Endocrinologic Adaptations to Wintertime Fasting in the Male American Mink (*Mustela vison*)

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The aim of this study was to investigate the endocrine response to wintertime starvation in the male American mink (*Mustela vison*) fasted for 16 hrs, 2 days, 3 days, 5 days, or 7 days ($n = 10$ per group). After 2 days of fasting, the plasma leptin concentrations decreased, along with the triiodothyronine, testosterone, and progesterone levels, and the blood monocyte counts. Leptin also seems to trigger the response to fasting in mustelids by inducing immunosuppression and downregulation of the reproductive and thyroid axes. The dramatic increase in the peptide YY concentrations after 3 days of fasting may be required to suppress gastrointestinal processes during food scarcity. The plasma insulin levels decreased, and those of glucagon increased after 5 days of fasting in association with efficient glucose sparing and lipid mobilization. Body energy stores cannot be wasted for growth during nutritional scarcity and, thus, the growth hormone levels of the minks decreased after 5 days of fasting. The plasma noradrenaline and cortisol concentrations also decreased after 3 and 7 days without food, respectively. The plasma ghrelin, adiponectin, resistin, thyroxine, adrenaline, or estradiol levels did not respond to fasting. The endocrine response to food deprivation is remarkably similar in divergent mammalian orders, indicating that the hormonal signals enhancing survival during nutritional scarcity must be evolutionarily old and well conserved. Exp Biol Med 230:612–620, 2005

Key words: Acrp30; catecholamines; fasting; leptin; *Mustela vison*

Introduction

The American mink, *Mustela vison* (Schreber, 1777), is a semi-aquatic mustelid predator originating from North America (1). In the 1920s, the species was introduced to European fur farms and soon it was accidentally and deliberately released into the wild. Because of its great colonizing ability, the mink presently thrives in Europe. The posterity of ‡individuals introduced to the former U.S.S.R. has spread to vast areas in the European part of Russia and Siberia. The northernmost mink populations inhabit the Palearctic and Nearctic tundra, where minks may experience involuntary food scarcity during harsh winters, because the species does not use a passive wintering strategy but forages actively throughout the year (2).

We have previously investigated the adaptation to fasting in the farm-bred mink by documenting the responses of energy metabolism and hematology to long-term food deprivation (2–7 days; Ref. 3). Previous studies show that, despite large body fat stores, the mink may have inadequate capacity for lipolysis and that it develops fatty liver syndrome during fasting (3, 4). This makes the species dependent on continuous foraging and food availability. A comprehensive screening of the endocrinologic response to fasting has not yet been conducted in the mink, although some studies have described particular endocrinologic parameters in the species during acute fasting or a negative energy balance (5, 6).

The aim of this study was to investigate the endocrine response to fasting in the male mink ($n = 50$) during long-term wintertime food deprivation (2–7 days). Because the mink is a widely used experimental animal in physiologic and ecotoxicologic studies, it is an attractive model to investigate the signals enabling seasonal weight control of wild carnivores, and the results of this study could have implications for understanding the evolution of mammalian and human weight regulation. Furthermore, the species is reared for the fur industry, and, to develop practical applications to avoid excessive obesity detrimental to the reproductive success of the mink (7), for instance, a complete investigation of the fasting response of the species has to be performed. This study investigated the effects of fasting on the endocrinology of the male mink. The fasting procedures were conducted in

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December, when the experimental animals had accumulated considerable fat stores and started to prepare themselves for the vernal mating season. The specific aims of the study were 1) to monitor the fasting-induced changes in plasma levels of weight-regulatory peptides, thyroid and stress hormones, and sex steroids, 2) to investigate the interactions of these fasting-induced changes with the previously documented fasting-induced modifications in the energy metabolism (3), and 3) to interpret the findings according to the life history of the species.

Materials and Methods

Fifty farm-bred brown male minks born between May 8, 2003, and May 18, 2003 were selected for the study. In addition to endocrinology, fasting-induced effects on carbohydrate and lipid metabolism were studied in these animals, and some of these data have been published previously (3). The minks were housed in standard wire cages (85 × 31 × 45 cm) with wooden nest boxes (27 × 31 × 38 cm). The cages were suspended above the ground in an unheated barn at the Juankoski Research Station fur farm, Juankoski, Finland (63°N; 28°E). The animals were kept at natural temperature and photoperiod, but the effects of wind were absent. They were fed with commercial fur animal diets (12.3% proteins, 8.7% fat, 13.8% carbohydrates, 1556 kcal/kg fresh weight) according to common farming practices, and water or ice was available *ad libitum*. The experiment was approved by the Animal Care and Use Committee of the University of Joensuu.

