J Comp Physiol B (2007) 177:787–795
DOI 10.1007/s00360-007-0175-2

ORIGINAL PAPER

Fasting in the American marten (*Martes americana*): a physiological model of the adaptations of a lean-bodied animal

Petteri Nieminen · Kirsti Rouvinen-Watt · Seppo Saarela · Anne-Mari Mustonen

Received: 5 March 2007 / Revised: 28 May 2007 / Accepted: 30 May 2007 / Published online: 29 June 2007

Abstract The American marten (*Martes americana*) is a boreal forest marten with low body adiposity throughout the year. The aim of this study was to investigate the adaptations of this lean-bodied species to fasting for an ecologically relevant duration (48 h) by exposing eight farm-bred animals to total food deprivation with seven control animals. Selected morphological and hematological parameters, plasma and serum biochemistry, endocrinological variables and liver and white adipose tissue (WAT) enzyme activities were determined. After 48 h without food, the marten were within phase II of fasting with depleted liver and muscle glycogen stores, but with active lipid mobilization indicated by the high lipase activities in several WAT depots. The plasma ghrelin concentrations were higher due to food deprivation, possibly increasing appetite and enhancing foraging behavior. The lower plasma insulin and higher cortisol concentrations could mediate augmented lipolysis and the lower triiodothyronine levels could suppress the metabolic rate. Fasting did not affect the plasma levels of stress-associated catecholamines or variables indicating tissue damage. In general, the adaptations to short-term fasting exhibited some differences compared to the related farm-bred American mink (*Mustela vison*), an example of which was the better ability of the marten to hydrolyze lipids despite its significantly lower initial fat mass.

Keywords Carbohydrate metabolism · Endocrinology · Fasting · Lipid metabolism · *Martes americana*

Abbreviations

A	Adrenaline
Acrp30	Adiponectin
Ala	Alanine
BM	Body mass
BMI	Body mass index
BUN	Blood urea nitrogen
Chol	Cholesterol
CK	Creatine kinase
FA	Fatty acid
G6Pase	Glucose-6-phosphatase
HDL	High-density lipoprotein
IAB	Intraabdominal
Ile	Isoleucine
LDL	Low-density lipoprotein
Leu	Leucine
MCHC	Mean corpuscular hemoglobin concentration
mes	Mesenteric
NA	Noradrenaline
om	Omental
PUFA	Polyunsaturated fatty acid
RIA	Radioimmunoassay
rp	Retroperitoneal
SC	Subcutaneous
T_3	Triiodothyronine
T_4	Thyroxine

Communicated by G. Heldmaier.

P. Nieminen (✉) · A.-M. Mustonen
Faculty of Biosciences, University of Joensuu,
P.O. Box 111, 80101 Joensuu, Finland
e-mail: pniemine@cc.joensuu.fi

K. Rouvinen-Watt
Department of Plant and Animal Sciences,
Nova Scotia Agricultural College,
P.O. Box 550, Truro, NS, Canada B2N 5E3

S. Saarela
Department of Biology, University of Oulu,
P.O. Box 3000, 90014 Oulu, Finland

TAG Triacylglycerol
Val Valine
WAT White adipose tissue

Introduction

Mustelids are small predators interesting from the point of view of nutritional physiology. Due to the high surface-to-volume ratio, their thermoregulatory costs are high (Brown and Lasiewski 1972; Iversen 1972; Gilbert and Gofton 1982). Mustelid adaptations to fasting have been previously investigated e.g., in the farmed American mink (*Mustela vison*) and the sable (*Martes zibellina*). Despite a body fat content close to 40%, the lipid mobilization of the farmed American mink may be limited and the species develops fatty liver syndrome after 5–10 days of food deprivation (Bjornvad et al. 2004; Mustonen et al. 2005a). While the mink can mobilize fatty acids (FA) from all its major adipose tissue depots, the relative decrease in the mass of its intraabdominal (IAB) fat is higher than that of the subcutaneous (SC) fat, probably due to the significance of the SC fat layer as insulation during aquatic predation (Mustonen et al. 2005a; Nieminen et al. 2006b). The fasted American mink also shows increased plasma transaminase activities, but remains normoglycemic after 5–7 days. However, the plasma urea concentrations increase after 3–7 days indicating phase III of fasting (Mustonen et al. 2005b). The farmed sable experiences diminished liver glycogen stores, increased fat mobilization and protein breakdown and increased plasma transaminase activities after 4 days of fasting (Mustonen et al. 2006a).

The American marten (*Martes americana*) is a boreal forest marten related to the Japanese marten (*M. melampus*), the sable and the European marten (*M. martes*; Anderson 1970; Buskirk 1994). The species inhabits forests or landscape mosaics with seasonal snow cover (Buskirk and Powell 1994; Gibilisco 1994) and feeds on voles, birds, fish, ungulate carcasses, insects, fruits and berries (Martin 1994). The American marten has a low body fat content (5%), possibly as an adaptation to maintain the lean body shape for hunting in the burrows of rodents (Harlow 1994) and does not experience clear seasonal cycles in its body fat content (Buskirk and Harlow 1989). Due to the small gastric ventricle, the capacity to consume large meals is limited. Yet the species can withstand at least 5 days of fasting by using fat and protein in a ratio of 1.56:1 (Harlow and Buskirk 1991). The plasma concentrations of alanine (Ala), isoleucine (Ile), leucine (Leu) and valine (Val) increase, while the excretion of urea decreases indicating high turnover of proteins and urea-N recycling (Harlow and Buskirk 1996). In addition, the American marten mobilizes FA from its SC and IAB fat depots equally during a 48 h period of food deprivation (Nieminen et al. 2006a).

The responses of morphology, hematology, carbohydrate metabolism and endocrinology to fasting remain uninvestigated in the American marten. Based on previous studies (Harlow and Buskirk 1991, 1996; Nieminen et al. 2006a), it can be hypothesized that the three phases of fasting would be short including phase I with rapid carbohydrate loss, phase II with lipid mobilization limited by the low initial fat mass followed soon by proteolysis during phase III. In nature, periods of fasting are presumably short for the wild American marten, as they leave their nests daily except during the harshest weather conditions (Buskirk et al. 1988). The specific aims of the study were to (1) determine the use of carbohydrates and lipids in the American marten that have fasted for an ecologically relevant period of 48 h, (2) measure the biochemical indicators of stress, (3) determine the concentrations of plasma weight-regulatory hormones, (4) investigate the hematological effects of fasting, and (5) compare the results with existing data on mustelid fasting and the natural history of the American marten.

