPA	DHA	n-3 PUFA	n-3 PUFA Pro: Pre
Fast	0.002	<0.001	**<0.001**	0.038	<0.001
Sex	0.210	0.068	0.072	0.128	0.709
Fast*Sex	0.596	0.001	0.349	0.171	0.158
Diet	<0.001	<0.001	**<0.001**	<0.001	<0.001
Fast*Diet	0.036	<0.001	0.051	**<0.001**	**<0.001**
Sex*Diet	0.339	<0.001	0.118	0.127	**0.043**
Fast*Sex*Diet	0.342	**0.032**	0.159	0.229	0.337
FI	0.302	0.171	0.459	0.936	0.423
Fast*FI	0.843	**0.021**	0.685	0.449	0.568
Sex*FI	0.947	0.061	0.644	0.513	0.538
Fast*Sex*FI	0.064	0.374	0.111	0.134	0.340
Diet*FI	0.001	0.586	0.894	0.766	**0.007**
Fast*Diet*FI	0.605	0.650	0.788	0.723	0.937
Sex*Diet*FI	0.706	0.298	0.761	0.704	0.654
Fast*Sex*Diet*FI	**0.042**	0.296	0.595	0.654	0.209

Table 4.23. Effect of feeding intensity on plasma fatty acid profile.

Mole %	80% RDA	120% RDA
SFA	33.26 ±0.53^{B}	35.13 ±0.53^{A}
AA	17.19 ±0.30^{B}	20.82 ±0.30^{A}
UFA: SFA	2.02 ±0.05^{A}	1.87 ±0.05^{B}
n-6 PUFA Pro: Pre	0.98 ±0.03^{B}	1.42 ±0.03^{A}

$^{A-B}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.24. Effect of fasting on plasma fatty acid profile.

Mole %	Non-Fasted	Fasted
SFA	33.75 ±0.53^{B}	34.63 ±0.53^{A}
AA	20.71 ±0.30^{A}	17.30 ±0.30^{B}
DHA	5.04 ±0.14^{B}	6.42 ±0.14^{A}
n-6 PUFA Pro: Pre	1.29 ±0.03^{A}	1.12 ±0.03^{B}

$^{A-B}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.25. Effect of fasting, feeding intensity and sex on plasma fatty acid profile.

		80% RDA		120% RDA	
Mole %		Male	Female	Male	Female
MUFA	Non-fasted	17.23 ±0.66^{AB*}	18.72 ±0.66^{A*}	17.14 ±0.66^{AB*}	16.61 ±0.66^{B*}
	Fasted	23.08 ±0.66^{A}	21.23 ±0.66^{AB}	19.72 ±0.66^{B}	20.87 ±0.66^{B}
LA	Non-fasted	21.77 ±0.75^{A*}	19.14 ±0.75^{B}	15.56 ±0.75^{C}	16.60 ±0.75^{C}
	Fasted	18.82 ±0.75^{A}	19.28 ±0.75^{A}	15.53 ±0.75^{B}	15.48 ±0.75^{B}
n-6 PUFA	Non-fasted	41.83 ±0.66^{A*}	39.83 ±0.66^{B*}	40.06 ±0.66^{AB*}	40.09 ±0.66^{AB*}
	Fasted	34.63 ±0.66	35.98 ±0.66	36.07 ±0.66	35.35 ±0.66

$^{A-C}$ Values differ within response rows (P <0.05).
* Values differ between non-fasted and fasted mink (P <0.05).
Lsmeans ± SEM are presented.

Table 4.26. Effect of diet on plasma fatty acid profile.

Mole %	n-3 PUFA	n-6 PUFA	n-9 MUFA
MUFA	22.10 ±0.41^{A}	14.61 ±0.41^{B}	21.26 ±0.41^{A}
AA	18.65 ±0.37^{B}	18.47 ±0.37^{B}	19.90 ±0.37^{A}
n-6 PUFA	31.98 ±0.41^{C}	44.46 ±0.41^{A}	37.51 ±0.41^{B}
n-6 PUFA Pro: Pre	1.55 ±0.04^{A}	0.78 ±0.04^{C}	1.27 ±0.04^{B}
DHA	7.29 ±0.18^{A}	4.59 ±0.18^{C}	5.32 ±0.18^{B}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.27. Effect of fasting and diet of plasma fatty acid profile.

Mole %		n-3 PUFA	n-6 PUFA	n-9 MUFA
n-3 PUFA	Non-Fasted	12.11 ±0.29^{A}	5.50 ±0.29^{E}	6.93 ±0.29^{D}
	Fasted	10.72 ±0.29^{B}	7.40 ±0.29^{CD}	7.96 ±0.29^{C}
n-6: n-3 PUFA	Non-Fasted	2.80 ±0.21^{D}	8.78 ±0.21^{A}	5.79 ±0.21^{B}
	Fasted	2.86 ±0.21^{D}	5.73 ±0.21^{B}	4.46 ±0.21^{C}
n-3 PUFA Pro: Pre	Non-Fasted	68.13 ±1.98^{A}	7.67 ±1.98^{D}	11.28 ±1.98^{C}
	Fasted	60.68 ±1.98^{A}	14.01 ±1.98^{B}	16.55 ±1.98^{B}

$^{A-E}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.28. Effect of sex and diet on plasma fatty acid profile.

Mole %		n-3 PUFA	n-6 PUFA	n-9 MUFA
n-6: n-3 PUFA	Male	2.84 ±0.21^{D}	6.68 ±0.21^{B}	5.24 ±0.21^{C}
	Female	2.82 ±0.21^{D}	7.83 ±0.21^{A}	5.02 ±0.21^{C}
n-3 PUFA Pro: Pre	Male	57.28 ±2.03^{A}	12.06 ±2.03^{BC}	13.05 ±2.03^{B}
	Female	71.53 ±2.03^{A}	9.62 ±2.03^{C}	14.78 ±2.03^{B}

$^{A-D}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.29. Effect of feeding intensity and diet of plasma fatty acid profile.

Mole %		n-3 PUFA	n-6 PUFA	n-9 MUFA
LA	80% RDA	13.07 ±0.61^{D}	27.64 ±0.61^{A}	18.55 ±0.61^{C}
	120% RDA	11.47 ±0.61^{E}	21.70 ±0.61^{B}	14.20 ±0.61^{D}
n-3 PUFA Pro: Pre	80% RDA	66.05 ±1.98^{A}	11.46 ±1.98^{CD}	12.14 ±1.98^{C}
	120% RDA	62.76 ±1.98^{A}	10.21 ±1.98^{D}	15.69 ±1.98^{B}

$^{A-E}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.30. Effect of diet, feeding intensity, sex and fasting on plasma ALA content.

ALA, Mole %			Non-Fasted	Fasted
n-3 PUFA	80% RDA	Male	0.16 ±0.05^{D}	0.20 ±0.05^{C}
		Female	0.14 ±0.05^{D}	0.14 ±0.05^{C}
	120% RDA	Male	0.18 ±0.05^{D}	0.17 ±0.05^{C}
		Female	0.14 ±0.05^{D}	0.13 ±0.05^{C}
n-6 PUFA	80% RDA	Male	0.58 ±0.05^{AB*}	0.39 ±0.05^{B}
		Female	0.45 ±0.05^{BC}	0.45 ±0.05^{AB}
	120% RDA	Male	0.53 ±0.05^{AB}	0.53 ±0.05^{A}
		Female	0.64 ±0.05^{A*}	0.43 ±0.05^{AB}
n-9 MUFA	80% RDA	Male	0.64 ±0.05^{A*}	0.44 ±0.05^{AB}
		Female	0.52 ±0.05^{AB}	0.48 ±0.05^{AB}
	120% RDA	Male	0.48 ±0.05^{BC}	0.40 ±0.05^{B}
		Female	0.39 ±0.05^{C}	0.35 ±0.05^{B}

$^{A-E}$ Values differ within response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P < 0.05).
Lsmeans ± SEM are presented.

Table 4.31. Effect of diet, sex and fasting on plasma EPA content.