The fasting experiments were conducted between December 10, 2003, and December 16, 2003. The minks were randomly assigned into five groups as follows: Group 1, fed control animals ($n = 10$); Group 2, animals fasted for 2 days ($n = 10$); Group 3, animals fasted for 3 days ($n = 10$); Group 4, animals fasted for 5 days ($n = 10$); and Group 5, animals fasted for 7 days ($n = 10$). The fed control group was fasted overnight (16 hrs) before sacrificing to avoid any chance of the results being affected by factors such as lipemic plasma because of a recent meal. The body masses (BMs) of the control group were measured before the last meal and 16 hrs after the removal of food at sampling. The fasted animals were weighed 22 hrs after the removal of the remains of their last meal on the first day of fasting and at the end of the fasting period at sampling. Water was available *ad libitum* during food deprivation.

After the fasting trials, the minks were sacrificed quickly with an electric shock and their blood samples were obtained with cardiac punctures. Electrocution leading to cardiac arrest is a recommended method for sacrificing fur animals (8). Blood samples were taken with aseptic needles into test tubes containing EDTA and centrifuged at 1000 *g* to obtain plasma. All visible subcutaneous (sc) and intra-abdominal (iab) fat depots were carefully dissected and weighed. Body fat percentage was calculated from these data by the formula: (total fat mass in grams/ BM in grams) × 100. Plasma glucose and free fatty acid (FFA) levels were measured with the Glucose Liquid Reagent Hexokinase Method and the NEFA Non Esterified Fatty Acids reagents (Randox Laboratories Ltd., Crumlin, UK). These data have been published previously (3).

Leptin concentrations were measured with the Multi-Species Leptin radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO; intraassay and interassay variations, 2.8–3.6% and 6.5–8.7% coefficient of variation [CV], respectively) and plasma ghrelin concentrations with the Ghrelin (Human) RIA kit (Phoenix Pharmaceuticals, Belmont, CA; < 5% and < 14% CV). Adiponectin (Acrp30) levels were determined with the Human Adiponectin RIA kit of Linco Research (1.78–6.21% and 6.90–9.25% CV) and peptide YY (PYY) and resistin levels with the PYY (rat, mouse, porcine) RIA kit (< 8.42% and < 14.52% CV) and the Human Resistin (43–65) RIA kit (< 5% and < 15% CV) from Phoenix Pharmaceuticals. Glucagon concentrations were determined with the Double Antibody Glucagon kit of DPC (Los Angeles, CA; 3.2–6.5% and 6.0–11.9% CV). Growth hormone (GH) levels were measured with the Porcine/Canine Growth Hormone RIA kit (5.36–6.27% and 8.18–10.67% CV) and insulin levels with the Human Insulin Specific RIA kit (2.2–4.4% and 2.9–6.0% CV) from Linco Research. Peptide assays not validated previously for the mink (9) were validated such that serial dilutions of the mink plasma showed linear changes in standard sample binding/maximum binding (BB_0^{-1}) values that were parallel with the standard curves produced with the standards of the manufacturers (Fig. 1a–f).

Plasma thyroxine (T_4), triiodothyronine (T_3), cortisol (C), and testosterone (T) concentrations were measured with the Spectria T4-, T3-, Cortisol-, and Testosterone [^{125}I] Coated Tube Radioimmunoassay kits from Orion Diagnostica (Espoo, Finland; T_4: 3.3–6.8% and 3.3–8.0% CV; T_3: 3.3–6.1 and 4.5–7.5; C: 2.6–5.4 and 6.5–7.3; T: 3.8–7.5 and 4.8–7.0% CV). Estradiol (E) and progesterone (P) levels were determined with the Spectria Estradiol [^{125}I] and Progesterone RIA Coated Tube Radioimmunoassay kits from Orion Diagnostica (E: 2.9–9.7 and 2.3–10.2; P: 2.9–5.8 and 4.7–5.1% CV). Hormonal measurements of all experimental animals were run within an assay.

