Original Review

Glucose homeostasis in mink (*Mustela vison*)

A review based on interspecies comparisons*)

Christian Friis Børsting and Anette Gade

Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele

Received: 20 July 1999 **Accepted: 13 December 1999**

Abstract

The scope of this review is to discuss glucose homeostasis in mink under varying dietary conditions and in relation to pregnancy and lactation.

As a result of evolutionary adaptation to diets which are low in carbohydrates, carnivores have higher activities of gluconeogenic enzymes than omnivores. The activity of liver enzymes involved in amino acid catabolism is high and unaffected by dietary protein level in carnivores. Furthermore, the activity of these enzymes is the same in the fed and fasted states. This enables the carnivorous species cats and mink to synthesise sufficient glucose primarily based on amino acids to allow glucose utilisation both in the fed and the fasted states. Mink and dogs can maintain glucose homeostasis when fed carbohydrate-free diets during pregnancy and lactation. However, this is not the case when there is a shortage of gluconeogenic precursors. Furthermore, mink are able to store excess glucose in the form of glycogen, which indicates that the risk of overloading mink with glucose under farmed conditions is low.

Keywords: Carnivorous, glucagon, gluconeogenesis, glycolysis, insulin, requirement.

Abbreviations: ALAT, alanine:2-oxoglutarate aminotransferase; ASAT, aspartate:2-oxoglutarate aminotransferase; DE, digestible energy; DM, dry matter; FBPase, fructose-1,6-diphosphatase; GIDH, glutamate dehydrogenase; GLP-1, glucagon-like peptide 1; G6Pase, glucose-6-phosphatase; GS, glycogen synthetase; LDH, lactate oxidoreductase; ME, metabolisable energy; PC, pyruvate carboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PFK1, phosphofructokinase1; PK, pyruvate kinase.

Introduction

The mink is a carnivorous species belonging to the marten family *(Mustelidae).* In its natural habitat it consumes mainly mammals, birds and fish and therefore its natural diet is rich in animal protein and fat whereas it is low in carbohydrates. During evolution this has resulted in a very simple digestive tract with low activities of the carbohydrate digestive enzymes (*Sangild & Elnif, 1996*).

Due to the low dietary intake of carbohydrates, the glucose demand of the mink has to a large extent to be covered by *de novo* glucose synthesis. It has been shown that females nursing 6 kits have to cover approximately 73% of their glucose demand in peak lactation via gluconeogenesis when fed 12% of the metabolisable energy (ME) from carbohydrates (*Børsting & Damgaard, 1995*).

Most lactating female mink are unable to cover their energy requirement by their energy intake, which leads to loss of body weight. Females nursing large litters are at a higher risk of developing the so-called nursing disease due to the extreme mobilisation of body reserves (*Clausen et al., 1992; Wamberg et al., 1992*). Nursing disease is a syndrome seen in lactating mink females around weaning (*Schneider et al., 1997*) and is characterised by extreme weight loss, loss of appetite, dehydration, progressive weakness, hyperglycemia in the late stages and a high mortality rate (*Clausen et al., 1992*). Some studies have indicated a higher risk of nursing disease to be related to a low dietary salt concentration (*Clausen et al., 1996*), and to a high proportion of carbohydrates in the diet (*Clausen & Olesen, 1991*).

Does the mink have a requirement for dietary carbohydrates or can it synthesise sufficient amounts of glucose *de novo* to maintain glucose homeostasis? Can there be negative effects of including excess amounts of carbohydrates in the diet? The scope of the present review is to discuss these questions in relation to glucose homeostasis under varying dietary conditions and in relation to pregnancy and lactation, based on knowledge obtained both in the mink and other species.

General aspects of glucose metabolism

Glucose homeostasis is maintained through a cascade of biochemical pathways. In the fed state, digestion of carbohydrates is followed by absorption of glucose, which stimulates insulin secretion but inhibits pancreatic glucagon secretion (*White et al., 1984*). These hormonal changes increase the process of glycogen synthesis (glycogenesis) and inhibit the conversion of non-glucose molecules to glucose (gluconeogenesis) (*Ganong, 1993; Stryer, 1988*).

Glycolysis

Glucose is metabolised (glycolysis) to pyruvate or lactate (Figure 1) under aerobic and anaerobic conditions, respectively (*Ganong, 1993; White et al., 1984*). Glycolysis is inhibited by a high cellular energy charge, which inhibits the activity of the key rate limiting enzyme phosphofructokinase1 (PFK1). In the final step of glycolysis, pyruvate is formed. Before pyruvate enters the citric acid cycle (TCA cycle), it is oxidised to acetyl-CoA, which is an irreversible process controlled by insulin.

