Lipid Metabolism in the Adipose Tissues of a Carnivore, the Raccoon Dog, During Prolonged Fasting

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Previous studies on laboratory rodents, rabbits, and humans have demonstrated that adipose tissue fatty acid (FA) mobilization is selective, and its efficiency is related to the molecular structure of FAs. This study was undertaken to find out whether such preferences of FA mobilization are a general feature of mammalian white adipose tissue (WAT) and are also manifested in carnivores. Fractional mobilization of a wide spectrum of FAs was studied by gas-liquid chromatography from six subcutaneous (scapular, rump, ventral) and intra-abdominal (omental, mesenteric, retroperitoneal) WAT depots of raccoon dogs (*Nyctereutes procyonoides*) fed or fasted for 2 months. Fasting stimulated the mobilization of shorter-chain saturated, monounsaturated (MUFAs), and polyunsaturated FAs (PUFAs). The effects of unsaturation and the position of the first double bond from the methyl end were more inconsistent. The effect of double-bond position may be due to chain shortening of longer-chain MUFAs and preferential utilization of n-3 PUFAs over n-6 PUFAs. Moreover, there were site-specific differences in fractional mobilization, the omental adipose tissue being the most divergent. The *in vivo* FA mobilization from the regional WAT depots of a carnivore was selective, and the molecular structure of the FA affected its efficiency. Exp Biol Med 232:58–69, 2007

Key words: double-bond position; fasting; fat depot location; fatty acid molecular structure; selective mobilization of fatty acids

Introduction

Seasonal food scarcity is a major challenge for arctic and boreal mammals. Many species collect extensive lipid stores in autumn to be utilized in winter and spring. Weight cycling is common also for humans, but it causes adverse health effects (1). Adipose tissues have several specialized functions, such as in hormonal signaling, eicosanoid production, and immune response (2–4). An important mediator of these functions is the quality of fatty acids (FAs) released from adipocytes and transported via circulation to other tissues. The composition of the FA pool depends on the diet and nutritional status of the individual (5). The traditional view of FA release is that it is either a random (6) or a selective (7) process that aims to preserve the biologically valuable essential FAs. In 1995, a study on fasted laboratory rats *Rattus norvegicus* demonstrated that FA mobilization is selective, and its efficiency is related to the molecular structure of FAs (5). Studies on rabbits (8), humans (9), and ruminants (10) supported this hypothesis. It is important for medical physiology to find out whether such preferences are a general feature of mammalian adipose tissue.

An excellent model for lipid research is the raccoon dog (*Nyctereutes procyonoides*), a medium-sized canid with profound but nonpathological autumnal fattening followed by natural weight loss in winter and spring (11). The raccoon dog has several large white adipose tissue (WAT) depots with pronounced seasonal changes in volume (12), allowing for good comparisons between anatomically different and physiologically specialized WAT depots (13). The species tolerates a total fast of at least 11 weeks without any deleterious effects (14, 15). The natural diet of wild raccoon dogs and the diet offered to farmed animals contain a large variety of FAs, and the FA composition of the WAT depots is homogenous, with only small vertical variations (16). Due to the long-term consumption of the

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Table 1. Fatty Acid Composition of the Diet (mol%; $n = 5$) of the Raccoon Dogs (Mean ± SE)[a]

Fatty acid	Diet mol%
14:0	2.7 ± 0.34
16:0	22.6 ± 0.68
17:0	0.4 ± 0.02
18:0	9.6 ± 0.98
ΣSFA	37.2 ± 0.97
16:1n-7	4.4 ± 0.56
18:1n-9	24.9 ± 1.26
18:1n-7	2.8 ± 0.04
20:1n-9	2.0 ± 0.32
22:1n-11	1.2 ± 0.24
ΣMUFA	37.9 ± 2.41
18:2n-6	12.1 ± 0.46
18:3n-3	1.7 ± 0.28
18:4n-3	0.5 ± 0.09
20:4n-6	2.3 ± 1.30
20:5n-3	1.9 ± 0.11
22:5n-3	0.6 ± 0.03
22:6n-3	3.8 ± 0.40
ΣPUFA	24.9 ± 1.44
Σn-6 PUFA	15.3 ± 1.68
Σn-3 PUFA	9.1 ± 0.68
n-3/n-6 PUFA	0.6 ± 0.07
UFA/SFA	1.7 ± 0.07

[a] Minor fatty acids not listed in the table also are included in the sums.

same diet, the FA composition of WAT is equilibrated prior to fasting. This means equal accessibility for mobilization of all FAs of different fat layers. As the raccoon dog is dietarily an omnivore (17), the data obtained have biomedical value and may have advantages over the data from traditional laboratory rodents.