Plasma catecholamine (noradrenaline, NA; adrenaline, A) concentrations were measured using an Agilent 1100-type high-performance liquid chromatography (HPLC), including autosampler, column oven, and electrochemical detector (Antec Leyden Decade 2; Zoeterwoude, the Netherlands). Agilent Chemstation software was used for device control, sample injection, and chromatogram analysis. Plasma proteins were precipitated with 4 *M* perchloric acid to plasma, 1:10 vol. Samples were purified by aluminium oxide (alumina) extraction. Catecholamines were adsorbed on alkaline alumina (1 *M* Tris, pH 8.65) and eluatedin an acidic solution (0.1 *M* perchloric acid). An internal standard of 20 ng/ml of dihydroxybenzylamine (DHBA) was added to the sample and standard (20 ng/ml of NA or A) test tubes before the purification procedure. As a stationary phase, an ESA Catecholamine HR-80 RP-C18 column (80 mm × 4.6 mm inside diameter; ESA Biosciences, Inc., Chelmsford, MA) was used. The mobile phase consisted of solutions A and B. Solution A consisted of 9.4 g

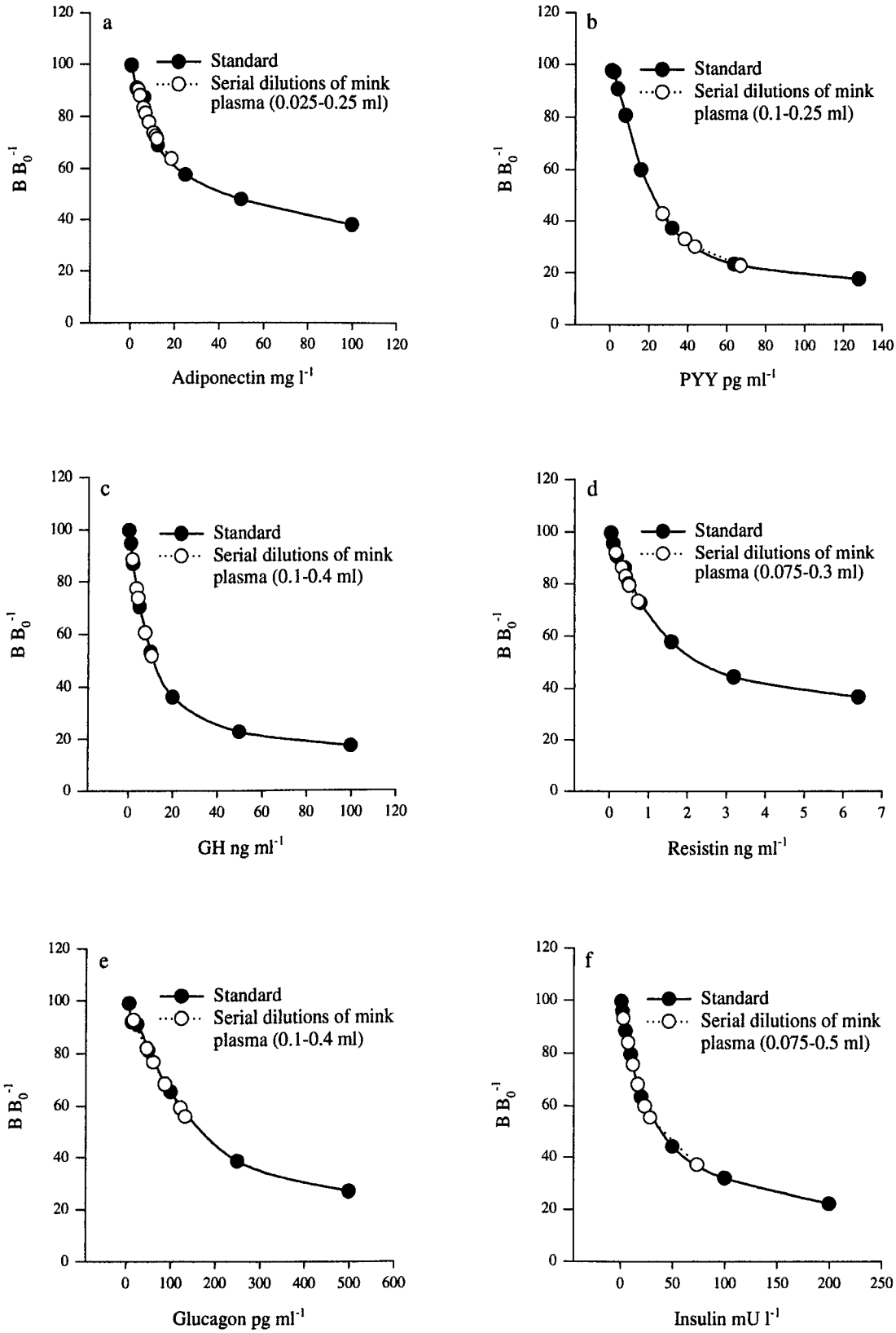

Figure 1. (a–f) Representative estimation of the percentage of cross-reactivity between mink plasma and adiponectin (a), peptide YY (b), growth hormone (c), resistin (d), glucagon (e), and insulin antibodies (f). B, standard or sample binding; B_0, maximum binding.