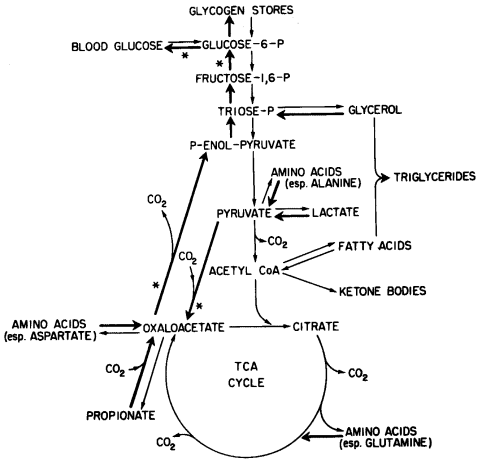

Figure 1. Major metabolic pathways in the liver and the kidney. Pathways for gluconeogenesis are shown as heavy arrows. Four major rate-limiting reactions are indicated by asterisks (Reprinted from Bergman, 1983 with permission from Elsevier Science).

Gluconeogenesis

Gluconeogenesis takes place in both the liver and the kidney; however, in mammals the major site of gluconeogenesis is the liver (*Donkin, 1999*). The function of gluconeogenesis is to maintain glucose homeostasis in the blood (*Stryer, 1988*). In the gluconeogenesis, glucose can be synthesised from pyruvate, however, this process is not just a reversal of glycolysis, since other pathways and enzymes are involved. Pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase) and fructose-1,6-diphosphatase (FBPase) are the rate limiting enzymes involved (*Stryer, 1988*). PC catalyses the conversion of pyruvate to oxaloacetate, and PEPCK catalyses the

decarboxylation and phosphorylation of oxaloacetate to phoshoenolpyruvate (Figure 1). FBPase converts fructose 1,6 diphosphate into glucose-6-phosphate. G6Pase catalyses the hydrolysis of glucose-6-phosphate to glucose which can leave the hepatocytes (*Stryer, 1988*). Endocrinologically, gluconeogenesis is stimulated by high plasma concentrations of glucagon and glucocorticoids and by low insulin concentrations (*White, 1984*).

Amino acid catabolism in the liver yields carbon skeletons which can be utilised in the TCA-cycle (Figure 1). Except for the strictly ketogenic amino acid leucine, all amino acids can to a varying degree be utilised as precursors in gluconeogenesis (*Stryer, 1988*). The main enzymes participating in the conversion of amino acids are alanine:2-oxoglutarate aminotransferase (ALAT), aspartate:2-oxoglutarate aminotransferase (ASAT) and glutamate dehydrogenase (GIDH) (*Stryer, 1988*).

Challenges to glucose homeostasis

Only few studies have been performed to study glucose homeostasis in mink during varying dietary and physiological conditions; however, some data are available on enzyme activities, quantitative glucose metabolism and regulating hormones. In non-pregnant, non-lactating female mink and in males, a 48 hour fast has been shown to cause a decline ($P<0.05$) in plasma glucose concentration from 9 mmol/l to 7 mmol/l (*Sørensen, 1999*). In females nursing 4 or 8 kits, plasma glucose was significantly ($P<0.001$) higher 2-3 hours after feeding (7.6 mmol/l) than after fasting (6.2 mmol/l), despite the rather low proportion of carbohydrates (12% of ME) in the diet (*Børsting & Damgaard, 1995*). In catheterised female mink nursing 4 or 8 kits, plasma glucose concentration (5.9 mmol/l) was unaffected by litter size (*Damgaard & Børsting, 1995*). In our laboratory we have studied glucose homeostasis in glucose tolerance tests by iv. injection of 0.5 g glucose per kg body weight. Glucose tolerance curves measured during the 2 hours immediately after the injection were not significantly different between lactating females with 4 or 8 kits (*Damgaard & Børsting, 1995*). However, total glucose production was slightly, but insignificantly higher in the females with the largest litters (*Børsting & Damgaard, 1995*). New data from our laboratory (*Damgaard, Fink & Børsting, unpubl.*) indicate that mink can maintain glucose homeostasis on a carbohydrate-free diet during pregnancy and lactation. However, this was only the case when the proportion of ME from protein was above approx. 45%.

