

Response to Fasting in an Unnaturally Obese Carnivore, the Captive European Polecat *Mustela putorius*

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The European polecat (*Mustela putorius*) is a naturally lean carnivore prone to excessive weight gain in captivity. This study assessed its suitability to investigate the natural history of the obese phenotype displayed in overweight humans, domestic animals, and seasonally obese wild mammals. Ten farm-bred polecats were subjected to a 5-day fast with 10 controls. Obesity (40% body fat) was associated with an unfavorable plasma lipid profile and high glucose and insulin concentrations. The polecats were in phase II of fasting with normoglycemia, low liver carbohydrate stores, and decreased plasma concentrations of urea and most amino acids. Although the plasma nonesterified fatty acid (NEFA) levels were elevated, the adipose tissue lipase activities suggested a blunted lipolytic response. Lipid mobilization was more efficient from intraabdominal fat. The animals developed hepatic lipidosis with elevated NEFA influx into the liver and losses of n-3 polyunsaturated fatty acids and arginine as hypothetical etiological factors. The plasma leptin, insulin, and triiodothyronine levels decreased but were not accompanied by reduced sex steroid or increased stress hormone concentrations. The blunted lipolytic response often encountered in obesity suggests that the organism is trying to defend the obese phenotype. Liver lipidosis and decreased insulin and triiodothyronine levels seem to be among the most

consistent responses to fasting manifested in diverse mammalian orders and different levels of body fatness. The polecat could be recommended as an easily accessible carnivore model to study the natural history of the obese phenotype and its comorbidities. Exp Biol Med 234:1287–1295, 2009

Key words: endocrine response to fasting; hepatic lipidosis; *Mustela putorius*; unnatural obesity

Introduction

The chronic imbalance between calorie intake and energy expenditure resulting in unnatural, often pathological obesity has become a major public health problem in Western and Asian countries as a primary cause of several comorbidities, such as type 2 diabetes, dyslipidemia, hepatic steatosis, hypertension, cardiovascular diseases, and cancers (1). Calorie restriction is the most apparent means for weight reduction, but dieting often leads to weight cycling with potentially deleterious health effects (2). The physiological responses to total fasting were previously compared between lean and obese humans and rodents (3–5). Naturally obese marine mammals experiencing regular seasonal fluctuations of body adiposity were also interesting study subjects, as fasting associated with molting, breeding, lactation, and/or weaning of pups is a part of their natural history (6, 7). In contrast, only a few studies were conducted in naturally lean carnivores not adapted to long-term fasting (8–11).

The fat storage capacity is limited in small members of the Mustelidae family (*Mustela erminea*, *M. nivalis*), as they benefit from a slim body shape to be able to hunt small mammalian prey in their burrows (12). Larger mustelids have longer fur and a more energetically advantageous body

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surface-to-volume ratio compared to smaller species. The body fat content of the American marten (*Martes americana*) averages <6% (13), but data are scanty for wild specimens of related species (14). This natural leanness can be lost in captivity, resembling the sedentary lifestyle of a significant part of the general population with overabundance of high-fat and high-carbohydrate foods and restricted exercise. High body fatness (20–38%) was documented in the farmed American mink (*Neovison vison*) (8, 11), and it can be assumed that fat reserves would be smaller in nature in comparison to those in mustelids raised on farms or kept as companion animals.

The ferret (*Mustela putorius furo*), domesticated either from the European polecat (*M. putorius*) or the steppe polecat (*M. eversmanni*), is widely used as a non-rodent experimental model in biomedicine, for instance, in the fields of respiratory, cardiovascular, and gastrointestinal diseases, carotenoid metabolism, and pharmaceutical drug development (15). Ferrets have become popular companion animals, and it was estimated that there might be several million pet ferrets in the USA. For this reason, improved knowledge on the nutritional and metabolic adaptations of the species is necessary. The farmed European polecat was chosen for the present study as a readily accessible predator that is thin in nature but can develop excessive obesity in captivity. The objectives were to evaluate the effects of total fasting in this unnaturally obese carnivore and to compare the data to previous results on lean and naturally obese mammals and overweight humans. The ultimate goal was to assess the suitability of the polecat in studying the natural history of the obese phenotype displayed (i) during seasonal body fat accumulation in wild mammals and (ii) in unnaturally obese domestic animals and humans with associated pathological conditions.