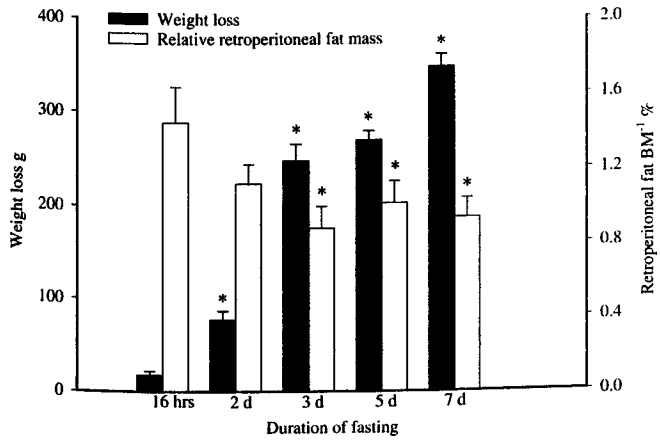

Figure 2. The weight loss (in grams) and the relative weight of retroperitoneal fat depot (percent of BM) of the minks during 16 hrs to 7 days of fasting (mean + SE). *, significant difference from the control group that was fasted for 16 hrs ($P < 0.05$).

of 0.1 *M* monochloric acetic acid, 3.3 g of NaOH, 1.0 g of Na_2EDTA, and 0.3 g of Na-dodecylsulphate, all added to 1 liter of water. Solution B consisted of 250 mg of NaOH, 18.62 mg of Na_2EDTA, 21 g of citric acid, 900 µl of diethylamine, and 90 ml of acetonitrile, all added to 1 liter of water. Solutions A and B were combined 1:1, pH was adjusted to 3.5, and the combination was vacuum-filtered through a membrane (pore size, 0.2 µm). All of the chemicals were HPLC-grade. An injection volume of 10 µl was used for samples and external standards. The detector voltage was adjusted to 0.65, and the column oven temperature to 38°C.

Multiple comparisons were performed with the SPSS (SPSS Inc., Chicago, IL) program using one-way analysis of variance (ANOVA) followed by a *post hoc* Duncan's test or, in the case of nonparametric variables, with the SigmaStat program using Kruskal-Wallis one-way ANOVA and a *post hoc* Dunn's test. Comparisons between two study groups were performed with Student's *t* test for independent samples. For nonparametric data, the Mann-Whitney *U* test was performed. Correlations were tested with the Spearman correlation coefficient (r_s). A $P < 0.05$ was considered statistically significant. The results are presented as the mean ± standard error (SE).

Results

The decrease in the BMs of the minks became significant after 2 days of fasting, and the minks that were fasted for 7 days lost approximately 350 g of their BMs (Fig. 2). The BMs of the fasted study groups at the beginning and at the end of the fasts were as follows: 2636 ± 89 g and 2559 ± 92 g (Group 2); 2623 ± 95 g and 2375 ± 100 g (Group 3); 2608 ± 99 g and 2338 ± 97 g (Group 4); and 2795 ± 90 g and 2448 ± 92 g (Group 5). There were no differences in the absolute masses of sc and omental adipose tissues between the study groups, but the amount of retroperitoneal fat decreased after 3–7 days of fasting (Table 1). The masses of mesenteric and total iab fat also decreased because of fasting (3–5 days). However, the overall body fat percentage was similar between the study groups. Fasting caused decreases in the plasma concentrations of leptin (2–7 days); T_3 (2–3 days; 7 days); T (2 days; 5–7 days); P (2 days; 5–7 days); NA (3–5 days); GH (5 days); insulin (5–7 days); and C (7 days), but the levels of PYY (3–7 days) and glucagon (5 days) increased because of fasting (Table 2; Fig. 3). Also, the T_3/T_4 ratios (2–3 days; 7 days) decreased during food deprivation (Table 2). The FFA levels increased after 7 days without food (Table 1). Fasting did not affect the concentrations of ghrelin, Acrp30, resistin, T_4, A, E, or glucose (Tables 1 and 2).