Materials and methods

For these experiments, 15 farm-bred American marten (9 males and 6 females) born between 1993 and 2004 were divided into two experimental groups by placing equal numbers of animals of both sexes and all age categories into each group. The marten colony was bred in captivity since 1990 and the animals used in the fasting experiments were all born captive, but can be considered genetically not different from wild marten. All animals were housed singly in standard cages (90 × 95–150 × 120 cm) with wooden nest boxes (35 × 18 × 18 cm) suspended above ground in an unheated shed at the Canadian Centre for Fur Animal Research, Nova Scotia Agricultural College, in Truro, Nova Scotia (45°22.15′N, 63°15.57′W). Before the experiment, the animals were fed for several months with a standard fur animal diet prepared by a local feed kitchen. Approximately, 200 g of wet feed or 1040 kJ (metabolizable energy 5000–5400 kJ kg fresh weight^{-1}; protein minimum 11.0–12.0%, fat 3.0–7.5%, carbohydrates maximum 10.0–11.0%; Rouvinen-Watt et al. 2005) was provided daily. In addition, they received a commercially prepared dry pelleted mink furring diet (Shur-Gain Mink Grow-Fur Ration, Maple Leaf Foods Inc., Guelph, Ontario, Canada; protein minimum 40.0%, fat 21.0–23.0%, fiber maximum 4.0%, estimated energy content 17.5 MJ kg^{-1} (90% dry matter)) as fed ad lib from a feed hopper. Occasionally, the marten were also provided with mice or pieces of poultry and fruits or berries. The marten are

estimated to require about 335 kJ day^{-1} when at rest (Strickland and Douglas 1999). The fasting experiments were conducted between 5th and 7th January 2005. Half of the animals (n = 8) were subjected to a 48 h fast, while the other half (n = 7) were fed using the diet described above. Water was available for both experimental groups ad lib. The average daily ambient temperature varied between –6.8 and –9.9°C during the study period (data provided by the Meteorological Service of Canada).

Body masses (BM) were recorded on the first day of the experiment and at sampling, and the body lengths were recorded at sampling (at 0830–1400 hours). Body mass indices (BMI) correlating with the body fat% (Nieminen et al. 2006a) were calculated with the formula BMI = BM (kg) [body $length^3$ (m)]$^{-1}$. The animals were anesthetized with intramuscular xylazine (Rompun^{TM} 3.4 mg kg^{-1}) and ketamine hydrochloride (Ketalean^{TM} 8.5 mg kg^{-1}). Blood samples were obtained by cardiac punctures. A small sample of whole blood was used for the complete blood count analysis and the rest was divided into plain tubes for the serum samples and into tubes containing EDTA as an anticoagulant for the plasma samples. The animals were euthanized with an intracardiac injection of pentobarbital (Euthanyl^{TM} 106 mg kg^{-1}). The blood was stored on ice until centrifugation at 1000 ×g for 15 min at 4°C, after which the serum and plasma were removed. The livers and white adipose tissue (WAT) samples were dissected. The WAT samples collected were as follows: SC scapular, SC rump, SC inguinal, IAB omental (om), IAB mesenteric (mes), IAB diaphragmatic, and IAB retroperitoneal (rp) and intramuscular. The intramuscular WAT sample was dissected between the gluteal muscles in the rump. Muscle and kidney samples were dissected and the spleen, pancreas, ovaries or testes, thyroid and adrenal glands, heart, gastric ventricles and intestines were weighed. All the samples were placed into vials, frozen immediately in liquid nitrogen and stored at –80°C. The carcasses were also frozen and the different WAT depots dissected and weighed at a later date.

The tissue glycogen and protein concentrations and the glucose-6-phosphatase (G6Pase), glycogen phosphorylase and lipase activities were measured spectrophotometrically as described in Mustonen et al. (2006a). The whole blood samples were analyzed for complete blood count using the Coulter T-890 analyzer (Model 442; Coulter Electronics of Canada Ltd., Burlington, Ontario). The serum clinical chemical parameters [calcium, phosphorus, magnesium, sodium, potassium, chloride, blood urea nitrogen (BUN), total protein, albumin, globulins, alkaline phosphatase] were measured using the Ciba Corning 550 Express Plus analyzer (Ciba Corning Canada Inc., Markham, Ontario). The above analyses were carried out at the Veterinary Services laboratory of the Nova Scotia Department of Agriculture. The plasma total cholesterol (Chol), low-density lipoprotein (LDL) Chol, high-density lipoprotein (HDL) Chol, triacylglycerol (TAG), glycerol, glucose, creatinine and bilirubin concentrations as well as the alanine aminotransferase, aspartate aminotransferase and creatine kinase (CK) activities and the liver TAG and Chol concentrations were determined as described previously (Mustonen et al. 2005a).

The plasma leptin concentrations were measured with the Multi-Species Leptin RIA kit (Linco Research, St Charles, MO; intraassay variation 2.8–3.6 % CV), the plasma ghrelin concentrations with the Ghrelin (human) RIA kit (Phoenix Pharmaceuticals, Belmont, CA; <5% CV), the plasma adiponectin (Acrp30) concentrations with the Human Adiponectin RIA kit (Linco Research; 1.78–6.21 % CV) and the plasma insulin concentrations with the Human Insulin Specific RIA kit (Linco Research; 2.2–4.4 % CV) each during a single run. The peptide assays were validated such that serial dilutions of the marten plasma showed linear changes in standard or sample binding/maximum binding (BB_0^{-1}) values that were parallel with the standard curves produced with the standards of the manufacturers (data not shown). The thyroxine (T_4), triiodothyronine (T_3) and cortisol concentrations were measured with the Spectria T4, T3 and CORTISOL [^{125}I] Coated Tube Radioimmunoassay kits of Orion Diagnostica (Espoo, Finland; T_4: 3.3–6.8; T_3: 3.3–6.1; cortisol: 2.6–5.4% CV). The plasma catecholamine (noradrenaline NA and adrenaline A) concentrations were measured as described previously (Nieminen et al. 2004; Mustonen et al. 2005c).