EPA, Mole %		Non-Fasted	Fasted
n-3 PUFA	Male	2.67 ±0.13^{B*}	1.57 ±0.11^{A}
	Female	3.74 ±0.11^{A*}	1.70 ±0.11^{A}
n-6 PUFA	Male	0.45 ±0.11^{D}	0.53 ±0.11^{B}
	Female	0.49 ±0.11^{D}	0.36 ±0.11^{B}
n-9 MUFA	Male	0.74 ±0.11^{CD}	0.64 ±0.11^{B}
	Female	0.82 ±0.11^{C}	0.54 ±0.11^{B}

$^{A-D}$ Values differ within response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P < 0.05).
Lsmeans ± SEM are presented.

Table 4.32. Effect of fasting and feeding intensity of plasma EPA content.

EPA, Mole %	80 % RDA	120% RDA
Non-Fasted	1.60 ±0.07^{A}	1.36 ±0.07^{B}
Fasted	0.86 ±0.07^{C}	0.92 ±0.07^{C}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

4.3.3.2 Liver Fatty Acid Profile

Significant differences in the liver fatty acid profile were found in response to diet, feeding intensity, sex and fasting (Table 4.33). Liver total SFAs decreased with fasting and tended to be the lowest in the mink fed the n-9 MUFA diet in both the NF and F mink (Table 4.34). The proportion of total MUFAs in the livers of the NF mink were the highest in the mink fed the n-9 MUFA diet at 120% RDA, followed by n-9 MUFA diet fed at 80% RDA, with lowest levels observed in the mink fed the n-6 PUFA diet. Fasting decreased total MUFAs, and MUFA percent in the liver was the lowest in the n-6 PUFA fed mink.

The liver UFA: SFA ratio was the highest in the NF mink fed the n-9 MUFA diet, especially in the mink fed the diet at 120% RDA. The UFA: SFA ratio increased with fasting. In the fasted mink the ratio was the highest in the mink fed the n-6 PUFA diet at 120% RDA or the n-9 MUFA diet. The n-6: n-3 PUFA ratio in the liver was the highest in the mink fed the n-6 PUFA diet and lowest in those fed the n-3 PUFA diet and increased with fasting (Table 4.35). The increase in response to fasting was particularly high in mink fed at 80% RDA (Table 4.36 and 4.37).

The proportion of total liver n-6 PUFAs was the highest in n-6 PUFA diet group and lowest in those fed the n-3 PUFA diet (Table 4.38). In the NF mink, the males had a higher proportion of n-6 PUFAs compared to the females when fed at 80% RDA (Table 4.39). Fasting decreased the liver's proportion of n-6 PUFAs in all the mink. Within the F mink, the female mink fed at 120% RDA had the lowest proportion of liver n-6 PUFAs. The mole % of LA was the highest in the mink fed the n-6 PUFA diet and lowest in the mink fed the n-3 PUFA diet (Table 4.40). No differences were detected in the NF mink for the liver AA percentage, while fasting decreased the proportion in the liver and was the highest in the mink fed the n-6 PUFA diet at 80% RDA. The n-6 PUFA product: precursor ratio in the livers of the NF mink was the highest in the n-3 PUFA group and decrease in all groups with fasting.

The total n-3 PUFAs were the highest in the livers of the NF mink fed the n-3 PUFA diet and lowest in those fed the n-9 MUFA diet (Table 4.41). Fasting decreased this proportion, and the mink fed the n-6 PUFA diet had higher liver total n-3 PUFAs

than mink fed the n-3 PUFA diet. Increased liver percentage of n-3 PUFA in the males fed at 120% RDA reflects differences observed in the n-6: n-3 PUFA ratio. The proportions of ALA were the highest in the mink fed the n-3 PUFA diet regardless of RDA (Table 4.42), and increased with fasting for mink fed the n-3 PUFA and n-9 MUFA diets. The increase of ALA with fasting was also observed in the sex and feeding intensity interaction, with the exception of males fed at 80% RDA, who had the lowest liver ALA percentage in both the NF and F groups. Fasting decreased the liver EPA levels (0.18 ±0.02% vs. 0.38 ±0.02%) and the lower feeding intensity of 80% RDA also related to lower liver EPA content (0.27±0.02% vs. 0.29 ±0.02%). The highest proportions of EPA were observed in the livers of the mink fed the n-3 PUFA diet. Fasting decreased the proportion of DHA, and again the proportions were the highest in the livers of the mink fed the n-3 PUFA diet and lowest in those fed the n-9 MUFA diet. The liver n-3 PUFA product: precursor ratio was higher in the females than the males, but fasting reduced both and eliminated the sex different (Table 4.43). Diet influenced the n-3 PUFA product: precursor ratio, which was the highest in the mink fed the n-3 PUFA diet and lowest in those fed the n-9 MUFA diet in the NF group. Fasting decreased the ratio in all the diet groups, and n-3 PUFA group had the highest ratio, followed by mink fed the n-6 PUFA diet at 80% RDA.

Table 4.33.a. P-values of the main effects and interactions for liver fatty acid profile. Highest order significant main effects and interactions are bolded.

Liver Effect	SFA	MUFA	UFA: SFA	n-6: n-3 PUFA	LA	AA	n-6 PUFA	n-6 PUFA Pro: Pre
Fast	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sex	0.713	0.287	0.719	0.879	0.084	0.909	0.039	0.868
Fast*Sex	0.913	0.969	0.911	0.228	0.638	0.713	0.632	0.446
Diet	<0.001	<0.001	<0.001	<0.001	**<0.001**	0.164	**<0.001**	<0.001
Fast*Diet	0.265	0.036	0.168	0.014	0.584	0.456	0.811	0.089
Sex*Diet	0.992	0.791	0.988	0.020	0.269	0.527	0.197	0.186
Fast*Sex*Diet	0.772	0.531	0.758	**0.032**	0.773	0.223	0.687	0.074
FI	0.028	0.049	0.026	0.020	0.015	0.535	0.168	0.709
Fast*FI	0.526	0.538	0.491	**0.013**	0.358	0.051	0.005	0.060
Sex*FI	0.973	0.312	0.962	**0.011**	0.651	0.084	0.242	0.163
Fast*Sex*FI	0.167	0.720	0.153	0.753	**<0.001**	0.665	**0.007**	0.595
Diet*FI	0.103	0.215	0.119	0.356	0.522	0.028	0.091	0.018
Fast*Diet*FI	**0.004**	**0.008**	**0.004**	0.356	0.638	**0.003**	0.291	**0.001**
Sex*Diet*FI	0.578	0.508	0.526	0.820	0.478	0.478	0.362	0.279
Fast*Sex*Diet*FI	0.060	0.399	0.053	0.082	0.084	0.831	0.059	0.884

Table 4.33.b. P-values of the main effects and interactions for liver fatty acid profile. Highest order significant main effects and interactions are bolded.

Liver Effect	ALA	EPA	DHA	n-3 PUFA	n-3 PUFA Pro: Pre
Fast	<0.001	**<0.001**	<0.001	<0.001	<0.001
Sex	0.137	0.472	0.438	0.606	0.402
Fast*Sex	0.096	0.067	0.479	0.460	**0.046**
Diet	<0.001	**<0.001**	<0.001	<0.001	<0.001
Fast*Diet	**<0.001**	0.350	<0.001	**<0.001**	0.068
Sex*Diet	0.052	0.184	0.741	0.536	0.174
Fast*Sex*Diet	0.913	0.154	0.216	0.269	0.146
FI	0.002	**0.004**	0.203	0.537	0.072
Fast*FI	0.558	0.089	0.812	0.402	0.584
Sex*FI	0.613	0.069	0.096	**0.004**	0.335
Fast*Sex*FI	**0.034**	0.943	0.733	0.405	0.488
Diet*FI	**0.046**	0.086	0.710	0.493	0.108
Fast*Diet*FI	0.082	0.290	**0.046**	0.243	**0.007**
Sex*Diet*FI	0.478	0.478	0.362	0.279	0.620
Fast*Sex*Diet*FI	0.084	0.831	0.059	0.884	0.051

Table 4.34. Effect of fasting, diet and feeding intensity on liver fatty acid profile.