There was a positive correlation between the plasma leptin concentrations and the sc fat mass ($r_s = 0.664$; $P < 0.01$); iab fat mass ($r_s = 0.671$; $P < 0.01$); mesenteric fat mass ($r_s = 0.674$; $P < 0.01$); retroperitoneal fat mass ($r_s = 0.668$; $P < 0.01$); fat percentage ($r_s = 0.624$; $P < 0.01$); T_3/T_4 ratio ($r_s = 0.324$; $P < 0.05$); white blood cell count ($r_s = 0.390$; $P < 0.01$); lymphocyte count ($r_s = 0.527$; $P < 0.01$); monocyte count ($r_s = 0.611$; $P < 0.01$); and liver glucose-6-phosphatase activity ($r_s = 0.331$; $P < 0.05$). The glucagon and FFA levels ($r_s = 0.417$; $P < 0.01$) as well as the Acrp30 concentrations and liver lipase activities ($r_s = 0.295$; $P < 0.05$) also correlated positively with each other. Moreover, there was an inverse correlation between the Acrp30 level

Table 1. Fat Depot Weights and Plasma Glucose and Free Fatty Acid (FFA) Concentrations of Male Minks According to the Duration of Fasting (Mean ± SE)

Duration of fasting (*n*)	16 hrs (10)	2 days (10)	3 days (10)	5 days (10)	7 days (10)
Subcutaneous fat (g)	860 ± 58	788 ± 60	720 ± 56	706 ± 57	763 ± 48
Omental fat (g)	47 ± 7	37 ± 5	35 ± 6	32 ± 4	34 ± 4
Retroperitoneal fat (g)	40 ± 6	29 ± 3	22 ± 3*	24 ± 3*	23 ± 3*
Mesenteric fat (g)	86 ± 10	70 ± 11	55 ± 11*	56 ± 7*	64 ± 9
Intra-abdominal fat (g)	180 ± 21	143 ± 19	118 ± 20*	118 ± 14*	128 ± 17
Total fat (g)	1040 ± 75	931 ± 76	837 ± 73	829 ± 70	891 ± 60
Body fat (%)	38.1 ± 1.52	35.9 ± 1.82	34.6 ± 2.00	35.3 ± 1.84	36.1 ± 1.20
Glucose (m*M*)	7.1 ± 0.52	7.2 ± 0.33	6.6 ± 0.28	9.2 ± 0.98	7.8 ± 0.26
FFA (m*M*)	0.5 ± 0.10	0.5 ± 0.12	0.7 ± 0.15	0.6 ±0.09	1.2 ± 0.18*

*Significantly different from the control group that was fasted for 16 hrs ($P < 0.05$).

Table 2. Plasma Hormone Concentrations of Male Minks According to the Duration of Fasting (Mean ± SE)

Duration of fasting (*n*)	16 hrs (10)	2 days (10)	3 days (10)	5 days (10)	7 days (10)
Leptin (ng/ml)	6.0 ± 0.91	3.9 ± 0.39*	3.0 ± 0.36*	2.8 ± 0.20*	2.9 ± 0.35*
Ghrelin (pg/ml)	65.0 ± 25.93	38.8 ± 16.64	20.9 ± 9.09	61.4 ± 31.59	58.4 ± 21.15
Adiponectin (μg/ml)	5.5 ± 0.87	5.4 ± 0.44	6.1 ± 0.67	4.8 ± 0.47	6.5 ± 0.65
PYY (pg/ml)	23.7 ± 18.53	4.6 ± 4.59	196.0 ± 53.74*	134.0 ± 52.56*	157.4 ± 52.49*
GH (ng/ml)	5.2 ± 1.67	1.6 ± 0.10	2.5 ± 0.68	1.5 ± 0.33*	2.4 ± 0.71
Resistin (ng/ml)	0.5 ± 0.09	0.3 ± 0.05	0.4 ± 0.10	0.4 ± 0.06	0.4 ± 0.09
Insulin (μU/ml)	35.3 ± 1.81	35.7 ± 7.17	28.2 ± 3.11	20.5 ± 1.90*	25.8 ± 2.82*
Glucagon (pg/ml)	52.1 ± 4.74	41.8 ± 3.77	56.5 ± 4.26	67.9 ± 5.80*	59.4 ± 6.84
T_3 (n*M*)	1.3 ± 0.12	0.9 ± 0.07*	1.0 ± 0.06*	1.5 ± 0.21	0.7 ± 0.04*
T_4 (n*M*)	24.4 ± 1.17	24.5 ± 0.63	25.6 ± 1.31	26.1 ± 1.70	21.5 ± 1.50
T_3/T_4 ratio (%)	5.5 ± 0.38	3.8 ± 0.24*	4.0 ± 0.30*	5.9 ± 0.69	3.5 ± 0.16*
Cortisol (n*M*)	38.7 ± 9.52	27.6 ± 6.72	37.4 ± 6.58	28.1 ± 3.50	14.4 ± 2.44*
Testosterone (n*M*)	13.2 ± 1.43	6.1 ± 1.36*	13.1 ± 3.18	6.6 ± 1.65*	7.3 ± 1.58*
Estradiol (p*M*)	49.7 ± 17.49	34.5 ± 5.57	43.5 ± 5.29	65.1 ± 10.30	64.6 ± 11.37
Progesterone (n*M*)	6.5 ± 0.59	4.2 ± 0.36*	5.7 ± 0.41	4.1 ± 0.24*	3.7 ± 0.26*