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 Inc., Chicago, IL). Significant changes within the time-series (before and after fasting) were analyzed with the paired t test for dependent samples. Correlations were calculated using the Spearman correlation coefficient (r_s). A P value less than 0.05 was considered to be statistically significant. The results are presented as mean ± SE.

Results

The American marten lost approximately 113 g or 13.4% of BM during the 48 h fasting period (Table 1). Many fasted animals exhibited stereotypical behavior and signs of stress, such as hair biting. The absolute or relative masses of the individual fat depots or the total SC or IAB fat masses did not differ between the experimental groups, while the total fat mass was 35% lower in the fasted animals. The BMI was also significantly lower in the fasted

Table 1 Body mass, body mass index and the absolute weights of the anatomically different fat depots of the American marten fed or fasted for 48 h (mean ± SE)

	Fed	Fasted
BM at start (g)	884 ± 70	849 ± 63
BM at end (g)	884 ± 70	734 ± 56
BMI at end [kg $(m^3)^{-1}$]	12.94 ± 0.70*	10.55 ± 0.22*
Scapular fat (g)	0.27 ± 0.05	0.15 ± 0.04
Rump fat (g)	0.16 ± 0.05	0.15 ± 0.05
Inguinal fat (g)	1.29 ± 0.57	0.61 ± 0.16
Total SC fat (g)	1.72 ± 0.53	0.92 ± 0.19
Diaphragmatic fat (g)	0.68 ± 0.27	0.33 ± 0.11
Omental fat (g)	4.94 ± 1.66	1.93 ± 0.61
Mesenteric fat (g)	2.44 ± 0.78	1.19 ± 0.24
Retroperitoneal fat (g)	5.58 ± 2.18	3.29 ± 1.79
Total IAB fat (g)	13.74 ± 4.46	6.77 ± 2.03
Intramuscular fat (g)	8.03 ± 0.85	7.52 ± 0.57
$\sum$ Body fat (g)	23.4 ± 4.38*	15.2 ± 2.24*
Body fat % BM	2.74 ± 0.56	2.06 ± 0.24

BM body mass, *BMI* body mass index, *SC* subcutaneous, *IAB* intra-abdominal

* Significant difference between the fed and the fasted animals ($P < 0.05$)

group at the end of the study. The absolute liver, kidney, splenic, pancreatic, ovarial and intestinal masses and the relative weights of the liver, spleen, testes and ovaries were lower in the fasted group (Table 2).

The mean corpuscular hemoglobin concentration (MCHC) and the percentages of lymphocytes and eosinophils were lower in the fasted animals, while the percentage of granulocytes was higher (Table 3). The serum total protein and globulin concentrations decreased and the magnesium concentrations increased (Table 4). The liver

Table 2 Absolute organ weights of the American marten fed or fasted for 48 h (mean ± SE)

	Fed	Fasted
Liver (g)	31.05 ± 2.25*	21.62 ± 1.29*
Kidneys (g)	6.29 ± 0.33*	5.15 ± 0.26*
Spleen (g)	3.35 ± 0.55*	1.66 ± 0.34*
Pancreas (g)	2.60 ± 0.24*	1.99 ± 0.10*
Testes (g)	0.76 ± 0.25	0.36 ± 0.05
Ovaries (g)	0.98 ± 0.13*	0.25 ± 0.03*
Gastric ventricle (g)	6.53 ± 0.70	4.92 ± 0.41
Intestines (g)	16.63 ± 2.02*	12.34 ± 0.73*
Heart (g)	7.75 ± 0.77	6.82 ± 0.53
Adrenal glands (mg)	38 ± 14	61 ± 12
Thyroid glands (mg)	224 ± 37	172 ± 18

* Significant difference between the fed and the fasted animals ($P < 0.05$)

Table 3 Complete blood count of the American marten fed or fasted for 48 h (mean ± SE)

	Fed	Fasted
WBC (10^9 l^{-1})	8.36 ± 0.95	6.75 ± 1.91
RBC (10^{12} l^{-1})	9.62 ± 0.15	8.82 ± 0.77
HGB (g l^{-1})	176 ± 2	158 ± 14
HCT (%)	47 ± 5	48 ± 4
MCV (fl)	54.1 ± 0.7	54.7 ± 0.9
MCH (pg)	18.31 ± 0.30	17.99 ± 0.31
MCHC (g l^{-1})	339 ± 2*	329 ± 1*
PLT (10^9 l^{-1})	670 ± 55	593 ± 45
Lymphocytes (%)	60.3 ± 5.3*	30.8 ± 5.8*
Monocytes (%)	0.9 ± 0.5	1.5 ± 0.5
Granulocytes (%)	32.1 ± 5.6*	66.6 ± 5.5*
Eosinophils (%)	6.3 ± 1.6*	0.4 ± 0.3*

WBC white blood cell count, *RBC* red blood cell count, *HGB* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *PLT* platelet count

* Significant difference between the fed and the fasted animals ($P < 0.05$)

and muscle glycogen concentrations, glycogen phosphorylase activities and the kidney G6Pase activity were lower in the fasted marten (Table 5) as were the plasma total

Table 4 Plasma and serum biochemistry and tissue protein concentrations of the American marten fed or fasted for 48 h (mean ± SE)