Studies of glucose homeostasis in relation to diet are much more frequent in another carnivorous species, the dog. In growing female dogs fed 6 diets ranging in composition from 0-62% of ME from carbohydrates and from 20 to 48% of ME from protein for 8 months, plasma glucose concentration was not affected by diet (*Romsos et al., 1976*). A similar result was found in racing sled dogs, where the plasma glucose level was not affected, when the dogs were fed a medium (38%), low (23%) or zero (0%) carbohydrate diet during a 2 to 24 week period (*Kronfeld et al., 1977*). Plasma glucose was similar in pregnant dogs fed 0% or 44% of ME from carbohydrates and 26% of ME from protein during pregnancy except in the last week prepartum, when plasma glucose tended to be reduced in bitches fed the carbohydrate-free diet (*Romsos et al., 1981*). This diet also caused higher incidence of stillborn pups and maternal hypoglycemia in the last week pre-partum (*Romsos et al., 1981*). However, Blaza et al. (1989) did not find extreme hypoglycemia before whelping or a higher pup mortality in bitches fed a carbohydrate-free diet containing 51% of ME from protein. Kienzle & Meyer (1989) found that pups born by bitches fed a carbohydrate-free, medium protein (26% of DE) diet during pregnancy had normal plasma glucose levels immediately after birth, but 12-72 hours after birth they developed severe hypoglycemia. However, hypoglycemia was not seen, when pups were born by bitches fed a carbohydrate-free diet with a high protein content (48% of DE) during pregnancy.

These studies indicate that both mink and dogs can maintain glucose homeostasis when fed carbohydrate-free diets. However, this is not the case when there is a shortage of gluconeogenic precursors in the form of amino acids.

Insulin

Increased plasma glucose concentration post-prandially, as found especially when high levels of carbohydrates are fed, is the major stimulus for the secretion of insulin (*Brand et al., 1998*). Insulin regulates the cellular glucose uptake and suppresses protein degradation in liver, cardiac and skeletal muscle, kidney and adipose tissue (*Mortimore & Pösö, 1987*).

When pregnant dogs were fed either a medium carbohydrate (30% of DE), medium protein (29% of DE) diet or a carbohydrate-free, high protein (48% of DE) diet, only small differences in plasma insulin concentrations were observed. On the other hand, dogs fed a carbohydrate-free, medium protein (26% of DE) diet had an extremely low plasma insulin level throughout pregnancy (*Kienzle & Meyer, 1989*).

In non-pregnant, non-lactating female mink, plasma insulin decreased significantly during a period of feed restriction and increased significantly during re-feeding in flush-fed mink (*Børsting et al., 1998; Fink & Tauson, 1998; Fink et al., 1998*). Lactating female mink fed 12% of ME from carbohydrates had a significantly ($P<0.01$) higher plasma insulin concentration 2 hours after feeding (21.1 mU/l) than 17 hours after feeding (7.8 mU/l) (*Børsting & Damgaard, 1995*). After glucose infusion (0.5 g per kg) in fasted, lactating mink, plasma insulin increased from 10 mU/l to 15 mU/l within 10 minutes ($P<0.01$), remained constant at this level until 40 minutes after infusion and returned to the basic level after 120 minutes (*Damgaard & Børsting, 1995*). Insulin response to feeding was increased with increased level of carbohydrates in the feed (*Gade & Børsting; Fink & Børsting, unpubl.*)

In a glucose tolerance test performed after a 24 hour fast in non-pregnant, non-lactating dogs, glucose concentration, insulin concentration and the glucose to insulin ratio were all higher for the first 30 minutes after glucose infusion in dogs previously fed a carbohydrate-free diet compared with dogs previously fed 27% or 62% of ME from carbohydrates (*Belo et al., 1976*). The insulin response was especially high in dogs fed a carbohydrate-free diet with a low level of protein (24% of ME) and a high level of fat (76% of ME) (*Belo et al., 1976*).

Litter size did not influence plasma insulin increment when lactating females with 4 or 8 kits were infused with 0.5 g glucose per kg (*Damgaard & Børsting, 1995*). However, lactating females with 8 kits had a slightly lower ($P<0.05$) plasma insulin concentration 2-3 hours post-prandially compared with females with 4 kits (*Børsting & Damgaard, 1995*). A 48 hour fast caused a decline ($P<0.005$) in plasma insulin from 40 mU/l (ad libitum) to 25 mU/l in both female and male mink *(Sørensen, 1999*). Lactating mink suffering from nursing disease and fasted for 8 hours had significantly ($P<0.01$) higher insulin concentrations (124 mU/l) compared with healthy lactating female mink (23 mU/l) (*Wamberg et al., 1992*).