Materials and Methods

The experiment was approved by the Animal Care and Use Committee of the University of Joensuu. Twenty farmed European polecats (12 males, 8 females) born in spring 2005 were randomly assigned into two experimental groups. The animals were housed in male-female or male-male pairs in a fur farm (62.559945 N, 29.125737 E) in cages under a roof at natural temperature and photoperiod and fed with a commercial diet (Super-Fox Kasvu rae, 33% protein, 24% fat, 27–30% carbohydrates, 16 MJ metabolizable energy/kg dry weight, Suomen Rehu Oy, Espoo, Finland) supplemented with moose (*Alces alces*) carcass offal (20%). On Nov 9th, 2005, the polecats were anesthetized with intramuscular ketamine (20 mg/kg) and xylazine (2 mg/kg) and sterile thermosensitive data loggers (iButton Thermochron, model DS1921H, Maxim Integrated Products, Sunnyvale, CA) were implanted into their abdominal cavities, as described previously (11). The loggers registered the core body temperature (T_b) at 60-min intervals, yielding 122 data points/animal during the

food deprivation experiment. A thermosensitive logger (iButton Thermochron, model DS1921G) registered the ambient temperature at the study site simultaneously ($-6.1 \pm 0.5^\circ\text{C}$).

On Jan 27th, 2006, the food-deprived group ($n = 10$) was subjected to a 5-day fast while water or snow was available *ad lib*. Body masses (BM) were recorded on day 1 of the experiment and at sampling. On Feb 1st, 2006, the animals were anesthetized as described above. Blood samples were obtained using cardiac punctures and the animals were euthanized with intracardial T 61 (Intervet Oy, Espoo). The fed group was food-deprived overnight (8, 10). One ml of whole blood with EDTA was refrigerated for the complete blood count (8, 10). The rest was centrifuged at $2000 \times g$ for 15 min, and the plasma was removed and stored at -80°C . The liver, kidneys, spleen, testes/ovaries and uterus, adrenals, thyroid glands, cardiac ventricles, and visible subcutaneous [sc, interscapular, rump, ventral], intraabdominal [iab, omental, mesenteric (mes), retroperitoneal (rp), diaphragmatic (dia)], and pericardial (pc) white adipose tissues (WAT) were dissected and weighed and muscle samples were obtained from the left thigh. Intermuscular fat samples were dissected from the fat pads situated between the flank muscle layers. The liver, WAT, muscle, and kidney samples were stored at -80°C . The tissue glucose-6-phosphatase, glycogen phosphorylase, and lipase activities as well as glycogen, total protein, triacylglycerol (TAG), and cholesterol (Chol) concentrations were measured with existing methods (8, 9), with some modifications. The lipase method is nonspecific, measuring the breakdown of ester bonds and the overall lipolytic activity within a tissue.

The plasma clinical-chemical variables and amino acid (AA) concentrations were assayed as described previously (8–10). The reagents used to measure the plasma total antioxidant status were purchased from Randox Laboratories Ltd (Crumlin, UK). The plasma leptin, insulin, adiponectin, growth hormone, cortisol, thyroxine (T_4), testosterone, estradiol, progesterone, and catecholamine concentrations were assessed with established methods (16). The triiodothyronine (T_3) and glucagon levels were measured with the Coat-A-Count Total T_3 and Double Antibody Glucagon kits of Siemens Medical Solutions Diagnostics (Los Angeles, CA). The ghrelin concentrations were determined with the Ghrelin (Rat, Mouse) RIA kit (Phoenix Pharmaceuticals, Burlingame, CA). All radioimmunoassays were validated such that serial dilutions of the polecat plasma showed linear changes in the sample binding/maximum binding values that were parallel with the standard binding/maximum binding curves produced with the standards of the manufacturers (data not shown). The possibility of insulin resistance was evaluated from the plasma insulin and glucose concentrations by the homeostasis model assessment (HOMA) index developed for humans (17) with the formula: insulin resistance = fasting insulin ($\mu\text{U/ml}$) $\times$ fasting glucose (mmol/l)/22.5.

Table 1. Effects of 5 Days of Food Deprivation on the Body Mass Loss, Absolute Organ Weights, and Body Temperature of the European Polecats (Mean $\pm$ SE)

	Fed	Food-deprived
Initial BM, kg males	2.0 $\pm$ 0.10	2.2 $\pm$ 0.11
Initial BM, kg females	1.3 $\pm$ 0.04	1.2 $\pm$ 0.03
Final BM, kg males	2.1 $\pm$ 0.10	1.9 $\pm$ 0.10
Final BM, kg females	1.3 $\pm$ 0.03*	1.1 $\pm$ 0.03*
Δ BM, g males	60 $\pm$ 9*	-280 $\pm$ 16*
Δ BM, g females	-5 $\pm$ 10*	-143 $\pm$ 9*
Liver mass, g	56.9 $\pm$ 3.93	45.6 $\pm$ 4.39
Kidneys mass, g	7.6 $\pm$ 0.60	6.1 $\pm$ 0.57
Spleen mass, g	10.2 $\pm$ 0.79	9.6 $\pm$ 1.45
Heart ventricles mass, g	5.0 $\pm$ 0.34	4.5 $\pm$ 0.33
Adrenals mass, mg	135 $\pm$ 20	153 $\pm$ 13
Thyroids mass, mg	51.1 $\pm$ 2.71	50.4 $\pm$ 5.47
Testes mass, g	4.2 $\pm$ 0.15	4.2 $\pm$ 0.19
Ovaries mass, mg	115 $\pm$ 14	93 $\pm$ 8
Uterus mass, mg	236 $\pm$ 15*	156 $\pm$ 15*
Mean 24-h T_b , °C	37.4 $\pm$ 0.04	37.4 $\pm$ 0.06
Min of 24-h T_b , °C	36.3 $\pm$ 0.07	36.4 $\pm$ 0.11
Max of 24-h T_b , °C	38.4 $\pm$ 0.04	38.4 $\pm$ 0.04
Range of 24-h T_b , °C	2.1 $\pm$ 0.05	2.0 $\pm$ 0.13