	Fed	Fasted
Bilirubin (μmol l^{-1})	5.23 ± 0.43	4.39 ± 0.51
Alanine aminotransferase (U l^{-1})	623 ± 154	496 ± 98
Aspartate aminotransferase (U l^{-1})	246 ± 56	333 ± 56
Alkaline phosphatase (U l^{-1})	122 ± 11	135 ± 17
Creatine kinase (U l^{-1})	280 ± 61	364 ± 60
Creatinine (μmol l^{-1})	64.7 ± 9.0	44.0 ± 2.5
Total protein (g l^{-1})	58.2 ± 1.7*	47.8 ± 4.0*
Albumin (g l^{-1})	36.1 ± 1.1	31.1 ± 2.5
Globulins (g l^{-1})	22.2 ± 0.7*	16.7 ± 1.6*
Blood urea nitrogen (mmol l^{-1})	15.0 ± 1.3	15.9 ± 1.6
Calcium (mmol l^{-1})	2.13 ± 0.04	1.94 ± 0.05
Phosphorus (mmol l^{-1})	1.57 ± 0.09	1.96 ± 0.17
Magnesium (mmol l^{-1})	0.86 ± 0.02*	1.17 ± 0.08*
Sodium (mmol l^{-1})	150 ± 1	150 ± 1
Potassium (mmol l^{-1})	4.24 ± 0.09	4.04 ± 0.18
Chloride (mmol l^{-1})	111.3 ± 0.5	110.5 ± 0.5
L protein (mg g^{-1})	45.2 ± 1.3	43.0 ± 1.9
K protein (mg g^{-1})	41.8 ± 1.3	39.9 ± 1.9
M protein (mg g^{-1})	52.4 ± 2.6	53.7 ± 3.4

L liver, *K* kidney, *M* muscle

* Significant difference between the fed and the fasted animals ($P < 0.05$)

Table 5 Carbohydrate metabolism of the American marten fed or fasted for 48 h (mean ± SE)

	Fed	Fasted
P glucose (mmol l^{-1})	10.37 ± 0.44	9.44 ± 1.05
L glycogen (μg mg^{-1})	7.68 ± 1.61*	2.57 ± 1.43*
L phosphorylase (μg P mg^{-1} h^{-1})	43.59 ± 2.24*	32.75 ± 1.49*
L glucose-6-phosphatase (μg P mg^{-1} h^{-1})	75.62 ± 3.92	72.56 ± 7.03
K glycogen (μg mg^{-1})	0.79 ± 0.06	1.14 ± 0.18
K phosphorylase (μg P mg^{-1} h^{-1})	6.49 ± 0.80	5.01 ± 0.81
K glucose-6-phosphatase (μg P mg^{-1} h^{-1})	41.43 ± 2.83*	31.17 ± 1.38*
M glycogen (μg mg^{-1})	1.09 ± 0.21*	0.43 ± 0.08*
M phosphorylase (μg P mg^{-1} h^{-1})	111.07 ± 7.06*	93.37 ± 4.10*

P plasma, *L* liver, *K* kidney, *M* muscle

* Significant difference between the fed and the fasted animals ($P < 0.05$)

Table 6 Lipid metabolism of the American marten fed or fasted for 48 h (mean ± SE)

	Fed	Fasted
P triacylglycerols (mmol l^{-1})	0.44 ± 0.07	0.39 ± 0.04
L triacylglycerols (mg g^{-1})	6.28 ± 2.92	11.58 ± 4.93
P total cholesterol (mmol l^{-1})	3.77 ± 0.14*	1.19 ± 0.21*
L total cholesterol (mg g^{-1})	3.83 ± 1.85	3.10 ± 0.55
P HDL-cholesterol (mmol l^{-1})	2.97 ± 0.09*	0.69 ± 0.17*
P LDL-cholesterol (mmol l^{-1})	0.15 ± 0.01*	0.07 ± 0.01*
P HDL-total cholesterol-ratio	0.79 ± 0.02*	0.55 ± 0.03*
P glycerol (μmol l^{-1})	295 ± 68	261 ± 45
L lipase (μg 2-naphthol mg^{-1} h^{-1})	27.17 ± 3.36	27.55 ± 2.66
K lipase	23.64 ± 1.39	22.40 ± 1.50
M lipase	7.09 ± 0.60	6.91 ± 0.86
Scapular WAT lipase	11.13 ± 2.93	23.10 ± 9.29
Rump WAT lipase	12.64 ± 2.89*	48.47 ± 10.64*
Inguinal WAT lipase	11.88 ± 2.98	41.84 ± 12.79
Mesenteric WAT lipase	15.20 ± 2.87	20.45 ± 3.02
Omental WAT lipase	11.70 ± 2.71*	35.20 ± 6.09*
Retroperitoneal WAT lipase	29.82 ± 7.54*	52.76 ± 6.97*
Average SC WAT lipase	12.40 ± 2.05*	38.33 ± 6.98*
Average IAB WAT lipase	18.91 ± 3.82*	36.14 ± 3.68*

P plasma, *L* liver, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *K* kidney, *M* muscle, *WAT* white adipose tissue, *SC* subcutaneous, *IAB* intraabdominal

* Significant difference between the fed and the fasted animals ($P < 0.05$)

Chol, HDL-Chol and LDL-Chol concentrations (Table 6). The lipase activities were significantly higher due to fasting in the following WAT depots: rump, om, rp, pooled SC and pooled IAB fats.

The plasma leptin and Acrp30 concentrations were unaffected by the fasting regime, while the plasma ghrelin concentrations were higher and the plasma insulin, T_3 (Fig. 1a, b) and T_4 concentrations (fed: 33.1 ± 1.66 vs. fasted: 15.5 ± 3.32 nmol l^{-1}) lower in the fasted marten. The plasma catecholamine concentrations did not differ between the experimental groups, but the plasma cortisol concentrations were higher in the fasted animals (Fig. 1c). The plasma ghrelin concentrations correlated negatively and the insulin concentrations positively with the final BMI (ghrelin: $r_s = -0.764$, $P < 0.01$; insulin: $r_s = 0.701$, $P < 0.01$), while the leptin and Acrp30 concentrations and the fat masses, BM or BMI did not correlate significantly with each other. The ghrelin concentrations correlated negatively with the relative masses of the mes and om fats ($r_s = -0.557$, $P < 0.05$).