		n-3 PUFA		n-6 PUFA		n-9 MUFA	
Mole %		80% RDA	120% RDA	80% RDA	120% RDA	80% RDA	120% RDA
SFA	Non-fasted	$40.79 \pm 0.83^{Aa*}$	$39.45 \pm 0.83^{A*}$	$39.23 \pm 0.83^{A*}$	$38.77 \pm 0.93^{A*}$	$34.35 \pm 0.83^{B*}$	$31.92 \pm 0.83^{C*}$
	Fasted	29.72 ± 0.83^{A}	30.79 ± 0.83^{A}	31.44 ± 0.83^{A}	26.70 ± 0.83^{B}	24.29 ± 0.83^{C}	25.58 ± 0.83^{BC}
MUFA	Non-fasted	$32.79 \pm 1.21^{C*}$	$32.97 \pm 1.21^{BC*}$	$22.57 \pm 1.21^{D*}$	$21.55 \pm 1.36^{D*}$	$36.35 \pm 1.21^{B*}$	$40.17 \pm 1.21^{A*}$
	Fasted	50.87 ± 1.21^{A}	50.07 ± 1.21^{A}	33.39 ± 1.21^{C}	39.70 ± 1.21^{B}	51.28 ± 1.21^{A}	51.39 ± 1.21^{A}
AA	Non-fasted	$5.81 \pm 0.36^{*}$	$7.06 \pm 0.36^{*}$	$5.66 \pm 0.36^{*}$	$7.21 \pm 0.40^{*}$	$5.64 \pm 0.36^{*}$	$5.41 \pm 0.36^{*}$
	Fasted	1.29 ± 0.36^{B}	1.42 ± 0.36^{B}	2.04 ± 0.36^{A}	1.15 ± 0.36^{B}	1.30 ± 0.36^{B}	1.38 ± 0.36^{B}
DHA	Non-fasted	$5.04 \pm 0.21^{A*}$	$5.01 \pm 0.21^{A*}$	$2.74 \pm 0.21^{BC*}$	$2.86 \pm 0.23^{BC*}$	$3.26 \pm 0.21^{B*}$	$2.66 \pm 0.21^{C*}$
	Fasted	1.89 ± 0.21^{A}	1.90 ± 0.21^{A}	1.76 ± 0.21^{AB}	1.23 ± 0.21^{B}	1.27 ± 0.21^{B}	1.43 ± 0.21^{AB}
UFA: SFA	Non-fasted	$1.45 \pm 0.09^{C*}$	$1.54 \pm 0.09^{C*}$	$1.56 \pm 0.09^{C*}$	$1.59 \pm 0.09^{C*}$	$1.93 \pm 0.09^{B*}$	$2.16 \pm 0.09^{A*}$
	Fasted	2.37 ± 0.09^{B}	2.25 ± 0.09^{B}	2.21 ± 0.09^{B}	2.75 ± 0.09^{A}	3.13 ± 0.09^{A}	2.92 ± 0.09^{A}
n-6 PUFA	Non-fasted	$0.48 \pm 0.03^{AB*}$	$0.61 \pm 0.03^{A*}$	$0.22 \pm 0.03^{C*}$	$0.29 \pm 0.03^{BC*}$	$0.34 \pm 0.03^{BC*}$	$0.35 \pm 0.03^{BC*}$
Pro: Pre	Fasted	0.09 ± 0.03^{A}	0.10 ± 0.03^{A}	0.07 ± 0.03^{B}	0.04 ± 0.03^{C}	0.07 ± 0.03^{B}	0.08 ± 0.03^{AB}
n-3 PUFA	Non-fasted	$20.77 \pm 0.62^{A*}$	$16.22 \pm 0.62^{A*}$	$2.09 \pm 0.62^{C*}$	$2.11 \pm 0.62^{C*}$	$2.83 \pm 0.62^{B*}$	$2.27 \pm 0.62^{B*}$
Pro: Pre	Fasted	4.64 ± 0.62^{A}	4.14 ± 0.62^{A}	1.43 ± 0.62^{B}	0.74 ± 0.62^{C}	0.77 ± 0.62^{C}	0.93 ± 0.62^{C}

$^{A-D}$ Values differ within response rows ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.35. Effect of fasting, diet and sex on liver n-6: n-3 PUFA ratio.

		n-3 PUFA		n-6 PUFA		n-9 MUFA	
Mole %		Male	Female	Male	Female	Male	Female
n-6: n-3	Non-Fasted	$2.71 \pm 0.32^{CD*}$	$2.49 \pm 0.32^{D*}$	$6.46 \pm 0.32^{A*}$	$6.37 \pm 0.32^{A*}$	$4.25 \pm 0.32^{B*}$	$4.24 \pm 0.32^{BC*}$
PUFA	Fasted	4.96 ± 0.32^{CD}	4.50 ± 0.32^{D}	7.21 ± 0.32^{B}	8.47 ± 0.32^{A}	5.52 ± 0.32^{C}	5.36 ± 0.32^{C}

$^{A-D}$ Values differ within response rows ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are presented

Table 4.36. Effect of fasting and feeding intensity of liver fatty acid profile.

n-6: n-3 PUFA	80 % RDA	120% RDA
Non-Fasted	4.41 ±0.20^{C}	4.43 ±0.20^{C}
Fasted	6.33 ±0.20^{A}	5.67 ±0.20^{B}

$^{A-D}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.37. Effect of feeding intensity and sex on liver fatty acid profile.

	80% RDA		120% RDA	
Mole %	Male	Female	Male	Female
22:5 n-3	0.42 ±0.04^{B}	0.50 ±0.04^{AB}	0.51 ±0.04^{A}	0.48 ±0.04^{AB}
n-3 PUFA	4.72 ±0.23^{B}	4.95 ±0.23^{AB}	5.19 ±0.23^{A}	4.64 ±0.23^{B}
n-6: n-3 PUFA	5.52 ±0.26^{A}	5.22 ±0.26^{AB}	4.85 ±0.26^{B}	5.25 ±0.26^{AB}

$^{A-B}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.38. Effect of diet on liver fatty acid profile.

Mole %	n-3 PUFA	n-6 PUFA	n-9 MUFA
n-6 PUFA	17.45 ±0.25^{C}	31.93 ±0.25^{A}	21.42 ±0.25^{B}
EPA	0.56 ±0.03^{A}	0.13 ±0.03^{B}	0.15 ±0.03^{B}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.39. Effect of fasting, feeding intensity and sex on liver fatty acid profile.

		80% RDA		120% RDA	
Mole %		Male	Female	Male	Female
n-6 PUFA	Non-fasted	$25.78 \pm 0.41^{A*}$	$24.28 \pm 0.41^{B*}$	$25.76 \pm 0.44^{A*}$	$25.21 \pm 0.41^{AB*}$
	Fasted	22.37 ± 0.41^{A}	22.80 ± 0.41^{A}	22.27 ± 0.41^{A}	20.34 ± 0.41^{B}
ALA	Non-fasted	1.13 ± 0.08^{A}	$0.94 \pm 0.08^{B*}$	$1.12 \pm 0.09^{A*}$	$1.13 \pm 0.08^{Ab*}$
	Fasted	1.12 ± 0.08^{B}	1.31 ± 0.08^{AB}	1.44 ± 0.08^{A}	1.29 ± 0.08^{AB}

$^{A-B}$ Values differ within response rows ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.40. Effect of diet, feeding intensity, sex and fasting on liver LA content.

LA, Mole %			Non-Fasted	Fasted
n-3 PUFA	80% RDA	Male	$12.61 \pm 0.70^{D*}$	14.61 ± 0.70^{E}
		Female	$11.46 \pm 0.70^{D*}$	13.78 ± 0.70^{E}
	120% RDA	Male	$12.18 \pm 0.70^{D*}$	14.65 ± 0.70^{E}
		Female	11.22 ± 0.70^{D}	12.86 ± 0.70^{E}
n-6 PUFA	80% RDA	Male	27.97 ± 0.70^{A}	27.45 ± 0.70^{B}
		Female	$23.96 \pm 0.70^{B*}$	29.67 ± 0.70^{A}
	120% RDA	Male	$23.74 \pm 0.70^{A*}$	28.92 ± 0.70^{AB}
		Female	27.17 ± 0.70^{B}	26.80 ± 0.70^{B}
n-9 MUFA	80% RDA	Male	17.46 ± 0.70^{C}	19.12 ± 0.70^{C}
		Female	$15.86 \pm 0.70^{C*}$	19.02 ± 0.70^{C}
	120% RDA	Male	16.75 ± 0.70^{C}	17.92 ± 0.70^{CD}
		Female	15.55 ± 0.70^{C}	16.65 ± 0.70^{D}

$^{A-E}$ Values differ within response columns ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.41. Effect of fasting and diet on liver fatty acid profile.