BM, body mass; T_b , body temperature.

* Significant difference between the fed and food-deprived animals ($P < 0.05$).

The average 24-h T_b was calculated for each date of each individual and the amplitude spectra were calculated with the Fast Fourier Transform using the Blackman window. Comparisons between the experimental groups were performed with the Student's t test for independent samples or, for nonparametric data, with the Mann-Whitney U test (SPSS v13.0 software package, SPSS Inc, Chicago, IL). Correlations were calculated using the Spearman correlation coefficient. $P < 0.05$ was considered to be statistically significant. The results are presented as mean $\pm$ SE.

Results

The rate of BM loss was $12.8 \pm 0.31\%$ ($2.6 \pm 0.06\%/d$) for the male polecats and $11.6 \pm 0.69\%$ ($2.3 \pm 0.14\%/d$) for the females (Table 1). The relative liver, kidneys, and uterine masses were lower in the food-deprived polecats, but the relative mass of the adrenals was higher. In the males, the relative mass of the thyroid glands and the absolute mass of pc fat decreased and, in the females, the masses of dia, mes, rp, total iab fat, and total fat decreased due to food deprivation (Table 2). The iab fat depots were mobilized more effectively than sc fat (-19 to -25% vs. -8 to -13%). The mean, minimum, or maximum 24-h T_b , T_b spectra, or the amplitude and range of T_b oscillations did not respond to fasting (Table 1).

Food deprivation decreased the liver glycogen concentrations but increased the hepatic TAG and Chol levels (Tables 2 and 3). The lipase activities increased in the liver and kidneys, while the opposite was observed for the ventral

and dia WAT (Table 2). The white blood cell (lymphocyte, monocyte, and granulocyte) counts decreased due to food deprivation, but the red blood cell count increased (Table 4). The plasma total Chol, high-density lipoprotein Chol, and total protein concentrations and the activities of creatine kinase and MB-fraction of creatine kinase decreased, while the nonesterified fatty acid (NEFA) concentrations increased (Table 5).

The ammonia, arginine (Arg), asparagine, carnosine, citrulline (Cit), cystathionine, glutamate (Glu), glycine (Gly), histidine, isoleucine (Ile), leucine (Leu), lysine, methionine, 1-methylhistidine, proline (Pro), serine (Ser), taurine, threonine (Thr), urea, valine (Val), total AA, total essential AA, and total nonessential AA concentrations and the urea-creatinine ratio were lower in the fasted animals, while the α -aminobutyrate (α -AB), cystine, and 3-methylhistidine (3-MH) levels were higher (Tables 5 and 6). Food deprivation decreased the leptin, insulin, glucagon, and T_3 concentrations; the T_3 - T_4 ratio; and the HOMA index (Table 7). The plasma catecholamine values were mostly below the detection limit (data not shown).

Discussion

Recurrent obesity is beneficial in nature, as due to extensive fat depots, obese mammals can prolong phase II of fasting with stimulated fat oxidation and protein conservation, permitting extended survival during nutritional scarcity (5, 6). Due to finite food resources, wild animals practically never develop persistent obesity with comorbidities, but the situation becomes different under domestication. Thus, although the natural body condition of the European polecat is lean, it has the potential for extensive weight gain and obesity analogous to humans. The high fat-% (40%) of the farmed polecats probably results from excessive and prolonged positive energy balance (18) and limited physical activity (19, 20)—crucial aspects in human obesity, as well. Artificial selection for a large body size (21) may also be a causative factor. To the best of our knowledge, there exist no published data on the body adiposity of wild polecats, but it can be assumed that they would be considerably thinner. In fact, the estimated fat-% of 2 wild-caught males was $9.8\% \pm 6.5\%$ in winter (Mustonen et al. unpubl. data).