Discussion

General remarks

The percentage of total fat of the American marten (2.1–2.7%) was similar to that in the previous report of Buskirk and Harlow (1989), who measured a 2.4% body fat content in Alaska and 4.6% in Wyoming in skinned American marten. The rate of BM loss in the fasted marten was 6.7% day^{-1}, slightly more than observed previously after 5 days of fasting (4.8% day^{-1}; Harlow and Buskirk 1991) and higher compared to the farmed sable (BM approximately 1.2 kg, body fat content 8%), which lost about 4.0% day^{-1} of its BM during a 4-day wintertime fast (Mustonen et al. 2006a). This presumably resulted from the facts that the fasting period was shorter in the present study and that the rate of BM loss is higher during phase I of fasting than during phase II. The percentage of body fat of the captive American marten was very different from the farmed American mink (BM approximately 2.7 kg), the body fat content of which can be 38% in winter (Mustonen et al. 2005a). Fasted American mink lost weight by 3.0% after 2 days, about 4.5 times less than the marten.

No data exist on the rate of weight loss of American marten fasting in the wild, but it is probable that the general body composition and the response to fasting of the marten in the present study were comparable to their wild relatives. The BM of the fasted marten decreased by 113 g, of which fat accounted for approximately 8.2 g. A major part of the BM loss was probably caused by the emptying of the intestine, although some body proteins could have been lost, too. However, the BUN levels as well as the tissue protein concentrations remained stable during fasting, while the glycogen stores decreased indicating that the marten were within phase II of fasting.

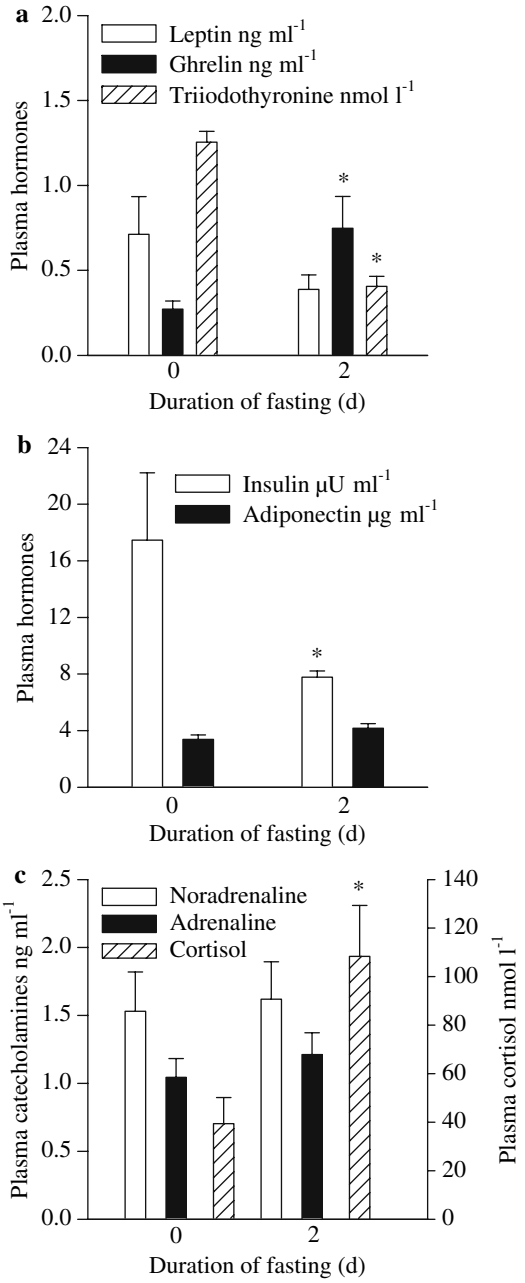

Fig. 1 The concentrations of **a** plasma leptin, ghrelin and triiodothyronine, **b** plasma insulin and adiponectin and **c** plasma noradrenaline, adrenaline and cortisol (mean + SE) in the fed and fasted marten. *Significant difference between the fed and the fasted animals ($P < 0.05$)

Health effects

The American marten tolerated food deprivation relatively well with the exception of the observed stress-related hair biting. No effects of fasting were observed on the plasma catecholamine concentrations. Previously, plasma NA levels increased in women fasted for 10 days (Beitins et al. 1985), while A and NA concentrations were stable in fasted raccoon dogs (*Nyctereutes procyonoides*; Nieminen et al. 2004) and American mink (Mustonen et al. 2005c). Similar to the American marten (Buskirk et al. 1988), these species experience wintertime food deprivation as a natural part of their seasonal physiology (Smal 1991; Asikainen et al. 2004). In contrast, the higher plasma cortisol concentrations could be an indicator of stress in fasting *Martes* spp. (Mustonen et al. 2006a). High physiological cortisol levels enhance proteolysis (Simmons et al. 1984) and whole body lipolysis, although inhibitory effects on lipolysis may occur in particular anatomic regions such as in the SC abdominal fat (Divertie et al. 1991; Samra et al. 1998). Stress could also increase the activity of the sympathetic nervous system and contribute to increased lipolysis (Jänig 1983) of the fasted animals.

The decreased lymphocyte count or percent has been observed also previously in mustelids after 4–7 days of fasting (Mustonen et al. 2005a, 2006a). The preferential mobilization of *n*-3 polyunsaturated FA (PUFA), established in fasted American marten and mink (Nieminen et al. 2006a, b), could cause a decrease in the *n*-3/*n*-6 PUFA ratio and lead to undesirable alterations in eicosanoid production and immune defense (Ackman and Cunnane 1992; Lessard et al. 2004) predisposing to e.g., viral diseases together with the reduced percentage of lymphocytes. In addition, the decrease in the percentage of eosinophils could cause increased susceptibility to parasitic infections. The higher percentage of granulocytes due to fasting is in common with the sable (Mustonen et al. 2006a) and may be induced by increased plasma cortisol concentrations (Toft et al. 1994). The lower MCHC could indicate incipient iron deficiency (Schnall et al. 2000).

The American mink experiences liver dysfunction and hepatic TAG accumulation after one week of fasting (Bjornvad et al. 2004; Mustonen et al. 2005a) and the sable after 4 days (Mustonen et al. 2006a). However, after 2 days, the mink did not yet show clear indications of hepatic lipidosis in concert with this study. Also, the plasma creatinine concentrations and CK activities remained unaffected in the fasted American marten suggesting that no kidney dysfunction or skeletal muscle damage were present (Adlercreutz et al. 1983).