The FA composition of WAT is used to monitor the long-term dietary habits of humans and the foraging ecology of top predators. In humans, the focus is on estimating the supply and reserves of essential polyunsaturated FAs (PUFAs) and the balance between n-3 and n-6 PUFAs (18). In foraging ecology, FAs accumulated in fat depots of predators give information on their diet (19). To interpret correctly the FA profiles, it is important to understand the selective changes in the FA composition of WAT and plasma caused by seasonal weight regulation of wild mammals or by weight cycling of humans. The goals of this study were to investigate (i) the adjustments of enzyme activities of energy metabolism to prolonged fasting, (ii) the consequences of fasting on the morphology of the raccoon dog, (iii) the mobilization efficiency of structurally different FAs during fasting, (iv) the quantitative and qualitative differences between several specific WAT depots in their FA release, and (v) the biochemical indicators of fasting in plasma.

Materials and Methods

Farm-bred female raccoon dogs ($n = 12$) born in spring 2002 were randomly divided into two groups ($n = 6$) as follows: Group 1 was fed throughout the winter, and Group 2 fasted for 8 weeks in the winter. They were housed singly under roof in cages (150 × 107 × 70 cm) with wooden nestboxes (75 × 43 × 37 cm) at natural temperature and photoperiod at the Juankoski Research Station fur farm, Juankoski, Finland (63°N, 28°E). To obtain initial blood samples on November 26, 2003, the animals were sedated with subcutaneous medetomidine (50 μg/kg) and butorphanol tartrate (0.5 mg/kg) and were further anesthetized with intramuscular (im) ketamine (5 mg/kg). The blood samples were taken from a superficial vein of the hind leg with sterile needles and syringes with EDTA as an anticoagulant and were centrifuged at 1500 *g*. Plasma was removed and stored at −80°C. During the 7-day recovery period the animals were fed with a commercial fur animal diet (1900 kJ per animal per day) consisting of Baltic herring (6%), freshwater fish (18%), rainbow trout offal (6%), slaughterhouse offal (16%), chicken offal (6%), barley (10%), protein mixture (8%), and vegetable oil (0.2%) and containing 35% to 43% protein, 14% to 20% fat, 36% to 40% carbohydrates, and 16,000 to 17,000 kJ metabolizable energy per kilogram dry matter (Ylä-Karjalan Rehu Oy, Valtimo, Finland; Table 1). On December 3, 2003, Group 2 was put to a total 56-day fast, which is a natural phenomenon for the species (11, 17). Group 1 was fed 1500 kJ per animal per day to avoid excessive obesity. Water or ice was continuously available *ad libitum*.

On November 26, 2003, the measurements of body length, for example, required anesthesia to avoid excessive stress. At the second blood sampling on December 30, 2003, no additional handling was necessary, and the sampling could be performed with minimal disturbance. On January 27, 2004, the animals were anesthetized with im ketamine (5 mg/kg) and xylazine (2 mg/kg). Blood samples were obtained by cardiac punctures, and the animals were euthanized with an intracardial injection of embutramide (60 mg/kg), mebezonium (15 mg/kg) and tetracaine hydrochloride (1.5 mg/kg). The complete blood count was determined with the Vet abc Animal Blood Counter (ABX Hematologie, Montpellier, France). Body masses (BMs) were determined on November 26, 2003, December 30, 2003, and January 27, 2004, and body lengths on November 26, 2003. Body mass indices (BMIs) were calculated by the formula: BM (kg) ÷ [body length^3 (m)] (Ref. 20). The livers and kidneys were dissected. The quadriceps muscle of the left hind thigh and subcutaneous (from scapular, rump, and ventral fat) and intra-abdominal (iab; omental, mesenteric, and retroperitoneal) adipose tissues were sampled. All samples were immediately frozen in liquid nitrogen and stored at −80°C. The carcasses were stored at −20°C until they were thawed and the adrenal glands, thyroid glands, spleens, vaginas, uteri, ovaries, and all visible fat depots were carefully dissected and weighed. The body fat content was calculated by the formula: [dissected subcutaneous and iab fats (g)] ÷ BM (g) × 100.