There are well-recognized associations of human obesity with an unfavorable plasma lipid profile and elevated transaminase and C-reactive protein levels (22, 23). Although the polecat has a different plasma lipoprotein profile and composition compared to humans (24), there were similar correlations of the plasma total and low-density lipoprotein Chol levels with the body fat-%. Overweight is also often associated with high rates of fat accumulation and lipolysis (22, 25, 26) and can be promoted further by decreased physical activity (27) and suppression of postprandial thermogenesis (28). Based on the data on nonobese (29) and overweight (30) humans and on lean (31) and

Table 2. Effects of 5 Days of Food Deprivation on the Fat Masses, Tissue Lipase Activities and Liver Triacylglycerol and Cholesterol Concentrations of the European Polecats (Mean $\pm$ SE)

		Fed	Food-deprived
Sc fat, g†	M	513 $\pm$ 37	474 $\pm$ 31
	F	340 $\pm$ 15	298 $\pm$ 25
Om fat, g	M	48 $\pm$ 3	40 $\pm$ 3
	F	39 $\pm$ 6	31 $\pm$ 3
Rp fat, g†	M	76 $\pm$ 8	61 $\pm$ 4
	F	55 $\pm$ 1*	41 $\pm$ 2*
Mes fat, g†	M	136 $\pm$ 14	108 $\pm$ 8
	F	80 $\pm$ 6*	59 $\pm$ 4*
Dia fat, g†	M	14 $\pm$ 1	12 $\pm$ 1
	F	10 $\pm$ 1*	7 $\pm$ 1*
Total iab fat, g†	M	274 $\pm$ 25	221 $\pm$ 16
	F	184 $\pm$ 11*	138 $\pm$ 7*
Pc fat, g	M	7 $\pm$ 1*	4 $\pm$ 1*
	F	9 $\pm$ 3	5 $\pm$ 1
Total fat, g†	M	794 $\pm$ 56	699 $\pm$ 44
	F	533 $\pm$ 23*	441 $\pm$ 27*
Body fat, % BM†	M	37.8 $\pm$ 1.20	36.6 $\pm$ 0.83
	F	41.7 $\pm$ 1.04	40.5 $\pm$ 1.53
Sc interscapular lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		1.6 $\pm$ 0.22	1.7 $\pm$ 0.40
Sc rump lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		4.4 $\pm$ 0.65	3.1 $\pm$ 0.24
Sc ventral lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		2.4 $\pm$ 0.21*	1.4 $\pm$ 0.18*
Om lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		2.6 $\pm$ 0.29	4.0 $\pm$ 1.59
Rp lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		4.0 $\pm$ 0.68	3.6 $\pm$ 0.41
Mes lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		4.1 $\pm$ 0.54	3.7 $\pm$ 0.68
Dia lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		2.8 $\pm$ 0.27*	1.4 $\pm$ 0.19*
Intermuscular lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		5.5 $\pm$ 0.59	4.1 $\pm$ 1.02
Liver lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		75.8 $\pm$ 3.84*	87.0 $\pm$ 1.91*
Kidney lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		11.9 $\pm$ 0.62*	14.8 $\pm$ 1.00*
Muscle lipase, μg 2-naphthol $\text{mg}^{-1} \text{h}^{-1}$		3.9 $\pm$ 0.20	4.2 $\pm$ 0.17
Liver triacylglycerols, mg g^{-1}		11.6 $\pm$ 1.47*	28.9 $\pm$ 2.87*
Liver cholesterol, mg g^{-1}		2.6 $\pm$ 0.49*	7.6 $\pm$ 2.56*

sc, subcutaneous; om, omental; rp, retroperitoneal; mes, mesenteric; dia, diaphragmatic; iab, intraabdominal; pc, pericardial; BM, body mass; M, males; F, females.

* Significant difference between the fed and food-deprived animals or † between the sexes ($P < 0.05$).

obese (8) mustelids, iab fat depots are mobilized more effectively than sc fat, which may be due to the higher responsiveness of lipid metabolism-related genes to calorie restriction in visceral fat (32). This is probably not valid in

all species, as there are also reports on lean (10) and obese (33) carnivores with higher rates of hydrolysis in sc fat.

In obesity, the utilization of fat and the clearance of fatty acids can be more pronounced (25, 34) and the rise in

Table 3. Effects of 5 Days of Fasting on the Tissue Glycogen and Protein Concentrations and Enzyme Activities of the European Polecats (Mean $\pm$ SE)