Mole %		n-3 PUFA	n-6 PUFA	n-9 MUFA
ALA	Non-fasted	0.33 ±0.07^{D}	1.50 ±0.07^{AB}	1.42 ±0.07^{B}
	Fasted	0.51 ±0.07^{C}	1.62 ±0.07^{AB}	1.74 ±0.07^{A}
n-3 PUFA	Non-fasted	7.47 ±0.21^{A}	5.26 ±0.21^{B}	5.46 ±0.21^{B}
	Fasted	3.39 ±0.21^{D}	3.93 ±0.21^{C}	3.73 ±0.21^{CD}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.42. Effect of feeding intensity and diet on liver ALA content.

ALA, Mole %	n-3 PUFA	n-6 PUFA	n-9 MUFA
80% RDA	0.38 ±0.07^{B}	1.42 ±0.07^{A}	1.57 ±0.07^{A}
120% RDA	0.45 ±0.07^{B}	1.70 ±0.07^{A}	1.59 ±0.07^{A}

$^{A-B}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.43. Effect of fasting and sex of the n-3 PUFA product: precursor ratio.

n-3 PUFA Pro: Pre	Male	Female
Non-Fasted	7.08 ±0.42^{B}	8.34 ±0.40^{A}
Fasted	2.12 ±0.40^{C}	2.10 ±0.40^{C}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

4.3.3.3 Mesenteric Fatty Acid Profile

P-values for the main effects and interactions for the fatty acid profile of the mesenteric fat depot are shown in Table 4.44. Total SFA percent was the highest in the mink fed the n-3 PUFA diet followed by n-6 PUFA diet and finally the n-9 MUFA diet (Table 4.45 and 4.46). Fasting decreased the SFA proportion within the mesenteric fat in both the n-3 PUFA and n-6 PUFA groups. A sex effect was noted in the n-9 MUFA group with the males having a higher proportion of SFAs. The proportion of MUFAs in the mesenteric fat was the lowest in mink fed the n-6 PUFA diet regardless of fasting treatment (Table 4.47).

The UFA: SFA ratio in the mesenteric fat increased with fasting, and was the highest in mink fed the n-9 MUFA diet group and lowest in the n-3 PUFA diet group in both F and NF mink groups (Table 4.48). The n-6: n-3 PUFA ratio increased with fasting in all treatment combinations with the exception the males fed the n-6 PUFA diet at 120% RDA. Generally, the n-6: n-3 PUFA ratio was the highest in mink fed the n-6 PUFA diet.

The n-6 PUFA proportion of the mesenteric fat depot was the highest in mink fed the n-6 PUFA diet and lowest in mink fed the n-3 PUFA diet. A sex difference was noted in the total n-6 PUFAs, where the percentage was higher in females when fed the n-3 PUFA or n-6 PUFA diets and the total n-6 PUFA reflected differences in the mesenteric 18:2n-6. In the F mink, total n-6 PUFAs was higher in the females than males when fed at 80% RDA (Table 4.49). The same pattern was also observed for the percentage of LA. In the NF mink the mesenteric proportion of AA was the highest in the mink fed the n-6 PUFA diet and decreased with fasting. Feeding intensity affected the percentage of AA differently depending on sex, with an increase in the males and a decrease in females with feeding at 120% RDA (Table 4.50). The mesenteric fat n-6 PUFA product: precursor ratio in the NF mink was the highest in those fed the n-3 PUFA diet and lowest in the n-6 PUFA group. The n-6 PUFA product: precursor ratio decreased with fasting, and mink fed the n-9 MUFA had the lowest ratio. A sex and feeding intensity effect was observed, with female mink fed 120% having the lowest n-6 PUFA product: precursor ratio in the mesenteric adipose tissue.

Fasting resulted in a decrease in total n-3 PUFAs in the mesenteric fat depot, except in males fed the n-9 MUFA diet at 120% RDA. The percentage of total n-3 PUFAs was the highest in the mink fed the n-6 PUFA diet and lowest in those fed the n-3 PUFA diet. The increase in the mesenteric n-3 PUFAs in mink fed the n-6 PUFA and decrease in the n-3 PUFA fed mink was a result of changes of ALA. A sex difference was noted in the n-9 MUFA group, with males having a higher proportion of ALA in the mesenteric depot than females. Fasting decreased the proportion of ALA in the mesenteric adipose tissue. Feeding intensity did not influence the percent of ALA in the NF mink, while in the F mink the percentage of ALA was higher in the males fed at 120% RDA and lowest in the males fed at 80% RDA. The mesenteric fat depots responded to the diets with increased percentage of EPA in the mink fed the n-3 PUFA diet, with the same levels found in the mink fed the n-6 PUFA or n-9 MUFA diets. Females had the lowest proportion of EPA, followed by the males fed at 80% RDA. Fasting resulted in a decrease in the mesenteric fat EPA for both sexes (Table 4.51). Increasing the feeding intensity reduced the proportion of EPA in NF mink, with fasting reducing the levels and eliminating the difference between the feeding intensities (Table

4.52). Levels of DHA were highest in the mink fed the n-3 PUFA diet, and higher in the males than females fed the n-6 PUFA and n-9 MUFA diet. Mink fed the n-3 PUFA diet at either feeding intensity and n-6 PUFA at 120% RDA had a decrease in the percent DHA with fasting (Table 4.53). In the F mink the highest proportion of DHA was observed in mink fed the n-3 PUFA diet at 80% RDA and lowest in those fed the n-6 PUFA or n-9 MUFA diet at 120% RDA. The n-3 PUFA product: precursor ratio was higher in the mink fed the n-3 PUFA diet at 80% RDA compared to 120% RDA, and the lowest ratios were observed in the mink fed the n-6 PUFA and n-9 MUFA diets (Table 4.54).

Table 4.44.a. P-values of the main effects and interactions for the fatty acid profile of the mesenteric fat. Highest order significant main effects and interactions are bolded.

Effect	SFA	MUFA	UFA: SFA	n-6: n-3	LA	AA	n-6 PUFA	Pro: Pre n-6
Fast	<0.001	<0.001	<0.001	<0.001	0.201	<0.001	0.038	<0.001
Sex	0.037	0.482	0.022	0.084	0.340	0.045	0.294	0.076
Fast*Sex	0.761	0.815	0.650	0.769	0.212	0.392	0.218	0.809
Diet	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Fast*Diet	**0.002**	0.114	0.223	0.004	0.838	**0.016**	0.923	**<0.001**
Sex*Diet	**0.014**	<0.001	<.0001	0.322	**<0.001**	0.121	**<0.001**	0.288
Fast*Sex*Diet	0.103	0.839	**0.003**	0.515	0.491	0.551	0.432	0.914
FI	0.887	0.487	0.371	0.155	0.886	0.627	0.601	0.514
Fast*FI	0.053	0.539	0.181	0.716	0.275	0.526	0.249	0.865
Sex*FI	0.437	0.098	0.109	<0.001	0.964	**<0.001**	0.593	**0.001**
Fast*Sex*FI	0.887	0.060	0.369	0.028	**0.041**	0.434	**0.044**	0.234
Diet*FI	0.294	0.565	0.636	0.003	0.254	0.324	0.435	0.149
Fast*Diet*FI	0.110	0.176	0.502	0.815	0.437	0.088	0.475	0.435
Sex*Diet*FI	0.302	0.264	0.306	0.422	0.247	0.632	0.405	0.161
Fast*Sex*Diet*FI	0.104	**0.008**	0.326	**0.034**	0.761	0.069	0.536	0.122

Table 4.44.b. P-values of the main effects and interactions for the fatty acid profile of the mesenteric fat. Highest order significant main effects and interactions are bolded.

Mesenteric Effect	ALA	EPA	DHA	n-3 PUFA	Pro: Pre n-3
Fast	<0.001	<0.001	<0.001	<0.001	0.187
Sex	0.422	0.003	0.028	0.221	0.443
Fast*Sex	0.413	0.005	0.581	0.545	0.754
Diet	<0.001	<0.001	<0.001	<0.001	<0.001
Fast*Diet	**<0.001**	0.224	0.250	0.273	0.084
Sex*Diet	**0.044**	**0.004**	**0.048**	0.110	0.264
Fast*Sex*Diet	0.921	0.203	0.346	0.533	0.920
FI	0.084	0.162	<0.001	0.162	0.003
Fast*FI	0.310	**0.033**	0.098	0.353	0.928
Sex*FI	0.063	**0.036**	0.033	0.004	0.117
Fast*Sex*FI	**0.001**	0.287	0.174	0.026	0.829
Diet*FI	0.120	0.709	0.099	0.057	**0.002**
Fast*Diet*FI	0.591	0.669	**0.025**	0.388	0.981
Sex*Diet*FI	0.513	0.332	0.452	0.570	0.064
Fast*Sex*Diet*FI	0.020	0.340	0.149	**0.002**	0.972

Table 4.45. Effect of fasting and diet on fatty acid profile of the mesenteric fat.