Glucagon

The gene encoding proglucagon, the biosynthetic precursor of glucagon, is expressed not only in the pancreatic islets, but also in endocrine cells of the gastro-intestinal mucosa from where enteroglucagon peptides are secreted (*Holst, 1997*). Generally, pancreatic glucagon is stimulated by starvation, low plasma glucose concentrations and ingestion of diets containing low carbohydrate or high protein levels (*Ganong, 1993*). On the other hand, all of the entero-glucagon peptides are secreted into the blood in response to ingestion of carbohydrates and lipids. The two enteroglucagon peptides oxyntomodulin and glucagon-like peptide 1 (GLP-1) have proven to be biologically active (*Holst, 1997*). GLP-1 is a potent insulinotropic hormone which inhibits glucagon secretion and therefore reduces blood glucose, whereas the effect of oxyntomodulin is probably to interact with GLP-1 and glucagon receptors. In lactating female mink fed 12% of ME from carbohydrates, pancreatic glucagon (4.1 pmol/l) ($P<0.01$) was significantly higher in the fed state than 17 hours post-prandially (1.1 pmol/l*) (Børsting & Damgaard, 1995*). In a recent study with lactating mink, we also found increases in pancreatic glucagon (*Gade & Børsting, unpubl.*) in response to feeding. In both cases the antibodies were monoclonal against the pancreatic glucagon. Therefore, the increase after

feeding can probably be ascribed to the high level of protein and the low level of carbohydrates in the diets. A similar result has been found in rats fed a high protein diet (*Eisenstein et al., 1974*).

Glycolytic enzymes

In the mink, the activity of the glycolytic enzyme PK in the liver was significantly higher in fed non-lactating, non-pregnant females (51 IU/g wet weight) than in fed males (38 IU/g) (Table 1). These PK activities in the mink were high compared with the cat (11 IU/g), but also compared with the omnivorous rat (28 IU/g). For another glycolytic liver enzyme, PFK1 values of 6.9 IU/g and 8.3 IU/g were found in male and female mink, respectively, compared with only 1.9 IU/g in the rat (Table 1). A 48 hour fast did not have any significant effect ($P>0.05$) on the activity of the two glycolytic enzymes, PFK1 and PK, in male mink. Minor effects on the activity of PFK1 were monitored in females after a 48 hour fast, where it decreased from 9 to 6 IU/g ($P<0.05$) (*Petersen et al., 1995*). In adult cats, a low protein (17.5% soy protein) or a high protein (70% soy protein) diet caused no significant differences in the activity of PK (*Roger et al., 1977*).

Table 1. Specific activities of hepatic enzymes in mink compared with activities in cats and rats. (Modifed from Sørensen et al., 1995).

	Mink		Rat	Cat
	Male	Female		
Enzyme	IU/g wet wt	IU/g wet wt	IU/g wet wt	IU/g wet wt
PC	4.7±0.6	-	5.8	-
PEPCK	27.0±5.6	30.1±5.1	5.3	2.7
FBPase	23.6±0.7	24.1±0.6	13.0	9.0
G6Pase	62.1±3.4***	91.6±7.5***	7.2	14.0
PFK1	6.9±0.7	8.3±0.8	1.9	-
PK	38.3±3.0*	50.8±3.0*	28.2	11.0
GIDH	105±8	136±19	210.0	29.0
ALAT	136±15	165±23	66.2	44.0
ASAT	133±10	165±12	193.2	120.0
GS (-G6P)	0.028±0.014	0.031±0.015	0.081	-
GS (+G6P)	0.519±0.144	0.574±0.339	0.525	-
LDH	333±33	-	230	214

+G6P and –G6P designate the presence or absence of glucose-6-phosphate in the assay mixture for GS. The deviations are given as SEM. * P<0.05, *** P<0.001.

Altogether, these findings infer that the carnivorous cat and mink are able to synthesise sufficient glucose to allow glucose utilisation (glycolysis) both in the fed and in the fasted states and both when fed high and low protein levels. In other words, the evolutionary adaptation to low carbohydrate levels has apparently not involved a reduced reliance on glucose utilisation compared with species absorbing large amounts of glucose.