		Fed	Food-deprived
L	Glycogen, $\mu\text{g mg}^{-1}$	19.6 $\pm$ 2.68*	5.1 $\pm$ 1.07*
L	Phosphorylase, $\mu\text{g P mg}^{-1} \text{h}^{-1}$	22.9 $\pm$ 0.89	20.2 $\pm$ 1.49
L	Glucose-6-phosphatase, $\mu\text{g P mg}^{-1} \text{h}^{-1}$	63.5 $\pm$ 4.27	58.7 $\pm$ 2.97
L	Protein, $\mu\text{g mg}^{-1}$	169 $\pm$ 5	171 $\pm$ 6
K	Glycogen, $\mu\text{g mg}^{-1}$	0.6 $\pm$ 0.03	0.7 $\pm$ 0.06
K	Phosphorylase, $\mu\text{g P mg}^{-1} \text{h}^{-1}$	4.8 $\pm$ 0.37	4.6 $\pm$ 0.26
K	Glucose-6-phosphatase, $\mu\text{g P mg}^{-1} \text{h}^{-1}$	58.3 $\pm$ 5.70	57.4 $\pm$ 2.38
K	Protein, $\mu\text{g mg}^{-1}$	134 $\pm$ 7	146 $\pm$ 5
M	Glycogen, $\mu\text{g mg}^{-1}$	4.0 $\pm$ 0.50	3.0 $\pm$ 0.44
M	Phosphorylase, $\mu\text{g P mg}^{-1} \text{h}^{-1}$	141.8 $\pm$ 8.17	135.0 $\pm$ 6.41
M	Protein, $\mu\text{g mg}^{-1}$	187 $\pm$ 12	181 $\pm$ 8

L, liver; K, kidney; M, muscle.

* Significant difference between the fed and food-deprived animals ($P < 0.05$).

Table 4. Effects of 5 Days of Food Deprivation on the Complete Blood Count of the European Polecats (Mean $\pm$ SE)

	Fed	Food-deprived
WBC, 10^3 (mm ³) ⁻¹	7.1 $\pm$ 0.51*	4.0 $\pm$ 0.39*
RBC, 10^6 (mm ³) ⁻¹	8.6 $\pm$ 0.14*	9.5 $\pm$ 0.10*
HGB, g l ⁻¹	142 $\pm$ 6	148 $\pm$ 2
HCT, %	42 $\pm$ 2	44 $\pm$ 1
PLT, 10^3 (mm ³) ⁻¹	538 $\pm$ 21	547 $\pm$ 37
MCV, μ m ³	46.2 $\pm$ 0.29	45.9 $\pm$ 0.26
MCH, pg	15.7 $\pm$ 0.14	15.4 $\pm$ 0.10
MCHC, g dl ⁻¹	33.8 $\pm$ 0.15	33.6 $\pm$ 0.10
RDW, %	15.9 $\pm$ 0.21	15.7 $\pm$ 0.22
MPV, μ m ³	11.8 $\pm$ 0.38	11.9 $\pm$ 0.22
LYM, 10^3 (mm ³) ⁻¹	1.4 $\pm$ 0.13*	0.8 $\pm$ 0.12*
MON, 10^3 (mm ³) ⁻¹	0.4 $\pm$ 0.04*	0.2 $\pm$ 0.02*
GRA, 10^3 (mm ³) ⁻¹	5.3 $\pm$ 0.44*	3.0 $\pm$ 0.34*
LYM, %	20.5 $\pm$ 1.36	21.9 $\pm$ 2.30
MON, %	5.9 $\pm$ 0.37	5.8 $\pm$ 0.41
GRA, %	73.6 $\pm$ 1.71	72.3 $\pm$ 2.66

WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; PLT, platelets; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; MPV, mean platelet volume; LYM, lymphocytes; MON, monocytes; GRA, granulocytes.

* Significant difference between the fed and food-deprived animals ($P < 0.05$).

Table 5. Effects of 5 Days of Food Deprivation on the Plasma Clinical Chemistry of the European Polecats (Mean $\pm$ SE)

	Fed	Food-deprived
Glucose, mmol l ⁻¹	10.4 $\pm$ 1.34	8.2 $\pm$ 0.58
Triacylglycerols, mmol l ⁻¹	1.1 $\pm$ 0.25	1.1 $\pm$ 0.11
Nonesterified fatty acids, mmol l ⁻¹	0.3 $\pm$ 0.04*	0.5 $\pm$ 0.07*
Glycerol, μ mol l ⁻¹	377 $\pm$ 138	215 $\pm$ 25
Total cholesterol, mmol l ⁻¹	3.5 $\pm$ 0.16*	2.9 $\pm$ 0.12*
HDL-cholesterol, mmol l ⁻¹	2.8 $\pm$ 0.18*	2.3 $\pm$ 0.11*
LDL-cholesterol, mmol l ⁻¹	0.5 $\pm$ 0.06	0.4 $\pm$ 0.04
Urea, mmol l ⁻¹	7.7 $\pm$ 0.72*	4.8 $\pm$ 0.41*
Ammonia, μ mol l ⁻¹	164 $\pm$ 18*	106 $\pm$ 6*
Uric acid, μ mol l ⁻¹	51.5 $\pm$ 10.53	55.3 $\pm$ 5.25
Total protein, g l ⁻¹	62.4 $\pm$ 1.11*	58.3 $\pm$ 1.34*
Bilirubin, μ mol l ⁻¹	6.2 $\pm$ 1.33	5.0 $\pm$ 0.37
ALT, U l ⁻¹	115 $\pm$ 18	133 $\pm$ 22
AST, U l ⁻¹	143 $\pm$ 14	146 $\pm$ 17
CK, U l ⁻¹	271 $\pm$ 65*	110 $\pm$ 11*
MB-fraction of CK, U l ⁻¹	365 $\pm$ 86*	117 $\pm$ 29*
Creatinine, μ mol l ⁻¹	61.5 $\pm$ 9.90	68.1 $\pm$ 4.89
Urea-creatinine ratio	121 $\pm$ 12*	77 $\pm$ 11*
Total antioxidant status, mmol l ⁻¹	1.5 $\pm$ 0.07	1.4 $\pm$ 0.04

HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase.