*Significantly different from the control group that was fasted for 16 hrs ($p < 0.05$).

and the omental ($r_s = -0.346$; $P < 0.01$); mesenteric ($r_s = -0.386$; $P < 0.01$); and retroperitoneal fat masses ($r_s = -0.297$; $P < 0.05$) and the body fat percentage ($r_s = -0.299$; $P < 0.05$). The ghrelin levels correlated negatively with the lipase activity levels of sc fat ($r_s = -0.358$; $P < 0.05$).

Discussion

As a small and totally carnivorous semiaquatic top predator, the mink is an attractive model to study comparative endocrinology of mammalian weight regulation. Because of the high surface-to-volume ratio, the metabolic rate and energy requirements of mustelids are elevated (10–12). The mink is dependent on continuous food availability and its adaptations to fasting are relatively inferior (3, 13). It can be hypothesized that its response to fasting must be rapid, containing both hormonal signals initiating energy preservation (e.g., the thyroid and gonadal axes), and signals stimulating foraging (e.g., low leptin levels). The mink has a great capacity for fat storage, as evidenced by the high body fat content (35–38%) of the experimental animals of the present study (3). Different fat depots function as energy sources, and sc fat also provides thermal insulation for the semiaquatic lifestyle. Farm-bred minks seem to have a limited capacity for lipid mobilization during fasting (3), which may be reflected in the hormonal milieu of the animals.

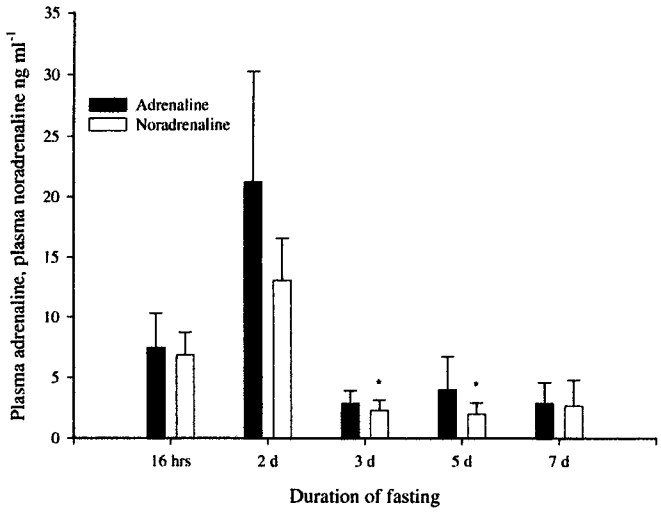

Figure 3. Effects of fasting on the plasma adrenaline and noradrenaline concentrations (ng/ml) of the male minks (mean + SE). *, significant difference from the control group that was fasted for 16 hrs ($P < 0.05$).

The plasma leptin concentrations of the minks decreased by 35% after 48 hrs of fasting. This fasting-induced decrease in circulating leptin levels has been previously observed in several nonseasonal (14) and seasonal (15) mammals. However, the leptin concentrations of some canids and pinnipeds have not always responded to food deprivation (16–18). The acute decrease in blood leptin levels of the mink is probably a signal initiating the endocrine response to fasting. For instance, the circulating leptin levels of fasting laboratory rodents decrease rapidly, together with their sex steroid and thyroid hormone levels, whereas stress hormone concentrations and the synthesis of the orexigenic neuropeptide Y in the hypothalamus increase (19). Exogenous leptin can blunt these changes and it can also reverse the immunosuppression induced by fasting (20).

From the evolutionary perspective, the ability to adapt to fasting is of fundamental importance for survival (21). During nutritional scarcity, it is not reasonable to waste energy on reproduction, growth, thermogenesis, or immune defense. Thus, the minks of this study could have saved energy by decreasing their plasma sex steroid concentrations, T_3 levels (see also Ref. 5), and blood monocyte counts (3). The decreasing plasma leptin level could have been the signal triggering these adaptations, supported by the positive correlation between the leptin levels and the T_3/T_4 ratios, and white blood cell, lymphocyte, and monocyte counts (3).