Gluconeogenic and amino acid catabolising enzymes

Carnivores are dependent on the rate limiting enzymes of gluconeogenesis and amino acid catabolism to maintain glucose homeostasis. Long-term regulation of gluconeogenesis in monogastrics has been characterised by changes in the expression of genes encoding glucoregulatory enzymes, mainly PEPCK and PK (*Donkin, 1999*). The activity of the gluconeogenic enzymes G6Pase and PEPCK has been shown to be about ten-fold higher (Table 1) in mink liver (40 IU/g wet weight) compared with corresponding figures from rats and cats (5.3 and 2.7 IU/g). Similarly, in the fed, carnivorous bird the black vulture, the activities of G6Pase and PEPCK were two and four times higher compared with fed chickens (*Migliorini et al., 1973; Veiga et al., 1978*). Furthermore, liver slices from fed black vultures synthesised glucose from alanine twice as efficiently as liver slices from fed chickens *(Migliorini et al., 1973; Veiga et al., 1978*).

The activity of PEPCK in liver cytosol was higher (P<0.01), when cats were fed a high protein (63% of DM) diet compared with a high carbohydrate diet (70% of DM) (*Kettelhut et al., 1980*). However, Roger et al. (1977) did not find any significant differences in the activity of PEPCK when adult cats were fed a low protein (17.5% soy protein) or a high protein (70% soy protein) diet. The activity of mitochondrial PEPCK increased when dogs were fed carbohydrate-free diets irrespective of protein level (24 or 48% of ME) (*Belo et al., 1976*).

Cats previously fed a high carbohydrate (70% of DM) diet had a significant (P<0.01) increase in the activities of cytosolic PEPCK in response to a 24

hour fast (*Kettelhut et al., 1980*). However, in fasted cats previously fed a high protein (63% of DM) diet, the activity of cytosolic and mitochondrial PEPCK was not affected by fasting. In mink, a 48 hour fast did not have any significant influence on the activity of PEPCK (*Sørensen, 1999*). Sørensen et al. (1995) observed higher G6Pase activity in non-lactating, non-pregnant female mink compared to males. In a carnivorous bird, 72 hours fasting did not affect the activity of either G6Pase or PEPCK (*Veiga et al., 1978*), whereas in chickens, 72 hours fasting greatly enhanced the activities of PEPCK and G6Pase compared with the fed state (*Veiga et al., 1978*).

No significant changes in the activity of the enzymes GIDH, ALAT and ASAT participating in the conversion of amino acids were seen in neither male nor female mink fasted for 48 hours compared with the ad lib fed state (*Petersen et al., 1995; Sørensen, 1999*). Sørensen et al. (1995) found that ALAT was the only amino acid catabolic enzyme that showed higher activity in fed mink than in fed rats (Table 1). The lack of increase in the catabolic enzymes GIDH, ALAT and ASAT found after a prolonged fast of carnivores is in accordance with results from Silva & Mercer (1992). In the cat, protein degradation was not stimulated by glucagon and glucocorticoids, which on the other hand stimulate proteolysis in the liver of other monogastric species like the rat *(Silva & Mercer, 1992*).

When comparing the activity of ALAT, GIDH and ASAT in fed mink with activities in fed rats, Sørensen et al. (1995) found that ALAT was the only amino acid catabolic enzyme that showed higher activity in the mink than in the rat (Table 1). Adult cats fed a low (17.5% soy protein) or a high protein (70% soy protein) diet showed no significant differences in the activity of ALAT, ASAT and GIDH (*Roger et al., 1977*). This is contrary to what is normally seen in the rat. The lack of influence of dietary protein level and of feeding versus fasting on the activity of these catabolic enzymes is indeed one of the key factors explaining the high dietary protein requirement of carnivorous species *(Roger et al., 1977*). Furthermore, carnivores have higher activities of gluconeogenic enzymes, compared with omnivores, as a result of evolutionary adaptation to diets which are low in carbohydrates. However, cats and dogs have to some extent retained the ability to reduce the activity of gluconeogenic enzymes, when high amounts of glucose are absorbed compared with when large amounts of amino acids are absorbed. Whether this is also the case in the mink has not yet been studied.

Gluconeogenic precursors

Lactate, pyruvate and amino acids (especially alanine and glutamine) are important precursors used for the synthesis of glucose in gluconeogenesis. Kienzle & Meyer (1989) found that the total fraction of gluconeogenic amino acids and plasma alanine decreased in two groups of dogs fed carbohydrate-free diets (protein: 48% of DE or 26% of DE) compared with dogs fed carbohydrate containing diets. In cats fed 17.5% or 70.0% soy protein, the protein level did not influence the *in vitro* rate of glucose synthesis from pyruvate, alanine or threonine in hepatocytes, whereas gluconeogenesis based on glutamine was significantly higher ($P<0.05$) in the cats fed the high protein diet (*Silva & Mercer, 1985*). Cats fed a high protein diet (63% of DM) had a higher ($P<0.01$) rate of glucose synthesis from alanine than cats fed a high carbohydrate diet (70% of DM) (*Kettelhut et al., 1980*).