Mole %		n-3 PUFA	n-6 PUFA	n-9 MUFA
SFA	Non-fasted	26.37 ± 0.32^{A}	22.82 ± 0.32^{B}	18.92 ± 0.32^{D}
	Fasted	23.34 ± 0.32^{B}	21.07 ± 0.32^{C}	18.20 ± 0.32^{D}
AA	Non-fasted	0.26 ± 0.01^{B}	0.30 ± 0.01^{A}	0.25 ± 0.01^{BC}
	Fasted	0.17 ± 0.01^{E}	0.22 ± 0.01^{CD}	0.21 ± 0.01^{D}
n-6 PUFA	Non-fasted	$0.03 \pm <0.01^{A}$	$0.01 \pm <0.01^{D}$	$0.02 \pm <0.01^{C}$
Pro: Pre	Fasted	$0.02 \pm <0.01^{B}$	$0.01 \pm <0.01^{E}$	$0.01 \pm <0.01^{F}$

$^{A-E}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.46. Effect of sex and diet on fatty acid profile of the mesenteric fat.

Mole %		n-3 PUFA	n-6 PUFA	n-9 MUFA
SFA	Male	25.31 ± 0.33^{A}	21.97 ± 0.33^{B}	19.51 ± 0.33^{C}
	Female	24.40 ± 0.33^{A}	21.93 ± 0.33^{B}	17.60 ± 0.33^{D}
LA	Male	8.57 ± 0.40^{D}	31.01 ± 0.40^{B}	15.35 ± 0.40^{C}
	Female	8.06 ± 0.40^{E}	33.68 ± 0.40^{A}	15.18 ± 0.40^{C}
n-6 PUFA	Male	9.04 ± 0.41^{D}	31.64 ± 0.41^{B}	15.86 ± 0.41^{C}
	Female	8.49 ± 0.41^{E}	34.22 ± 0.41^{A}	15.61 ± 0.41^{C}
ALA	Male	0.34 ± 0.07^{D}	2.48 ± 0.07^{A}	2.21 ± 0.07^{B}
	Female	0.30 ± 0.07^{D}	2.58 ± 0.07^{A}	1.99 ± 0.07^{C}
EPA	Male	0.18 ± 0.01^{A}	0.05 ± 0.01^{C}	0.05 ± 0.01^{C}
	Female	0.16 ± 0.01^{B}	0.03 ± 0.01^{D}	0.03 ± 0.01^{D}
DHA	Male	0.58 ± 0.03^{A}	0.30 ± 0.03^{B}	0.30 ± 0.03^{B}
	Female	0.58 ± 0.03^{A}	0.22 ± 0.03^{C}	0.24 ± 0.03^{C}

$^{A-E}$ Values differ within responses ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.47.a. Effect of diet, feeding intensity, sex and fasting on fatty acid profile of the mesenteric fat.

			MUFA, Mole %	
			Non-Fasted	Fasted
n-3 PUFA	80% RDA	Male	$62.07 \pm 1.09^{B*}$	66.22 ± 1.09^{AB}
		Female	$62.65 \pm 1.09^{B*}$	68.63 ± 1.09^{A}
	120% RDA	Male	$61.93 \pm 1.09^{B*}$	65.63 ± 1.09^{AB}
		Female	64.41 ± 1.09^{A}	66.27 ± 1.09^{AB}
n-6 PUFA	80% RDA	Male	$41.07 \pm 1.09^{C*}$	46.65 ± 1.09^{D}
		Female	39.82 ± 1.09^{C}	39.58 ± 1.09^{E}
	120% RDA	Male	41.57 ± 1.09^{C}	43.81 ± 1.09^{D}
		Female	$38.73 \pm 1.09^{C*}$	45.01 ± 1.09^{D}
n-9 MUFA	80% RDA	Male	$60.15 \pm 1.09^{B*}$	63.17 ± 1.09^{BC}
		Female	62.97 ± 1.09^{AB}	64.49 ± 1.09^{BC}
	120% RDA	Male	61.77 ± 1.09^{B}	62.12 ± 1.09^{C}
		Female	63.67 ± 1.09^{A}	66.10 ± 1.09^{AB}

$^{A-C}$ Values differ within response rows ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.47.b. Effect of diet, feeding intensity, sex and fasting on fatty acid profile of the mesenteric fat.

			n-3 PUFA, Mole %		n-6: n-3 PUFA	
			Non-Fasted	Fasted	Non-Fasted	Fasted
n-3 PUFA	80% RDA	Male	$1.79 \pm 0.16^{E*}$	1.26 ± 0.16^{E}	$5.14 \pm 0.45^{C*}$	7.19 ± 0.45^{DE}
		Female	$2.22 \pm 0.16^{DE*}$	1.22 ± 0.16^{E}	$3.92 \pm 0.45^{C*}$	6.99 ± 0.45^{DE}
	120% RDA	Male	$1.88 \pm 0.16^{E*}$	1.17 ± 0.16^{E}	$4.93 \pm 0.45^{C*}$	7.66 ± 0.45^{CD}
		Female	$1.42 \pm 0.16^{F*}$	0.95 ± 0.16^{E}	$6.18 \pm 0.45^{C*}$	8.93 ± 0.45^{C}
n-6 PUFA	80% RDA	Male	$3.54 \pm 0.16^{A*}$	2.30 ± 0.16^{BCD}	$9.08 \pm 0.45^{B*}$	13.40 ± 0.45^{A}
		Female	$3.21 \pm 0.16^{AB*}$	2.77 ± 0.16^{A}	$10.62 \pm 0.45^{A*}$	12.80 ± 0.45^{AB}
	120% RDA	Male	$3.47 \pm 0.16^{A*}$	2.78 ± 0.16^{A}	9.18 ± 0.45^{B}	11.56 ± 0.45^{B}
		Female	$3.60 \pm 0.16^{A*}$	2.36 ± 0.16^{ABC}	$9.91 \pm 0.45^{AB*}$	13.57 ± 0.45^{A}
n-9 MUFA	80% RDA	Male	$3.28 \pm 0.16^{AB*}$	2.08 ± 0.16^{CD}	$5.11 \pm 0.45^{C*}$	7.40 ± 0.45^{D}
		Female	$2.78 \pm 0.16^{C*}$	2.30 ± 0.16^{BCD}	$5.71 \pm 0.45^{C*}$	7.05 ± 0.45^{DE}
	120% RDA	Male	2.98 ± 0.16^{BC}	2.58 ± 0.16^{AB}	$5.36 \pm 0.45^{C*}$	6.15 ± 0.45^{E}
		Female	$2.62 \pm 0.16^{CD*}$	1.90 ± 0.16^{D}	$5.91 \pm 0.45^{C*}$	8.09 ± 0.45^{CD}

$^{A-E}$ Values differ within response columns ($P < 0.05$).

* Values differ between non-fasted and fasted mink ($P < 0.05$).

Lsmeans ± SEM are presented.

Table 4.48. Effect of diet, sex and fasting on the UFA: SFA ratio in the mesenteric fat.

UFA: SFA		Non-Fasted	Fasted
n-3 PUFA	Male	2.73 ±0.08^{C*}	3.21 ±0.08^{D}
	Female	2.86 ±0.08^{C*}	3.20 ±0.08^{D}
n-6 PUFA	Male	3.33 ±0.08^{B*}	3.81 ±0.08^{C}
	Female	3.45 ±0.08^{B*}	3.70 ±0.08^{C}
n-9 MUFA	Male	4.14 ±0.08^{A}	4.13 ±0.08^{B}
	Female	4.46 ±0.08^{A*}	4.93 ±0.08^{A}

$^{A-D}$ Values differ within response columns ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are present

Table 4.49. Effect of fasting, feeding intensity and sex on fatty acid profile of the mesenteric fat.