* Significant difference between the fed and food-deprived animals ($P < 0.05$).

Table 6. Effects of 5 Days of Food Deprivation on the Plasma Nitrogenous Compounds (μ mol l⁻¹) of the European Polecats (Mean $\pm$ SE)

	Fed	Food-deprived
Alanine	261 $\pm$ 24	239 $\pm$ 11
α -Aminobutyrate	22.0 $\pm$ 2.73*	32.0 $\pm$ 0.82*
Arginine	135 $\pm$ 8*	76 $\pm$ 7*
Asparagine	39.4 $\pm$ 1.29*	29.7 $\pm$ 1.27*
Aspartate	7.7 $\pm$ 0.87	6.5 $\pm$ 0.31
Carnosine	37.3 $\pm$ 3.48*	22.8 $\pm$ 1.23*
Citrulline	33.3 $\pm$ 3.03*	8.7 $\pm$ 0.97*
Cystathionine	6.2 $\pm$ 0.42*	4.8 $\pm$ 0.40*
Cystine	10.2 $\pm$ 0.89*	15.8 $\pm$ 2.08*
Glutamate	71.7 $\pm$ 7.12*	49.6 $\pm$ 2.56*
Glutamine	719 $\pm$ 13	703 $\pm$ 38
Glycine	240 $\pm$ 11*	169 $\pm$ 8*
Histidine	120 $\pm$ 4*	87 $\pm$ 2*
Isoleucine	80.6 $\pm$ 7.31*	52.6 $\pm$ 2.80*
Leucine	131 $\pm$ 13*	72 $\pm$ 3*
Lysine	164 $\pm$ 10*	125 $\pm$ 6*
Methionine	53.6 $\pm$ 4.46*	36.0 $\pm$ 1.12*
1-Methylhistidine	11.2 $\pm$ 0.44*	7.6 $\pm$ 0.72*
3-Methylhistidine	10.3 $\pm$ 0.93*	24.3 $\pm$ 2.72*
Ornithine	14.8 $\pm$ 4.67	17.4 $\pm$ 6.08
Phenylalanine	60.8 $\pm$ 4.50	53.2 $\pm$ 2.30
Phosphoserine	6.4 $\pm$ 1.22	4.0 $\pm$ 0.85
Proline	107 $\pm$ 26*	24 $\pm$ 2*
Serine	127 $\pm$ 8*	83 $\pm$ 3*
Taurine	389 $\pm$ 83*	248 $\pm$ 14*
Threonine	170 $\pm$ 7*	110 $\pm$ 5*
Tyrosine	41.2 $\pm$ 2.88	35.0 $\pm$ 1.84
Valine	198 $\pm$ 16*	110 $\pm$ 4*
Essential AA	1502 $\pm$ 137*	969 $\pm$ 22*
Nonessential AA	1730 $\pm$ 64*	1451 $\pm$ 45*
Essential/nonessential AA	0.86 $\pm$ 0.05*	0.67 $\pm$ 0.02*
Total amino acids	3232 $\pm$ 188*	2420 $\pm$ 47*

AA, amino acid.

* Significant difference between the fed and food-deprived animals ($P < 0.05$).

NEFA during fasting less prominent (4, 5). In the polecats, the NEFA concentrations doubled after 5 days of food deprivation. Although the lipase activities were elevated in the livers and kidneys, they decreased in some fat tissues. A similar response was documented previously in the WAT lipase of obese carnivores (8, 33), but a stimulated response was noted in leaner species (10, 31). Lipolysis was also clearly stimulated in normal-weight humans during fasting, but the increase was blunted in obese individuals (35, 36). The impaired ability to downregulate the activity of WAT lipoprotein lipase (LPL) due to obesity (37) also suggests an attempt of the organism to preserve the obese phenotype. The responsiveness of lipases to fasting can also differ between unnaturally and naturally obese mammals, as LPL activity failed to decrease in the American mink after 12 hrs without food (38) but was suppressed in fasting ursids during winter sleep (39).