The lower ovarial and testicular masses of the fasted marten were not observed previously in the American mink or in the sable (Mustonen et al. 2005a, 2006a, b). Decreased reproductive effort during nutritional scarcity could allow targeting energy reserves for individual survival. In fact, decreased plasma testosterone and progesterone concentrations have been observed in the male American mink after 2 days without food in winter (Mustonen et al. 2005c). The American marten has its mating period from July to early August and delayed implantation until February (Mead 1994). The lengthening

photoperiod triggers prolactin secretion leading to increased circulating progesterone levels and implantation. The decrease in progesterone concentration could thus prevent implantation or predispose females to spontaneous abortion during postimplantational gestation (Durfee and Pernoll 1991), which could be a survival strategy for mustelids during severe food shortage.

Use of different body energy reserves

The liver and muscle carbohydrate stores of the fasted American marten were depleted after 48 h. Similar to this, the muscle glycogen levels of the fasted American mink were lower than in the fed animals after 2 days and the liver glycogen concentrations after 5 days (Mustonen et al. 2005a). The same was observed in the fasted sable after 4 days (Mustonen et al. 2006a). Yet the American marten and mink could maintain euglycemia after 2 days of fasting. Previously, the glucose levels of the fasted American marten were lower after 5 days (Harlow and Buskirk 1991) and those of the fasted sables after 4 days (Mustonen et al. 2006a), while no effects of fasting on the plasma glucose levels of the American mink were evident even after 7 days (Mustonen et al. 2005a). As the mink is more exclusively carnivorous compared to *Martes* spp., it can presumably maintain its blood glucose levels stable for longer periods by efficient gluconeogenesis from amino acids at the expense of muscle tissue (Mustonen et al. 2005a, b).

The levels of plasma total, HDL- and LDL-Chol were lower in the fasted American marten probably due to the cessation of lipid intake, while the lipase activities were 2–4-fold higher in several SC and IAB WAT depots indicating active hydrolysis of TAG. This fits well into the pattern of the American marten being able to mobilize FA from both SC and IAB WAT (Nieminen et al. 2006a) and is different from the American mink with stable SC and rp WAT lipase activities after 2 days of fasting (Mustonen et al. 2005a). In contrast, the more closely related sable experiences a two-fold increase in its rp fat lipase activities after 4 days without food (Mustonen et al. 2006a) and utilizes SC and IAB FA equally for energy (Nieminen and Mustonen 2007). Although the percentage of body fat of the marten is much smaller than that of the farmed mink, the marten seems to be able to mobilize lipids more efficiently from its SC fat depots than the mink during acute fasting (Nieminen et al. 2006a, b). As the marten is strictly terrestrial, unlike the mink, which has a preference for aquatic predation, especially in winter, it is conceivable that the insulatory demands for the SC fat layer of the mink restrict its use as an energy reserve. As a consequence, the relative mobilization of SC fat during energy shortage would be more effective in the marten.

Decreases in the serum total protein and globulin concentrations are well-known effects of acute fasting (Taylor et al. 1948). These were probably caused by the cessation of food intake and not by muscle proteolysis as indicated by the stable BUN and tissue protein concentrations. A previous study on the American marten fasted for 5 days showed increases in the plasma levels of Ala, Val, Leu and Ile (Harlow and Buskirk 1996), of which Ala, Val and Ile are glucogenic (Harris 1986). Similar changes in the concentrations of Val, Leu and Ile were observed in the fasted American mink after 2 days (Mustonen et al. 2005b), while no significant changes were observed in the sable fasted for 4 days (Mustonen et al. 2006a).

Endocrine effects of fasting

The adipocyte hormone leptin (Zhang et al. 1994) inhibits food intake (Pelleymounter et al. 1995). Its plasma concentrations decrease rapidly in fasting humans (Kolaczynski et al. 1996), rodents (Hardie et al. 1996; Schneider et al. 2000) and American mink (Mustonen et al. 2005c), but not in sables (Mustonen et al. 2006a) and some other studied Carnivora (Ortiz et al. 2001; Nieminen et al. 2004) including the American marten of the present study. Also the adipocyte-derived Acrp30 concentrations (Scherer et al. 1995) were unchanged in the marten similar to the American mink after 2–7 days of fasting (Mustonen et al. 2005c), while the blue fox (*Alopex lagopus*) experienced elevated plasma Acrp30 levels after 8–22 days (Mustonen et al. 2005d). It has been previously suggested for humans that the serum Acrp30 concentrations are not strongly regulated by acute fasting, but reflect the long-term nutritional status of the individual (Gavrila et al. 2003).

In contrast, the plasma ghrelin concentrations of the fasted American marten were higher than in the fed animals as observed previously in several other mammals (Tschöp et al. 2000; Mustonen et al. 2006a). Ghrelin increases food intake (Tschöp et al. 2000), memory retention and behavior related to anxiety (Carlini et al. 2002), enhancing survival during food deprivation. Increased ghrelin concentrations may also contribute to the euglycemic state (Broglio et al. 2001). The ghrelin concentrations correlated negatively with the BMI of the marten similar to humans (Haqq et al. 2003). The amounts of the om and mes fat could be the strongest indicators of the amount of energy stores present in the marten, as their relative masses correlated inversely with the plasma ghrelin levels. In addition, the relative decrease (%) in the masses of the fat depots was the highest for the om fat of the fasted marten. Buskirk (1983) also noted that the weight of the om fat is the best morphological indicator of body condition of the wild American marten.

The plasma insulin concentrations of the American marten reacted rapidly to food deprivation as opposed to the American mink, in which the decrease was observed only after 5–7 days of fasting (Mustonen et al. 2005c). The lowered insulin levels are a common response to a negative energy balance promoting the sparing of glucose, lipid mobilization, gluconeogenesis and ketogenesis (Cahill 1976). The lower plasma thyroid hormone concentrations of the fasted marten could suppress their metabolic rate, as suggested previously for humans and some carnivores (Brück 1983; Nieminen et al. 2004; Mustonen et al. 2005c, 2006a). This can be of benefit e.g., during snowstorms when marten are forced to stay in dens.