		80% RDA		120% RDA	
Mole %		Male	Female	Male	Female
LA	Non-fasted	18.73 ±0.45	18.82 ±0.45	18.40 ±0.45	19.37 ±0.45*
	Fasted	17.76 ±0.45^{A}	19.54 ±0.45^{B}	18.35 ±0.45^{AB}	18.16 ±0.45^{B}
n-6 PUFA	Non-fasted	19.29 ±0.45	19.36 ±0.45	19.01 ±0.45	19.85 ±0.45
	Fasted	18.25 ±0.45^{B}	20.02 ±0.45^{A}	18.83 ±0.45^{AB}	18.53 ±0.45^{AB}
ALA	Non-fasted	1.98 ±0.07*	1.81 ±0.07*	1.94 ±0.07*	1.92 ±0.07*
	Fasted	1.19 ±0.07^{C}	1.44 ±0.07^{AB}	1.59 ±0.07^{A}	1.32 ±0.07^{BC}

$^{A-C}$ Values differ within response columns ($P < 0.05$).
* Values differ between non-fasted and fasted mink ($P < 0.05$).
Lsmeans ± SEM are presented.

Table 4.50. Effect of feeding intensity and sex on fatty acid profile of the mesenteric fat.

	80% RDA		120% RDA	
Mole %	Male	Female	Male	Female
AA	0.23 ±0.01^{B}	0.23 ±0.01^{B}	0.27 ±0.01^{A}	0.21 ±0.01^{C}
EPA	0.08 ±0.01^{B}	0.08 ±0.01^{C}	0.10 ±0.01^{A}	0.07 ±0.01^{C}
n-6 PUFA Pro: Pre	0.02 ±<0.01^{A}	0.02 ±<0.01^{A}	0.02 ±<0.01^{A}	0.01 ±<0.01^{B}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.51. Effect of fasting and sex on mesenteric fat EPA content.

EPA, Mole %	Male	Female
Non-Fasted	0.13 ±0.01^{A}	0.11 ±0.01^{B}
Fasted	0.06 ±0.01^{C}	0.04 ±0.01^{D}

$^{A-D}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.52. Effect of fasting and feeding intensity on mesenteric fat EPA content.

EPA, Mole %	80 % RDA	120% RDA
Non-Fasted	0.12 ±<0.01^{A}	0.11 ±<0.01^{B}
Fasted	0.05 ±<0.01^{C}	0.05 ±<0.01^{C}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

Table 4.53. Effect of diet, feeding intensity and fasting on mesenteric fat DHA content.

DHA, Mole %		Non-Fasted	Fasted
n-3 PUFA	80% RDA	0.82 ±0.04^{A*}	0.55 ±0.04^{A}
	120% RDA	0.57 ±0.04^{A*}	0.39 ±0.04^{B}
n-6 PUFA	80% RDA	0.29 ±0.04^{BC}	0.33 ±0.04^{BC}
	120% RDA	0.24 ±0.04^{CD*}	0.17 ±0.04^{E}
n-9 MUFA	80% RDA	0.34 ±0.04^{B}	0.28 ±0.04^{CD}
	120% RDA	0.24 ±0.04^{D}	0.24 ±0.04^{DE}

$^{A-E}$ Values differ within response columns (P <0.05).
* Values differ between non-fasted and fasted mink (P <0.05).
Lsmeans ± SEM are presented.

Table 4.54. Effect of feeding intensity and diet on mesenteric fat n-3 PUFA product: precursor ratio.

n-3 PUFA Pro: Pre	n-3 PUFA	n-6 PUFA	n-9 MUFA
80% RDA	2.89 ±0.14^{A}	0.16 ±0.14^{C}	0.17 ±0.14^{C}
120% RDA	1.91 ±0.14^{B}	0.09 ±0.14^{C}	0.13 ±0.14^{C}

$^{A-C}$ Values differ within responses (P <0.05).
Lsmeans ± SEM are presented.

4.4 Discussion

In carnivores such as the mink, feeding history can alter the weight of the body's fat reserves and significantly influence their fatty acid profiles (Rouvinen and Kiiskinen 1989; Iverson 2004). Fasting has been shown to be selective with preferential mobilization of individual fat depots and fatty acids in mink and polecats (Nieminen et al. 2006, 2009). Prior feeding history was shown to alter the development of fatty liver in mink (see chapter 3). However, the role of the individual fat depots and the mobilization of the fatty acids in the development of fatty liver is not known in the mink. This study examined how prior feeding history and fasting altered the weight of the fat depots in relation to liver lipid content.

4.4.1 Body Composition

Controlling caloric intake, through feeding intensity, altered the body weight and body composition of the mink in this study, similar to results reported earlier in mink (Rouvinen-Watt et al. 2004). In both studies, increasing the feeding intensity to 100-120% RDA generally increased the body weights of the mink compared to the mink fed at 80% RDA. This was also reflected in the estimated total body fat content of the animals in the current study. In the 120% RDA group, a larger percent of the increase in the total body weight was deposited as subcutaneous fat, the most significant fat depot by mass in mink (Mustonen et al. 2005a, Nieminen et al. 2006). Increases were also noted in the intermuscular fat deposits and intra-abdominal fat, which reflects increases in the omental and mesenteric reserves, in the mink fed at 120% RDA compared to those fed at 80% RDA. In relation to the total body fat, an increase in the proportion of mesenteric fat was observed with the increased feeding intensity compared to the other fat depots. This suggests that the mesenteric fat depot increases disproportionately more in mass with increased adiposity and may be a concern regarding liver health. It should be noted that, like other studies, the reported weights of the fat depots underestimate the actual depot mass since only visible fat is dissected and weighed (Mustonen et al. 2005a).

Preferential deposition of fat into the various depots is also likely caused by differences in the activity of enzymes involved in the uptake of fatty acids by adipocytes,

such as LPL. Boivin et al. (2007) examined the LPL activity in normal and obese men and found increased LPL activity in the omental fat compared to subcutaneous suggesting increased capacity for fat deposition in this depot. More pronounced LPL expression in the mink mesentery would allow for increased deposition in the intra-abdominal fat compared to subcutaneous fat, resulting in an increase in the proportion of visceral adipose tissue with obesity.

Feeding intensity also affected lean body mass and organs weights, both being larger in mink fed at 120% RDA compared to mink fed at 80% RDA. This indicates that not only did feeding intensity alter body fat reserves, but it may have altered the physiological development of the mink leading to fatter, bigger and bulkier mink. By early September, mink kits achieve 85 to 90% of the adult body frame size (Rouvinen-Watt et al. 2005). Feeding intensity during the fall months could alter the remaining development of mink, which could include larger organs. This was not the case in a previous study where no differences in organ weights were found (Muise 2008), but the feeding intensities used in that study did not include a restricted feeding intensity, such as the 80% RDA used here. No clear indication of an effect of feeding intensity on body length was found, suggesting that the feeding intensities used either did not influence skeletal growth or the feeding regimes were started after most of the longitudinal skeletal growth had been finished.

It is noteworthy that the male mink fed the n-3 PUFA diet were larger than males fed the n-6 PUFA or n-9 MUFA diets. In a study by Muise (2008), no effect was found for diet between the experimental feeding intensities of 100 % and 120 % RDA, which was supported by no differences in feed intake. Another study has noted that dietary oil source can significantly affect growth rate in mink, however they did not evaluate body condition or adiposity directly (Käkelä et al. 2001). In this study the males fed the n-3 PUFA diet consumed more feed towards the end of the study (Table 3.7). Whether this is due to a taste preference in mink for herring oil or a physiological effect of herring oil is not clear. The increased feed intake in these animals did result in heavier mink, with increased adiposity and as a consequence a proportionate decrease in lean body mass. Along with having higher levels of n-3 LCPUFAs in the n-3 PUFA diet, an increase in the proportion of SFAs was also observed compare to the other diets. In chickens

increasing dietary SFA intake has been shown to alter body composition and fat deposition, with increased SFA: UFA ratio increasing the amount of abdominal fat (Sanz et al. 1999; Newman et al. 2002). This may also affect mink, as mink fed beef or pork fat, which is high in SFAs, were found to gain more weight during the fall compared to mink fed fish or vegetable oils (Käkelä et al. 2001).