Human obesity and the metabolic syndrome are often associated with nonalcoholic fatty liver disease (NAFLD), a

Table 7. Effects of 5 Days of Food Deprivation on the Endocrinology of the European Polecats (Mean $\pm$ SE)

	Fed	Food-deprived
Leptin, ng ml ⁻¹	10.1 $\pm$ 1.68*	0.9 $\pm$ 0.20*
Adiponectin, μ g ml ⁻¹	3.6 $\pm$ 0.18	3.8 $\pm$ 0.15
Leptin-adiponectin ratio	2.7 $\pm$ 0.40*	0.3 $\pm$ 0.06*
Ghrelin, ng ml ⁻¹	1.2 $\pm$ 0.14	1.1 $\pm$ 0.10
Insulin, μ U ml ⁻¹	26.3 $\pm$ 9.42*	10.0 $\pm$ 1.05*
Glucagon, pg ml ⁻¹	40.2 $\pm$ 4.42*	22.0 $\pm$ 2.68*
Insulin-glucagon ratio	0.7 $\pm$ 0.23	0.6 $\pm$ 0.07
HOMA index	10.4 $\pm$ 2.79*	3.5 $\pm$ 0.23*
Growth hormone, ng ml ⁻¹	3.9 $\pm$ 1.22	8.1 $\pm$ 2.87
T ₄ , nmol l ⁻¹	16.9 $\pm$ 1.28	16.1 $\pm$ 1.77
T ₃ , nmol l ⁻¹	0.6 $\pm$ 0.06*	0.4 $\pm$ 0.03*
T ₃ -T ₄ ratio	0.04 $\pm$ 0.004*	0.03 $\pm$ 0.003*
Testosterone, nmol l ⁻¹ †	M 26.9 $\pm$ 7.35	37.4 $\pm$ 4.80
	F 0.7 $\pm$ 0.21	0.8 $\pm$ 0.51
Estradiol, pmol l ⁻¹	M 299 $\pm$ 22	234 $\pm$ 41
	F 365 $\pm$ 58	441 $\pm$ 198
Progesterone, nmol l ⁻¹ †	M 0.4 $\pm$ 0.03	0.4 $\pm$ 0.04
	F 0.6 $\pm$ 0.06	0.8 $\pm$ 0.19
Cortisol, nmol l ⁻¹	7.2 $\pm$ 2.19	10.5 $\pm$ 2.97

HOMA, homeostasis model assessment; T₄, thyroxine; T₃, triiodo-thyronine; M, males; F, females.

* Significant difference between the fed and food-deprived animals or † between the sexes ($P < 0.05$).

common liver abnormality also encountered, e.g., in starvation and after by-pass surgery (40). NAFLD is characterized by the loss of n-3 polyunsaturated fatty acids (PUFA) and increased n-6/n-3 PUFA ratio in the liver and WAT (41). Ferrets and polecats are susceptible to the development of fatty liver with similarities to NAFLD when fed a high-fat diet (42) or deprived of food (43). Common characteristics shared by NAFLD and polecat fatty liver are, for instance, micro- and macrovesicular steatosis, a decreased n-3 PUFA sum, and a derangement of the hepatic n-6/n-3 PUFA ratio (43). Also other carnivores (44) and, e.g., rodents (45) and ruminants (46) can be prone to liver lipidosis due to food deprivation. In addition to increased influx of plasma NEFA into the liver (40), losses of n-3 PUFA may promote fat accumulation and trigger an inflammatory response via overrepresentation of n-6 PUFA-derived eicosanoids (47). NAFLD is often characterized by insulin resistance, abnormal concentrations of obesity-related hormones, and increased plasma lipid concentrations and aminotransferase values (23, 41, 48), while only the plasma TAG level correlated with the degree of lipidosis in the polecats (43).

Plasma concentrations of glucose and insulin often increase in obesity (4, 22) and, as a result of peripheral insulin resistance, the responses of LPL and hormone-sensitive lipase to insulin action are postprandially suppressed in WAT (22). Also in the polecats, the plasma glucose and insulin levels correlated with the body fat-% and total fat mass. However, the animals were probably insulin sensitive during fasting as their HOMA indices

decreased—a common response to negative energy balance (49, 50), although there also exist reports demonstrating insulin resistance (51, 52). Naturally obese seals are interesting examples, as they maintain high circulating glucose concentrations and endogenous glucose production during natural fasts in a diabetes-like condition insensitive to the action of glucoregulatory hormones (7). Moreover, particular rodents show hyperinsulinemia and peripheral insulin resistance during fattening in preparation for hibernation (53). The polecats remained normoglycemic during fasting, which is a typical feature of carnivores (8, 54). This may be attributed to the high activities of gluconeogenic enzymes in mustelids (55). Although glucagon concentrations generally increase due to fasting (56), the polecats maintained normoglycemia without elevated glucagon levels. Stable or decreased glucagon concentrations during fasting were previously observed in naturally and unnaturally obese mammals (4, 5, 7, 57).