Furthermore, the falling leptin levels remove the satiety signal function of this peptide and can lead to increased foraging behavior. Leptin also promotes hepatic gluconeogenesis (22) and skeletal muscle glycogen synthesis (23). Thus, the decreases in the liver glucose-6-phosphatase activities and in the muscle glycogen concentrations observed in minks after 48 hrs of fasting (3) may be related to the low leptin levels. This is also supported by the positive correlation between the leptin levels and the hepatic glucose-6-phosphatase activities.

The plasma leptin concentrations have been shown to correlate positively with the BM of the mink (24, 25). During weight gain and gestation, the leptin levels increase whereas restricted feeding and lactation cause a decrease in leptin concentrations (24–26). In the present study, the plasma leptin concentrations of the minks decreased after 48 hrs of fasting, whereas the weights of their different fat depots had not yet decreased significantly (3). It has also been observed in other mammals (15) that the fasting-induced decrease in leptin levels is not directly proportional to the amount of lost fat. However, the leptin levels of the fasted minks remained 35–53% lower than the levels of the control animals and correlated positively with the weights of the sc and iab depots as well as with the body fat percentage (3). These results indicate that the plasma leptin level can function as an indicator of the body-fat content of the mink, although a positive correlation cannot be always demonstrated between leptin concentrations and the BM index of the species (27). In this study, the strongest correlations were observed between the leptin levels and the mesenteric and retroperitoneal fat masses. Because of the minor importance of iab fat in thermal insulation, these fat depots seem to be more readily mobilized during fasting. This could mean that the amount of iab fat would be an important contributor to the plasma leptin concentrations and regulate satiety more than the superficial fat depots. Because more than 80% of the total fat mass of the mink is sc fat, fasting did not change the overall body fat percentage of the animals, although it decreased the amounts of specific iab fat depots.

Peptide YY is a gastrointestinal peptide released by ingestion of a meal, by intraduodenal administration of fat and carbohydrates, and by intracolonic administration of fat, amino acids, and proteins (28). Previously, PYY levels of humans have decreased during a 3-day fast (29). On the contrary, 5.7-fold to 8.3-fold higher PYY concentrations were observed in the plasma of the minks that had fasted for 3–7 days than in their controls. Elevated plasma PYY concentrations have been observed in the blue (arctic) fox (*Alopex lagopus*) after 15 days of fasting (30). PYY can suppress gastric acid secretion, intestinal motility, blood flow in the gastrointestinal tract, as well as the exocrine and endocrine secretions of the pancreas (31–34). For this reason, the higher release rate of PYY in fasted carnivores could downregulate these processes during fasting and direct physiologic and behavioral processes to only those crucial for survival. In fact, PYY has orexigenic effects (35), and its levels may increase in fasting animals to stimulate food intake together with the low leptin concentrations.

In contrast to humans (29), the plasma NA concentrations of the minks decreased during Days 3–5 of food deprivation. This was unexpected, because food withdrawal could have been expected to increase the levels of plasma catecholamines, which stimulate fat mobilization (36). In fact, slight rises were observed in the NA and A concentrations after 48 hrs of fasting, but they did not reach significance. It can, however, be suggested that these increases were a part of the acute fasting-induced stress-response in the species. The following decrease in the NA concentrations, on the other hand, may be related to decreased stimulation of the sympathetic nervous system and, thus, to energy saving during fasting.

Peptide YY is known to have a suppressive effect on plasma insulin concentrations (33), which decreased by 27–42% during Days 5–7 of fasting. A similar fasting-induced decrease in circulating insulin levels has been previously reported in several canids (37–39). It has been also observed that negative energy balance has a suppressive effect on insulin concentrations of the female mink (5). The low insulin levels were observed simultaneously with decreases in the liver glycogen concentrations and glycogen phosphorylase activities and with the increases in plasma triacylglycerol, FFA, and glycerol concentrations (3), suggesting a role for insulin in glucose sparing and fat mobilization (40). Insulin may also participate in the control of factors such as gluconeogenesis, ketogenesis (41), and metabolic rate (42) during food deprivation.

A 30% increase and a 71% decrease were observed in the plasma glucagon and GH concentrations of the minks after 5 days of fasting. Glucagon is able to stimulate glycogenolysis, gluconeogenesis (43), lipolysis (44), and ketogenesis (45). The liver glycogen concentrations of the minks did not decrease significantly until the fifth day of fasting (3), and it is possible that the increase in the glucagon levels could have eventually stimulated the degradation of liver glycogen stores. Although the liver glucose-6-phosphatase activities of the minks were low during the entire fasting period, the minks that were fasted for 5 days had significantly higher plasma glucose concentrations than the animals that were fasted for 3 days, which may be also connected to the increased glucagon concentrations. Moreover, the increases in the plasma triacylglycerol levels after 5 days of fasting and the positive correlation between the glucagon and FFA concentrations during the entire study period could indicate that glucagon plays an important role in the adaptation to fasting in the mink.