Fasting decreased body weight, which was caused by a reduction in both the lean body mass and fat reserves. Both these reserves would provide energy sources during the fasting period, with fat providing fatty acids and the lean body mass, particularly muscle tissue providing amino acids. This is supported by the decreased protein content of locomotory muscle in observed in fasted mink (Mustonen et al. 2005b). A greater decrease in the proportion of body fat compared to lean body mass was observed, suggesting that body fat could be the principal metabolic fuel during fasting in mink. Within the fat depots, a decrease was observed in the perirenal fat depot, a depot previously shown to strongly respond to fasting (Mustonen et al. 2005a). Comparing the percent body weight of the omental and subcutaneous fat depots demonstrated that omental fat is more readily mobilized, decreasing in the proportion of body weight by 18% versus the 10% in the subcutaneous depot. The mesenteric fat depot did not respond to fasting as consistently as the other intra-abdominal fat depots. It decreased in relation to body weight in fasted males fed the n-3 and n-6 PUFA diets and females fed the n-9 MUFA diets, while males fed the n-9 MUFA diets and females fed the n-3 and n-6 PUFA diets groups did not show significant change. It has been suggested that subcutaneous fat helps mink maintain thermal insulation during the colder winter months, and is supported by an increased mobilization of the subcutaneous fat depots in summer compared to winter (Mustonen et al. 2005a; Nieminen et al. 2006). Preserving the subcutaneous fat depot for insulation rather than as an energy source would provide energetic savings for the mink because of the mink's long body shape (Mustonen et al. 2005a). Female mink are at a further disadvantage, because of their smaller body size (Korhonen and Niemelä 1998), and as such would have more energetic benefits from mobilizing intra-abdominal fat instead of subcutaneous fat. However, in this study there is no indication that there is a sex difference in the mobilization of the fat reserves in relation to body weight or total body fat.

Preferential mobilization of the intra-abdominal fat depots may be a result of differences in the activity of enzymes involved in the mobilization of fatty acids from adipocytes such as HSL. Lipase activity tends to be higher in ventral fat depots than subcutaneous fat in polecats (Mustonen et al. 2009), however it is unclear if this occurs in the mink due to the lower number of specific ventral and subcutaneous depots tested in mink (Mustonen et al. 2005a). One factor known to influence lipolysis and HSL activity in adipocytes is cell size. Reynisdottir et al. (1997) found that lipolytic capacity and levels of HSL are at least partly determined by adipocyte size, with increasing cell size increasing HSL expression in humans. Differences in the size of the adipocytes of the different depots could be present and explain why mink mobilize visceral fat disproportionately more than subcutaneous. Increasing body fat would result thus in higher lipolysis levels during periods of severe negative energy balance. Whether mink show differences in adipocyte size between the different fat depots is currently unknown and would warrant further study.

For all the mink, fasting resulted in a decrease in small intestine and pancreas weights, and only mink fed the n-6 PUFA and n-9 MUFA diets demonstrated a decrease in stomach weight. This is similar to results obtained by Rouvinen-Watt et al. (2010) except for no changes to kidney or female reproductive tract weights in response to fasting were observed, but differs from other studies in mink and polecats where no alterations to organ weights were found (Mustonen et al. 2005a, 2009). Decreases in thyroid gland weights or increased adrenal gland weights have not been observed in previous studies (Mustonen et al. 2005a, 2009; Rouvinen-Watt et al. 2010), although were found in this study. Changes in the weights of the adrenal glands could suggest endocrinological changes as a result of fasting. Increased adrenal gland weight has been documented previously in rats fasted for 48 hours with increases in adrenal volume and cortisol levels (Kmiec et al. 2006). This study observed no effect of fasting on cortisol levels (Table 3.31), but increased adrenal volume could indicate increased cortisol production but not increased secretion. Increased adrenal gland mass in males fed at 120% RDA (62.87 nmol/L) compared to 80% RDA (37.04 nmol/L) could be linked to the increased cortisol production observed in mink fed at 120% RDA.

4.4.2 Alterations to Fatty Acid Profiles

The fatty acid signatures of mammals reflect the dietary history of the animal, but are also modified by metabolically demanding events such as fasting (Rouvinen and Kiiskinen 1989; Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). The interaction that dietary fat and fasting may have on the development of fatty liver in the mink may be related to changes in the fatty acid profiles of the liver, plasma and body fat depots.

In this study, like others, an increase in MUFA and decreases in SFA were noted in the livers of fasted mink compared to non-fasted counterparts (Nieminen et al. 2009; Rouvinen-Watt et al. 2010). The role of diet was also apparent in the mink with differences in the n-3 PUFA and n-6 PUFA content of the diet reflecting the fatty acid profiles of the liver. Within the n-3 PUFA class, the livers of mink fed the n-6 PUFA and n-9 MUFA had the highest levels of ALA, reflecting the higher levels of this fatty acid in the diet compared to the n-3 PUFA diet. The opposite was true for the n-3 LCPUFAs, EPA and DHA, which increased as a result of the higher levels in the n-3 PUFA diet. Fasting resulted in a decrease in total n-3 PUFAs, and more significantly a decrease in n-3 LCPUFAs. A decrease in total liver n-3 PUFAs along with EPA and DHA has been reported in previous fasting studies in mink and polecats (Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). Increased liver n-6: n-3 PUFA ratio reflected the decrease in DHA and EPA caused by fasting. Furthermore the dietary influence on the n-6: n-3 PUFA ratio was also observed, with the highest ratios in the mink fed the n-6 PUFA diet and the lowest in mink fed the n-3 PUFA diet.

Changes to the fatty acid profile of the liver caused by fasting could result from the mobilization of fatty acids from the fat depots into the plasma and subsequent uptake by the liver. The plasma fatty acid profile can represent the net mobilization of fatty acids from the fat reserves during fasting. In this study plasma SFAs and MUFAs increased. This differs from previous studies and is most likely a result of differences in fatty acid profiles between the diets used in those experiments and the diets in this study (Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). In the n-6 PUFA class, diet did influence the plasma concentration, with the highest levels being found in the n-6 PUFA diet and lowest in the n-3 PUFA diet. AA decreased with fasting, and increased with feeding

intensity. The n-3 PUFAs showed fasting differences based on diet. Mink fed the n-3 PUFA diet had a decrease in total n-3 PUFAs and EPA and an increase in DHA from fasting, while mink fed the n-6 PUFA and n-9 MUFA diets had higher total n-3 PUFAs as a result of the higher ALA content in the diets compared to the n-3 PUFA diet. Fasting did not affect plasma levels of EPA in mink fed the n-6 PUFA or n-9 MUFA diets. The plasma n-6: n-3 PUFA ratios decreased with fasting only in the mink fed the n-6 PUFA or n-9 MUFA diets. Differences in the mobilization of the fatty acids from the adipose tissue depots shows that preferential mobilization is controlled not only by fatty acid chain length, degree of desaturation and location of double bonds (Raclot 2003), but also by the dietary history as it in turn influences fatty acid mobilization.

Due to preferential deposition and mobilization of fatty acids from the different fat depots (Mustonen et al. 2007; Nieminen et al. 2006), examining the fatty acid profile of the depots helps clarify changes in the fatty acid reserves from increased adiposity or fasting. Of these depots, the mesenteric fat is the largest visceral fat depot in mink, which is a concern in the development of fatty liver in humans (Westphal 2008). Thus, examining the changes in this dynamic depot could help better understand the physiological role visceral fat plays in the development of fatty liver in the mink. The mesenteric fat depot and the other intra-abdominal depots are known to contain higher proportions of SFA and lower proportions of total PUFAs compared to the subcutaneous fat depots (Mustonen et al. 2007). In this study, the composition of this depot was modified by diet, indicating that this depot can increase its levels of n-3 PUFA which may play a role as a reserve during periods of food deprivation. Fasting caused a decrease in SFAs, AA, and ALA regardless of diet. The other n-3 PUFAs demonstrate a different picture where fasting decreased EPA and DHA in mink fed the n-3 PUFA diet, but not the n-6 PUFA or n-9 MUFA diet. Decreasing proportions of n-3 PUFA represent increased mobilization of these fatty acids into the blood circulation. This could indicate that mink fed the n-6 PUFA or n-9 MUFA diets did not have adequate dietary EPA and DHA to build up enough reserves to show the same changes in n-3 PUFAs as did the mink fed the n-3 PUFA diet. This is supported by other studies by Nieminen et al. (2006, 2009), who found differences in the mesenteric profile following fasting showing higher EPA and/or DHA levels than mink fed the n-6 PUFA and n-9 MUFA diets. Of the n-3

and n-6 PUFA classes, EPA and AA are preferentially mobilized over the other fatty acids in human adipose tissue (Raclot 1997, Raclot et al. 2001).