Conclusions

1. American marten, food-deprived for 48 h, are within phase II of fasting with decreased tissue carbohydrate stores, but active lipid mobilization indicated by the high lipase activities in several WAT depots.
2. The 48-h fasting period does not affect the plasma levels of stress-associated catecholamines or any of the measured variables indicating tissue dysfunction or damage. The plasma cortisol concentrations, however, are elevated due to fasting.
3. The plasma ghrelin concentrations are higher due to food deprivation possibly increasing appetite and enhancing foraging behavior, while the insulin, T_3 and T_4 concentrations are lower. The ghrelin concentrations correlate negatively with the relative amounts of the om and mes fats.
4. The lower percentages of blood lymphocytes and eosinophils in the fasted animals may increase susceptibility towards viral and parasitic infections. The higher percentage of granulocytes could be linked to the elevated plasma cortisol concentrations.
5. Compared to the farmed American mink, the American marten seems to have a better ability to hydrolyze lipids during acute fasting despite its significantly lower initial fat mass. Enhanced lipid mobilization can be partly mediated by the decreased plasma insulin and increased cortisol concentrations.

Acknowledgments This study was supported financially by the Academy of Finland (project #109884), Otto A. Malm's Donation Fund, Alfred Kordelin Foundation, Helve Foundation and the Natural Sciences and Engineering Research Council (NSERC) of Canada (Discovery Grant to KRW). We wish to thank Tanya Morse and Rena Currie, the staff of the Canadian Centre for Fur Animal Research, Rauni Kojo, Marja-Liisa Martimo-Halmetoja, Danielle Collins, Judy Grant, Cindy Crossman, Jody Muise and Margot White for logistical support during this research. The experiment complies with the current laws of Canada and Finland.

References

Ackman RG, Cunnane SC (1992) Long-chain polyunsaturated fatty acids: sources, biochemistry and nutritional/clinical applications. In: Padley FB (ed) Advances in applied lipid research, vol 1. JAI Press, London, pp 161–215

Adlercreutz H, Saris N-E, Vihko R (1983) Analyses of clinical chemistry (in Finnish), 3rd edn. Lääketieteen kandidaattiseura/Kandidaattikustannus Oy, Helsinki

Anderson E (1970) Quaternary evolution of the genus *Martes* (Carnivora, Mustelidae). Acta Zool Fenn 130:1–132

Asikainen J, Mustonen A-M, Hyvärinen H, Nieminen P (2004) Seasonal physiology of the wild raccoon dog (*Nyctereutes procyonoides*). Zoolog Sci 21:385–391

Beitins IZ, Barkan A, Klibanski A, Kyung N, Reppert SM, Badger TM, Veldhuis J, McArthur JW (1985) Hormonal responses to short term fasting in postmenopausal women. J Clin Endocrinol Metab 60:1120–1126

Bjornvad CR, Elnif J, Sangild PT (2004) Short-term fasting induces intra-hepatic lipid accumulation and decreases intestinal mass without reduced brush-border enzyme activity in mink (*Mustela vison*) small intestine. J Comp Physiol B 174:625–632

Broglio F, Arvat E, Benso A, Gottero C, Muccioli G, Papotti M, van der Lely AJ, Deghenghi R, Ghigo E (2001) Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. J Clin Endocrinol Metab 86:5083–5086

Brown JH, Lasiewski RC (1972) Metabolism of weasels: the cost of being long and thin. Ecology 53:939–945

Brück K (1983) Functions of the endocrine system. In: Schmidt RF, Thews G (eds) Human physiology. Springer, Berlin, pp 658–687

Buskirk SW (1983) The ecology of marten in southcentral Alaska. PhD dissertation, University of Alaska, Fairbanks

Buskirk SW (1994) Introduction to the genus *Martes*. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA (eds) Martens, sables and fishers: biology and conservation. Cornell University Press, Ithaca, pp 1–12

Buskirk SW, Harlow HJ (1989) Body-fat dynamics of the American marten (*Martes americana*) in winter. J Mammal 70:191–193

Buskirk SW, Powell RA (1994) Habitat ecology of fishers and American martens. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA (eds) Martens, sables and fishers: biology and conservation. Cornell University Press, Ithaca, pp 283–296

Buskirk SW, Harlow HJ, Forrest SC (1988) Temperature regulation in American marten (*Martes americana*) in winter. Natl Geogr Res 4:208–218

Cahill GF Jr (1976) Starvation in man. J Clin Endorinol Metab 5:397–415

Carlini V, Monzon ME, Varas MM, Cragnolini AB, Schioth HB, Scimonelli TN, de Barioglio SR (2002) Ghrelin increases anxiety-like behavior and memory retention in rats. Biochem Biophys Res Commun 299:739–743

Divertie GD, Jensen MD, Miles JM (1991) Stimulation of lipolysis in humans by physiological hypercortisolemia. Diabetes 40:1228–1232

Durfee RB, Pernoll ML (1991) Early pregnancy risks. In: Pernoll ML (ed) Current obstetric & gynecologic diagnosis & treatment. Prentice Hall, Englewood Cliffs, pp 300–325

Gavrila A, Chan JL, Yiannakouris N, Kontogianni M, Miller LC, Orlova C, Mantzoros CS (2003) Serum adiponectin levels are inversely correlated with overall and central fat distribution but are not directly regulated by acute fasting or leptin administration in humans: cross-sectional and interventional studies. J Clin Endocrinol Metab 88:4823–4831

Gibilisco CJ (1994) Distributional dynamics of modern *Martes* in North America. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA (eds) Martens, sables and fishers: biology and conservation. Cornell University Press, Ithaca, pp 59–71

Gilbert FF, Gofton N (1982) Heart rate values for beaver, mink and muskrat. Comp Biochem Physiol A 73:249–251

Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, Purnell JQ (2003) Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 88:174–178

Hardie LJ, Rayner DV, Holmes S, Trayhurn P (1996) Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochem Biophys Res Commun 223:660–665

Harlow HJ (1994) Trade-offs associated with the size and shape of American martens. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA (eds) Martens, sables and fishers: biology and conservation. Cornell University Press, Ithaca, pp 390–403