Energy stores cannot be wasted for growth during nutritional scarcity, and because of this, it is reasonable to decrease the release of GH (46). However, GH also promotes gluconeogenesis (47) and lipolysis (48), for instance, and GH levels increase during food deprivation

in some species (49). According to the results of the present study, the mink may not exhibit stimulated GH secretion during fasting. Because GH is secreted in a pulsatile fashion and the animals of the present study were sampled only once, the differences in the GH levels between the study groups could have been caused by the diurnal secretion rhythm of GH as well. In laboratory rodents, the fasting-induced decrease in leptin concentrations is responsible for the simultaneous suppression of GH secretion (46). Consequently, exogenous leptin is able to reverse the decrease in GH release. Because the decreases in the leptin and GH concentrations were not simultaneous in the present study, and because no positive correlation was observed between these variables, the transient drop in the GH levels after 5 days of fasting was not necessarily induced by low leptin levels.

The only novel endocrinologic change recorded first after 7 days of fasting was the 63% decrease in the plasma C concentrations. Because C has lipolytic activity (50) and the lipase activities in the sc and retroperitoneal fat depots of the minks decreased simultaneously with the decrease in the C concentrations (3), C may play a role in the regulation of fat metabolism in the fasting mink. Between Days 3 and 5 of fasting, several variables related to protein catabolism, that is, plasma urea, ammonia, uric acid, and total protein levels, were elevated in the fasting minks, however, most of them normalized after 7 days of fasting (13). Because C stimulates proteolysis (51), it can be speculated that the lowered plasma C concentrations may be associated with limited protein catabolism at this point of fasting. It is possible that as fasting continues, the mink preferably reduces lipolytic activity and even enters proteolysis to ensure adequate sc insulation to enable aquatic predation.

Fasting did not affect the concentrations of ghrelin, Acrp30, resistin, T_4, E, or A in the minks. Previously, circulating ghrelin levels have increased in nonseasonal (52) and seasonal rodents (53) during fasting. High ghrelin levels stimulate the appetite (52) and, thus, increase the chances to survive through nutritional scarcity. The ghrelin levels of the minks correlated negatively with the lipase activities measured from sc fat (3), which fits well with the data of ghrelin decreasing fat use in rodents (52). The Acrp30 concentrations of the minks did not change during fasting (see also Ref. 54 for humans) but they correlated positively with the liver lipase activities and inversely with the masses of different iab fat depots and with the body fat percentage (3). This confirms previous human data with a reduction in BM index increasing the plasma Acrp30 levels (55). In contrast to rodents (56), but similar to humans (57), the plasma resistin levels of the minks did not decrease because of the 7-day fast.

It has been previously documented in the mink that a negative energy balance can decrease its blood T_4 concentrations (5), although the T_4 levels of the minks in the present study remained unaltered during the 7 days of fasting. T_4 can be considered a prohormone for T_3 production (36). For this reason, the T_3 levels may be more responsive to nutritional status than the T_4 concentrations. Although the plasma T and P levels of the male minks decreased because of fasting, the plasma E levels remained at the level of the control males. Testosterone is usually synthesized from cholesterol via pregnenolone, dehydroepiandrosterone, and androstenedione (36). However, because both the T and P concentrations of the minks decreased during fasting, it is possible that the testosterone production of the mink occurs partly through P. Down-regulation of P synthesis can also explain the decrease in the plasma C concentrations at the end of the fast, because P can function as a precursor for C (36). Nutritional scarcity prevents reproductive processes of many animals, and, thus, it is reasonable that fasting had already decreased the T levels of the male minks after 48 hrs of food deprivation.

The response to fasting is rapid in the mink, and the species seems to have the capacity to cope with short-term food deprivation. The mink is, for instance, able to maintain its plasma glucose at constant levels for 7 days without food (3). In contrast to seasonal carnivores with a higher BM and lower energy requirements, the mink is more dependent on constant food availability (3). Fasting procedures have no practical use on mink farms because of the modest decreases in body fat stores and the fasting-induced liver dysfunction. Restricted feeding is presumably a safer and a more efficient way to diet farm-bred minks during the winter. The endocrine response to fasting seems to be remarkably constant in carnivores, rodents, and primates with particular species-specific peculiarities indicating that the adaptations to cope with seasonal food scarcity are of fundamental importance for survival in nature and evolutionarily well conserved.

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