Overweight can be associated with high levels of protein synthesis and catabolism and utilization of alanine (Ala) for gluconeogenesis (34, 58). The circulating concentrations of Ala and some essential AA, particularly Ile, Leu, and Val, can be also elevated in obesity (3, 4, 58). In the polecats, the plasma Ala levels correlated with the body fat-%, too. Obese mammals are able to prolong phase II of fasting, as they have a lower contribution of protein oxidation to energy expenditure than leaner individuals (4, 5, 34, 59). Based on the variables of nitrogen metabolism, the polecats were in phase II of fasting after 5 days without food. Although protein breakdown characteristic of phase III was not stimulated, proteins were presumably catabolized to a lesser extent for gluconeogenesis in agreement with the observed normoglycemia, the increased 3-MH (9, 60), and decreased Arg concentrations (9, 61). The lower Arg level can be associated with proteolysis in carnivores and may predispose to hepatic lipidosis via increased synthesis of orotic acid (61). In accordance, the Arg concentrations correlated inversely with the liver TAG levels of the polecats.

Although the patterns of change in plasma concentrations of individual AA were similar between normal and obese humans during a 2-week fast (3), the responses of particular AA to fasting were diverse in previous studies, possibly depending on the species, initial body fat reserves, and duration and phase of fasting (3, 9, 10, 44, 57, 60, 62–67). However, in several cases the concentrations of Ala, Arg, Cit, and Pro decreased and those of α -AB, branched-chain AA (BC-AA), glutamine (Gln), and 3-MH increased. The polecats differed from this pattern in several regards. Their Ala concentrations remained stable, as observed previously for a lean mustelid (10). Ala is an important substrate for gluconeogenesis and its release from muscle and hepatic uptake generally decrease during phase II of fasting (58). Although circulating Ala concentrations decreased in obese subjects during food deprivation (5, 9, 44, 57, 67), increased levels were recorded in lean and obese

carnivores (60, 64). Neither Ala, Gln, nor glycerol levels responded to food deprivation in the polecats, while Glu, Gly, Ser, and Thr decreased in concentrations. Thus, further studies are required to elucidate the significance of different glucogenic substrates in mustelids.

Gln is a key player in ammoniogenesis, urea synthesis, and gluconeogenesis (68). Its circulating concentrations increased during food deprivation in fasting-adapted obese canids (57, 67) and in obese felids (44), but decreased or remained stable in lean and obese mustelids (9, 10) and obese rodents (4, 5). Particular AA supplied by protein breakdown in muscle can be converted into Gln that is released into the bloodstream (69), but increased plasma Gln levels are not necessarily derived from muscle, as the liver can become a net producer of Gln during fasting (70). It is possible that high Gln levels of hepatic origin could be required to limit muscle proteolysis during fasting and species incapable of this would depend more on muscle proteins for energy. The studied mustelid species with high protein requirements (71) and limited conservation of proteins in negative energy balance (9, 10) fit this model, but it is not supported by the decreased Gln levels in obese pinnipeds presumably not entering phase III of fasting under normal circumstances (6, 66). The decreased plasma BC-AA levels of the polecats could have resulted from the lack of protein intake (72). In this respect, they differed from the previously tested lean mustelids with stable or increased BC-AA levels (10, 64), but resembled obese rodents, canids, and juvenile pinnipeds (57, 64, 66).

Similar to previous studies (30), the weight reduction-induced decrease in the plasma leptin concentrations (−91%) was not proportional to the loss of fat mass (−15%). Leptin is considered a signal initiating the neuro-endocrine response to fasting and reduced leptin concentrations can be associated with increased stress hormone levels, decreased thyroid and sex hormone concentrations, and immunosuppression (73, 74). These responses would be energetically advantageous during nutritional scarcity. Low leptin concentrations can also stimulate appetite via neuropeptide Y and increase physical activity (73, 75), promoting foraging and increasing the probability of survival. The endocrinologic response of the polecat shared similarities with the American mink, another mustelid capable of developing excessive obesity (low leptin, stable ghrelin, ref. 16), but differed from lean mustelids (stable leptin, high ghrelin, refs. 10, 31). Food-deprived lean and obese mustelids both displayed a decreased lymphocyte count or % (8, 10, 31), not necessarily associated with the ability of leptin to stimulate lymphopoiesis (76). The unchanged T_b of the fasted polecats and previous data on the increased T_b and physical activity in other fasted mustelids (11) argue against a role for the thyroid gland in the induction of metabolic depression in smaller mustelids, as these species that are active year-round have evolved different physiological and behavioral strategies to survive food shortage compared to, e.g., species with carnivorean lethargy.

In conclusion, prolonged positive energy balance and restricted physical activity are the key factors leading to excessive obesity in captive polecats, similar to humans. The polecat shared several common features in addition to some peculiarities in its response to fasting with previously studied species displaying excessive obesity. It could be an easily accessible and useful carnivorean model to study the natural history of the obese phenotype and associated pathological conditions.

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