Harlow HJ, Buskirk SW (1991) Comparative plasma and urine chemistry of fasting white-tailed prairie dogs (*Cynomys leucurus*) and American martens (*Martes americana*): representative fat- and lean-bodied animals. Physiol Zool 64:1262–1278

Harlow HJ, Buskirk SW (1996) Amino acids in plasma of fasting fat prairie dogs and lean martens. J Mammal 77:407–411

Harris RA (1986) Carbohydrate metabolism I: major metabolic pathways and their control. In: Devlin TM (ed) Textbook of biochemistry with clinical correlations. Wiley, NY, pp 261–328

Iversen JA (1972) Basal energy metabolism of mustelids. J Comp Physiol 81:341–344

Jänig W (1983) The autonomic nervous system. In: Schmidt RF, Thews G (eds) Human physiology. Springer, Berlin, pp 111–144

Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF (1996) Responses of leptin to short-term fasting and refeeding in humans, a link with ketogenesis but not ketones themselves. Diabetes 45:1511–1515

Lessard M, Gagnon N, Godson DL, Petit HV (2004) Influence of parturition and diets enriched in *n*-3 or *n*-6 polyunsaturated fatty acids on immune response of dairy cows during the transition period. J Dairy Sci 87:2197–2210

Martin SK (1994) Feeding ecology of American martens and fishers. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA (eds) Martens, sables and fishers: biology and conservation. Cornell University Press, Ithaca, pp 297–315

Mead RA (1994) Reproduction in *Martes*. In: Buskirk SW, Harestad AS, Raphael MG, Powell RA (eds) Martens, sables and fishers: biology and conservation. Cornell University Press, Ithaca, pp 402–422

Mustonen A-M, Pyykönen T, Paakkonen T, Ryökkynen A, Asikainen J, Aho J, Mononen J, Nieminen P (2005) Adaptations to fasting in the American mink (*Mustela vison*): carbohydrate and lipid metabolism. Comp Biochem Physiol A 140:195–202

Mustonen A-M, Puukka M, Pyykönen T, Nieminen P (2005) Adaptations to fasting in the American mink (*Mustela vison*): nitrogen metabolism. J Comp Physiol B 175:357–363

Mustonen A-M, Saarela S, Pyykönen T, Nieminen P (2005) Endocrinologic adaptations to wintertime fasting in the male American mink (*Mustela vison*). Exp Biol Med 230:612–620

Mustonen A-M, Pyykönen T, Nieminen P (2005) Adiponectin and peptide YY in the fasting blue fox (*Alopex lagopus*). Comp Biochem Physiol A 140:251–256

Mustonen A-M, Puukka M, Saarela S, Paakkonen T, Aho J, Nieminen P (2006) Adaptations to fasting in a terrestrial mustelid, the sable (*Martes zibellina*). Comp Biochem Physiol A 144:444–450

Mustonen A-M, Pyykönen T, Aho J, Nieminen P (2006) Hyperthermia and increased physical activity in the fasting American mink *Mustela vison*. J Exp Zool A 305:489–498

Nieminen P, Mustonen A-M (2007) Uniform fatty acid mobilization from anatomically distinct fat depots in the sable (*Martes zibellina*). Lipids (in press). doi:10.1007/s11745-007-3061-5

Nieminen P, Saarela S, Pyykönen T, Asikainen J, Mononen J, Mustonen A-M (2004) Endocrine response to fasting in the overwintering captive raccoon dog (*Nyctereutes procyonoides*). J Exp Zool A 301:919–929

Nieminen P, Rouvinen-Watt K, Collins D, Grant J, Mustonen A-M (2006) Fatty acid profiles and relative mobilization during fasting in adipose tissue depots of the American marten (*Martes americana*). Lipids 41:231–240

Nieminen P, Käkelä R, Pyykönen T, Mustonen A-M (2006) Selective fatty acid mobilization in the American mink (*Mustela vison*) during food deprivation. Comp Biochem Physiol B 145:81–93

Ortiz RM, Wade CE, Ortiz CL (2001) Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. Am J Physiol 280:R790–R795

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F (1995) Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269:540–543

Rouvinen-Watt K, White MB, Campbell R (2005) Mink feeds and feeding. Ontario Ministry of Agriculture and Food, through the Agricultural Research Institute of Ontario and the Nova Scotia Agricultural College, Truro

Samra JS, Clark ML, Humphreys SM, MacDonald IA, Bannister PA, Frayn KN (1998) Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue. J Clin Endocrinol Metab 83:626–631

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270:26746–26749

Schnall SF, Berliner N, Duffy TP, Benz EJ Jr (2000) Approach to the adult and child with anemia. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, McGlave P (eds) Hematology: basic principles and practice, 3rd edn. Churchill Livingstone, Oxford, pp 367–382

Schneider JE, Blum RM, Wade GN (2000) Metabolic control of food intake and estrous cycles in Syrian hamsters. I. Plasma insulin and leptin. Am J Physiol 278:R476–R485

Simmons PS, Miles JM, Gerich JE, Haymond MW (1984) Increased proteolysis: an effect of increase in plasma cortisol within the physiologic range. J Clin Invest 73:412–420

Smal CM (1991) Population studies on feral American mink *Mustela vison* in Ireland. J Zool 224:233–249

Strickland MA, Douglas CW (1999) Marten. In: Novak M, Baker JA, Obbard ME, Malloch B (eds) Wild furbearer management and conservation in North America, CD-ROM. Ontario Fur Managers Federation under license from the Ontario Ministry of Natural Resources. Queens Printer, Ontario, pp 530–546

Taylor HL, Mickelsen O, Keys A (1948) The effects of induced malaria, acute starvation and semi-starvation on the electrophoretic diagram of the serum proteins of normal young men. J Clin Invest 28:273–281

Toft P, Helbo-Hansen HS, Tønnesen E, Lillevang ST, Rasmussen JW, Christensen NJ (1994) Redistribution of granulocytes during adrenaline infusion and following administration of cortisol in healthy volunteers. Acta Anaesthesiol Scand 38:254–258

Tschöp M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407:908–913

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432