Glycogen and lipid storage

Storage and mobilisation of glycogen in the liver as well as storage of lipids in extrahepatic tissues are other mechanisms involved in glucose homeostasis and storage of excess dietary energy. Insulin activates both glycogen synthetase (GS) resulting in storage of glucose as glycogen (*White, 1984*) and lipogenic enzymes leading to lipid storage (*Donkin, 1999*). Sørensen et al. (1995) found that the GS activity was almost equal in mink and rats (Table 1). This infers that the mink through evolution has maintained the ability to store glucose in the form of glycogen. This has two implications. Firstly, the mink has a depot of readily available energy, and secondly glycogen synthesis is an efficient way of clearing glucose from the blood stream, when the mink is fed substantial amounts of carbohydrates.

Cats fed a high carbohydrate (70% of DM) diet had a significantly (P<0.01) higher liver glycogen content compared with cats fed a high protein diet (63% of DM) (*Kettelhut et al., 1980*). In fasted cats previously fed the high carbohydrate diet, the liver glycogen content decreased drastically during fasting, while liver glycogen mobilisation was much slower in cats fed a high protein diet *(Kettelhut et al., 1980*). Kienzle & Meyer (1989) have shown that puppies born from dog bitches fed carbohydrate-free diets (26% or 48% DE from protein) during pregnancy had low liver glycogen content and small liver sizes at birth.

In mink, a 48 hour fast caused a decline in liver glycogen (P<0.001) from 6 mg/g wet weight to 0.5 mg/g wet weight (*Petersen et al., 1995*). These results show that during fast, liver glycogen is inevitably decreasing. However, the rate at which liver glycogen is decreasing is very dependent on the diet previously fed.
The content of triglycerides in adipocytes is the balance of the reciprocal regulation of lipogenesis and lipolysis. Insulin promotes metabolism of glucose in lipogenesis, whereas lipolysis during fast is stimulated by glucagon (*Donkin, 1999*). Lipolysis yields glycerol, which can be utilised in gluconeogenesis, and fatty acids, which can not be utilised in this pathway.

Glucose homeostasis during pregnancy and lactation

During both pregnancy and lactation there is an extra glucose demand for growth of the fetuses and milk lactose, respectively. Consequently, plasma glucose has been demonstrated to decrease during pregnancy in a number of species. Kienzle & Meyer (1989) have shown that pregnant dogs expecting large litters can become extremely hypoglycemic prior to whelping. In a recent mink study, we have found a lower glucose concentration of 6.6 mmol/l (SEM=0.2) in late pregnancy compared with early pregnancy, where glucose concentration was 7.5 mmol/l (SEM=0.3) (*Gade & Børsting, unpubl.*).

According to Romsos et al. (1981), lactating dog bitches have a relatively low requirement for glucose for lactose synthesis because their milk has a low concentration of lactose. Pups and kittens ingest about 5-6 g lactose/kg/day from the milk (*Meyer, 1992; Romsos et al., 1981*). The daily output of lactose and other sugars in the milk of a female mink nursing a litter of 8 kits is at least 9 g per day, which is within the same range as the total absorption of glucose (*Børsting & Damgaard, 1995*). Therefore, the synthesis of glucose via gluconeogenesis is essential for supplying the mink with sufficient glucose, especially during the lactation period. This is supported by Børsting & Damgaard (1995), who found that in lactating mink fed a diet with 12% of ME from carbohydrates, gluconeogenesis accounted for about 73% of the glucose requirement. In a recent study (*Gade & Børsting, unpubl.*), an increase in the proportion of carbohydrates in the feed from 11% to 21% of ME did not increase glucose utilisation during the interval 2 to 3 hours post-prandially. Hence, 11% of ME from glucose combined with 59% of ME from protein yielded sufficient gluconeogenic substances to make the same amount of glucose available to the mink as when glucose absorption was almost doubled (21% of ME) and protein was slightly reduced (53% of ME). Altogether, this demonstrates the high ability of the mink to synthesise glucose, when sufficient precursors are available in the form of amino acids.