The type of dietary n-3 fatty acids provided to mink also needs to be considered. A lack of the Δ6 desaturase enzyme in cats prevents the elongation of ALA or LA (Rivers et al. 1975) and this may also be the case in mink. In this study dietary fatty acid source greatly influenced the product: precursor ratios, with mink fed n-3 PUFAs having the highest proportions due to the higher content of AA, EPA and DHA and lower content of LA and ALA compared to the other diets. This is supported by earlier findings by Käkelä et al. (2001) who showed that the mink are likely unable to adequately produce EPA and DHA from ALA, and will require a dietary source of EPA and DHA in order to accumulate these fatty acids in their tissues.

4.4.3 Role of Lipid Mobilization in the Development of Fatty Liver

One of the most common traits associated with the development of fatty liver is excessive body fat in mink, cats, and humans (Hunter and Barker 1996; Marceau et al. 1999; Armstrong and Blanchard 2009). Visceral fat has been identified as a depot of concern for the development of fatty liver and other metabolic disorders in humans (Jeong et al. 2008). Mink readily mobilize this depot during fasting while also accumulating fat within the liver (Mustonen et al. 2005a; Rouvinen-Watt et al. 2010). The disproportionate mobilization of lipids from the visceral depots is concerning since the NEFAs released will enter the portal circulation to the liver where hepatocytes can absorb them (Smith and Schenk 2000). The liver helps buffer increasing plasma NEFAs from body fat mobilization by increasing absorption (Bradbury 2006). The net effect though can be damaging to the liver by causing the development of fatty liver. Feeding intensity increases the quantity of body fat in both the visceral and subcutaneous depots and the development of obesity. The effects of fasting were augmented by feeding at the higher feeding intensity. The consequential increased mobilization of the visceral fat depot could further overwhelm the liver leading to the increased liver lipid content seen in the fasted mink fed at 120% RDA compared to those fed at 80% RDA.

Dietary fatty acids likely play a role in fatty liver disease, especially the n-3 PUFAs (El-Badry et al. 2007). Decreases in n-3 LCPUFAs, EPA and DHA were

observed in the livers of fasted mink. Increasing the dietary intake of EPA and DHA increased the levels found in the liver and the levels of EPA and DHA remained relatively high despite decreases caused by fasting. Increased feeding of the n-3 PUFAs did alter the composition of the mesenteric depot, and served as a source of DHA and EPA during fasting. Mink fed diets low in DHA and EPA, seen in this study with the n-6 PUFA and n-9 MUFA diets did not show decreases in the proportion of EPA and DHA in the mesenteric fat. The low levels found in these depots suggest that there was not a large enough reserve before fasting to allow for the preferential mobilization during fasting to occur or be observed. Other studies have noted a decrease in n-3 PUFAs and increased n-6: n-3 PUFA ratio in the liver and fat depots of mink and polecats (Nieminen et al. 2006, 2009; Rouvinen-Watt et al. 2010). Maintaining a good dietary source of EPA and DHA is important, as human fatty liver disease is associated with the same changes in n-3 PUFAs as seen in this study and in polecats (Araya et al. 2004; Nieminen et al. 2009). Furthermore, mink are likely unable to sufficiently elongate ALA, the precusor fatty acid for EPA and DHA and would require a dietary source of EPA and DHA (Käkelä et al. 2001). The benefit of n-3 PUFAs in increasing fatty acid oxidation and decreasing fatty acid synthesis, possibly through increasing PPAR-α levels, has been shown to prevent or ameliorate fatty liver in humans (Lindén et al. 2002; Levy et al. 2004; Alwayn et al. 2005; Capanni et al. 2006; El-Badry et al. 2007). The role of n-3 LCPUFA and dietary need being taken into consideration together may explain why the mink fed at 120% RDA did not develop as severe fatty liver when given the n-3 PUFA diet, compared to the mink fed the n-6 PUFA or n-9 MUFA diets.

The mobilization of lipids in mink is preferentially from the visceral depots (Mustonen et al. 2005a; Rouvinen-Watt et al. 2010) which could mean that increasing the n-3 LCPUFA content of these depots is beneficial in maintaining a delivery of EPA and DHA to the liver during fasting. Examining the fatty acid profiles of the other fat depots in the mink, such as subcutaneous, intermuscular, omental and perirenal would give a better understanding of the deposition and mobilization of fatty acids in mink in relation to feeding intensity, diet and fasting. This will also clarify the possible role of increasing dietary EPA and DHA supply to increase the availability of n-3 PUFAs during fasting.

4.5 Conclusion

Increasing feeding intensity increased mink body fat content, with a disproportionate increase in the visceral fat. This can negatively affect the liver since the mobilized fatty acids from the omental and mesenteric fat will enter the portal circulation and be delivered directly to the liver. By preventing the excessive accumulation of visceral fat through restricted feeding the severity of the fatty liver could be decreased, likely from decreased fatty acid delivery. Dietary fatty acids were strongly reflected in the fatty acid profiles of the plasma, liver and mesenteric fat. Feeding higher levels of EPA and DHA increased the content in the liver and mesenteric fat depot, and helped maintain higher levels during food deprivation. Higher liver EPA and DHA levels likely contributed to decreasing liver lipid accumulation in overweight mink fed the n-3 PUFA diet. In this study, feeding mink at 80% RDA decreased body fat and the severity of fasting-induced fatty liver compared to those fed at 120% RDA. Additionally, increased EPA and DHA levels of the liver and mesenteric fat depot was observed in the mink fed the n-3 PUFA diet, with these levels maintained during fasting. The n-3 LCPUFAs, EPA and DHA, in the n-3 PUFA diet may have helped the liver by promoting fatty acid oxidation resulting in the lower lipid accumulation in these mink compared to the mink fed the n-6 PUFA or n-9 MUFA diets when the diets were fed at 120% RDA. In conclusion, maintaining an ideal body condition and increasing the dietary intake of n-3 LCPUFAs may help during periods in the production cycle when the mink may have an increased risk of developing fatty liver, such as autumnal fattening, conditioning for breeding, and lactation.

CHAPTER 5. Conclusion

In this study, altering the feeding intensity affected the weight of the mink. Mink fed at 80% RDA lost weight during the fall period, and only the males fed at 120% RDA gained weight. Changes in the body weights of the mink were directly reflected in the changes in their body fat content. The mink remained normoglycemic throughout the feeding trial. No difference in the liver lipid content of the mink fed at 80% or 120% RDA was detected among the non-fasted animals.

Fasting for five days resulted in the development of fatty liver in mink. However in this study, other than the lipid accumulation, the haematology and clinical chemistry indicated that mink were otherwise healthy. Fasting resulted in the mobilization of the body fat reserves, with a disproportionate increase in the mobilization of the visceral fat. The increased lipolysis caused by fasting increased plasma NEFAs. The rapid mobilization of the fat reserves is likely the principal mechanism in the development of fasting-induced fatty liver in mink. The increased adiposity of the mink fed at 120% RDA contributed to increased lipid accumulation during fasting, demonstrating a role for obesity in the development of fatty liver, possibly due to augmented lipolysis of the fat depots.

Increasing dietary intake of n-3 LCPUFAs, EPA and DHA, increased the content of these fatty acids in mink livers and mesenteric fat. The proportions of n-3 LCPUFAs remained relatively high despite decreases in these fatty acids caused by fasting. This was found to be beneficial to the mink, as the animals fed the n-3 PUFA diet at 120% RDA had lower liver lipid accumulation than the rest of the mink fed at the same feeding intensity. The role of n-3 PUFAs could involve increasing PPAR-α, which may increase the expression of enzymes involved in β-oxidation and VLDL secretion.

In conclusion, the findings in this study indicate that the development of fasting-induced fatty liver in mink can be modulated through dietary management. It is recommended to avoid overconditioning of mink during the fall to prevent the development of fatty liver. Furthermore, increasing dietary intake of EPA and DHA may promote fatty acid oxidation in the liver, which can help decrease the severity of fatty liver in mink. The most important factor in preventing the development of fatty liver in

mink is avoiding disruption of normal feed intake as this leads to rapid mobilization of body fat.

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