Litter size seems to influence the production rate of glucose as shown by Børsting & Damgaard (1995), who found slightly higher glucose production rates in female mink nursing 8 kits compared with females nursing 4 kits. This may be part of the etiological background for the development of nursing disease which is characterised by a disrupted glucose homeostasis. Hence, Wamberg et al. (1992) found that lactating mink suffering from nursing disease had a very high plasma glucose level (23.4 mmol/l) compared with 5.3 mmol/l in healthy, lactating females fed a diet consisting of 12% of ME from carbohydrates, which was in agreement with results from Schneider & Hunter (1997). Results from Wamberg et al. (1992) indicate that the peripheral cells of mink suffering from nursing disease develop lower responsiveness to insulin resulting in a reduced cellular uptake of glucose.

Conclusions

Compared with omnivores, carnivores have higher activities of gluconeogenic enzymes as a result of evolutionary adaptation to diets which are low in carbohydrates. The activity of liver enzymes involved in amino acid catabolism is high in carnivores, and it is unaffected by dietary protein level and equal in the fed and fasted states. This enables the carnivorous species cats and mink to synthesise sufficient glucose primarily based on amino acids to allow glucose utilisation both in the fed and the fasted state. Therefore, the evolutionary adaptation to low carbohydrate levels has apparently not involved a reduced reliance on glucose utilisation compared with species absorbing large amounts of glucose. Mink and dogs can maintain glucose homeostasis when fed carbohydrate-free diets. However, this is not the case when there is a shortage of gluconeogenic precursors in the form of amino acids. The risk of hypoglycemia is highest during late pregnancy and lactation, when glucose requirement is highest. Furthermore, mink are able to store excess glucose in the form of glycogen, which indicates that the risk for overloading mink with glucose under farmed conditions is low.

References

Belo, P.S., D.R. Romsos & G.A. Leveille, 1976. Influence of diet on glucose tolerance, on the rate of glucose utilisation and on gluconeogenic enzyme activities in the dog. J. Nutr. **106** (10), 1465-1474.

Bergman, E.N., 1983. The pools of cellular nutrients: Glucose. In: Riis, P.M. (ed.), Dynamic Biochemistry of Animal Production, World Animal Science A3, Elsevier, 173-189.

Blaza, S.E., D. Booles & I.H. Burger, 1989. Is carbohydrate essential for pregnancy and lactation in dogs? In: I.H. Burger & J.P.W. Rivers (eds.), Nutrition of the dog and cat. 7th Waltham Symposium, Cambridge University Press, 229-243.

van den Brand, H., N.M. Soede, J.W. Schrama & B. Kemp, 1998. Effects of dietary energy source on plasma glucose and insulin concentration in gilts. J. Anim. Physiol. Anim. Nutr. **79**(1), 27-32.

Børsting, C.F. & B. Damgaard, 1995. The intermediate glucose metabolism in the nursing period of the mink. NJF-Seminar no. 253, Göteborg, Sweden, NJF-report no. 106, 104-111.

Børsting, C.F., B.M. Damgaard, & R. Fink. 1998. Effects of different energy supply prior to the breeding season on reproductive performance and metabolism in female mink. NJF-Seminar no. 295, Bergen, Norway, NJF-Report (ISSN 0333-1350), 8 pp..

Clausen, T.N. & C.R. Olesen, 1991. Relationship between feeding and nursing sickness in mink. [In Danish]. In: Glem-Hansen, N. (ed.) Technical Year Report 1990, Danish Fur Breeders Association, 204-213.

Clausen, T.N., C.R. Olesen & S. Wamberg, 1992. Nursing sickness in lactating mink (*Mustela vison*) I. Epidemiological and pathological observations. Can. J. Vet. Res. **56**, 89-94.

Clausen, T.N., S. Wamberg & O. Hansen. 1996. Incidence of nursing sickness and biochemical observations in lactating mink with or without dietary salt supplementation. Can. J. Vet. Res. **60**, 271-276.

Damgaard, B.M. & C.F. Børsting, 1995. Glucose tolerance test in mink. [In Danish]. NJF-Seminar no. 253, Göteborg, Sweden, NJF-report no. 106, 179-182.

Donkin, S.S. 1999. Role of the endocrine pancreas in animal metabolism, growth and performance. In: Pierzynowski, S & Zabielski, R. (eds.), Biology of the pancreas in growing animals, Elsevier Science, 315-328.

Eisenstein, A.B., I. Strack & A. Steiner, 1974. Glucagon stimulation of hepatic gluconeogenesis in rats fed a high-protein, carbohydrate-free diet. Metabolism **23**, 15-23.

Fink, R. & A.H. Tauson, 1998. Flushing of mink (*Mustela vison*): effects on energy metabolism and some blood metabolites. Anim. Sci. **66**, 277-284.

Fink, R., A.H. Tauson & M. Forsberg, 1998. Influence of different planes of energy supply prior to the breeding season on some blood metabolites in the mink (*Mustela vison*). Reprod. Nutr. Develop. **28**, 107-116.

Ganong, W.F., 1993. Review of Medical Physiology. 16th ed. Pretice-Hall Int. Inc. Connecticut, USA, 572 pp.

Holst, J.J., 1997. Enteroglucagon. Annu. Rev. Physiol. **59**, 257-271.

Kettelhut, I.C., M.C. Foss & R.H. Migliorini, 1980. Glucose homeostasis in a carnivorous animal (cat) and in rats fed a high-protein diet. Amer. J. Physiol. **239**, R437-R444.

Kienzle, E. & H. Meyer, 1989. The effects of carbohydrate-free diets containing different levels of protein on reproduction in the bitch. In: I.H. Burger & J.P.W. Rivers (eds.) Nutrition of the dog and cat. 7th Waltham Symposium, Cambridge University Press, 243-257.

Kronfeld, D.S., E.P. Hammel, C.F. Ramberg & H.L. Dunlap, 1977. Hematological and metabolic responses to training in racing sled dogs fed diets containing medium, low or zero carbohydrate. Amer. J. Nutr. **30**, 419-430.

Meyer, H., 1992. Laktosefütterung bei Fleischfressern. Tierärtz. Mschr. **79**, 236-

241.

Migliorini, R.H., C. Linder, J.L. Moura & J.A.S. Veiga, 1973. Gluconeogenesis in a carnivorous bird (black vulture). Amer. J. Physiol. **225** (6), 1389-1392.

Mortimore, G.E. & Pösö, A.R. 1987. Intracellular Protein Catabolism and its Control during Nutrient Deprivation and Supply. Ann. Rev. Nutr. **7,** 539-564.

Petersen, I.M., O. Sand & P.G. Sørensen, 1995. Effect of starvation on the activities of key enzymes of the glycolysis and the gluconeogenesis in liver of mink. NJF-Seminar no. 253, Göteborg, Sweden, NJF-Report no 106, 196.

Roger, Q.R., J.G. Morris & R.A. Freedland, 1977. Lack of hepatic enzymatic adaptation to low and high levels of dietary protein in the adult cat. Enzyme **22**, 348-356.

Romsos, D.R., P.S. Belo, M. R. Bennink, W. G. Berger & G.A, Leveille, 1976. Effects of dietary carbohydrate, fat and protein on growth, body composition and blood metabolite levels in the dog. J. Nutr. **106** (10), 1452-1464.

Romsos, D.R., H. J. Palmer, K. L. Muiruri and M. R. Bennink, 1981. Influence of a low carbohydrate diet on performance of pregnant and lactating dogs. J. Nutr. **111**, 678-689.

Sangild, P.T. & J. Elnif, 1996. Intestinal hydrolytic activity in young mink (*Mustela vison*) develops slowly postnatally and exhibits late sensitivity to glucocorticoids. J. Nutr. **126**, 2061-2068.

Schneider, R.R. & D.B. Hunter, 1997. Nursing disease in mink: Clinical and post mortem findings. Blue Book of Fur Farming 1997, 12-30.

Silva, S.V.P.S. & J.R. Mercer, 1985. Effect of protein intake on amino acid catabolism and gluconeogenesis by isolated hepatocytes from the cat (*Felis Domestica*). Comp. Biochem. Physiol. **80B**(3), 603-607.

Silva, S.V.P.S. & Mercer, J.R. 1992. The control of protein degradation in monolayer cultures of cat hepatocytes. Int. J. Biochem. **24**(10), 1651-1656.

Stryer, L., 1988. Biochemistry. 3rd ed. Freeman and Company, San Francisco, USA, 1089 pp.

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

Sørensen, P.G. 1999. Inst. of Biochem., Odense University, Denmark. Pers.Comm.

Veiga, J.A.S., E.S. Roselino & R.H. Migliorini, 1978. Fasting, adrenalectomy, and gluconeogenesis in the chicken and a carnivorous bird. Amer. J. Physiol. **234** (3), R115-R121.

Wamberg, S., T.N. Clausen, C.R. Olesen & O. Hansen, 1992. Nursing sickness in lactating mink (*Mustela vison*). II Pathophysiology and changes in body fluid composition. Can. J. Vet. Res. **56**, 95-101.

White, D.A., B. Middleton & M. Baxter (eds.), 1984. Hormones and metabolic control. Edward Arnold Ltd, London, England. 103 pp.