Plasma total cholesterol (Chol) was determined with the

Table 2. Effects of 56 Days of Fasting on the BM loss, BMI, and Absolute Organ Weights of the Raccoon Dogs (Mean ± SE)

	Fed	Fasted
BM loss, g	757 ± 230[a]	3142 ± 152[a]
BMI, kg $(m^3)^{-1}$	30.7 ± 0.87[a]	24.9 ± 0.91[a]
Liver mass, g	235 ± 19[a]	172 ± 16[a]
Kidney mass, g	45 ± 4[a]	32 ± 1[a]
Splenic mass, g	19 ± 2	15 ± 1
Adrenal mass, mg	428 ± 50	434 ± 26
Thyroid mass, mg	470 ± 52	427 ± 46
Ovary mass, mg	392 ± 31[a]	303 ± 27[a]
Uterus mass, mg	1087 ± 93	1025 ± 169
Vagina mass, mg	415 ± 50	434 ± 63

[a] Significant difference between the fed raccoon dogs and the animals fasted between December 3, 2003, and January 27, 2004 (*t*-test, Mann-Whitney *U* test, $P < 0.05$).

cholesterol enzymatic endpoint method, and plasma low-density lipoprotein (LDL) Chol and high-density lipoprotein (HDL) Chol were determined with the direct LDL- and HDL-cholesterol reagents (Randox Laboratories Ltd., Crumlin, UK). Plasma triacylglycerol (TAG) and glucose levels were measured with the triglycerides GPO-PAP and the glucose liquid reagent hexokinase methods, and creatinine and glycerol concentrations were measured with the creatinine and glycerol colorimetric methods. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured with the ALT (GPT) alanine aminotransferase EC 2.6.1.2 ECCLS and AST (GOT) aspartate aminotransferase EC 2.6.1.2 ECCLS reagents, respectively. Also, the total protein biuret method, the urea enzymatic kinetic method, the ammonia enzymatic UV-method, the uric acid enzymatic colorimetric method, the bilirubin DCA method, and the CK NAC-activated creatine kinase EC 2.7.3.2 reagents used were purchased from Randox Laboratories Ltd. The Technicon RA-XT analyzer (Swords, Dublin, Ireland) was used for the measurements. Glucose-6-phosphatase (G6Pase), glycogen phosphorylase, and lipase activities, as well as glycogen and total protein concentrations were measured from tissue samples (21–24). Also, activities of liver alkaline phosphatase were measured spectrophotometrically with *p*-nitrophenyl phosphate as substrate in the presence of magnesium (pH 10.5, at 37°C).

FA composition was determined from total lipids. Subsamples of WAT and liver were transmethylated according to Christie (25). The formed FA methyl esters (FAMEs) were extracted with hexane. The dried and concentrated FAMEs were analyzed by a gas-liquid chromatograph equipped with two injectors and flame ionization and mass detectors (GC-FID and GC-MS, 6890N network GC system with autosampler, FID detector and 5973 mass selective detector; Agilent Technologies Inc., Palo Alto, CA). The peaks were reintegrated manually, and the mass spectra were extracted using the Agilent ChemStation software (Agilent Technologies Inc.). The FAMEs were identified based on the retention time, mass spectrum, and comparisons with authentic (Sigma-Aldrich Inc., St. Louis, MO) and natural standards of known composition and published reference spectra (http://www.lipidlibrary.co.uk/masspec.html). Quantifications were based on flame ionization detector responses. The peak areas of the FID chromatograms were converted to mol% using the theoretical response factors (26) and calibrations with quantitative authentic standards. The FAs were marked by using the abbreviations: [carbon number]:[number of double bonds] n-[position of the first double bond calculated from the methyl end]. If not stated otherwise, PUFAs were methylene interrupted. Double-bond index (DBI) and total average chain length (TACL) indicating the mean numbers of double bonds or carbon atoms per molecule were calculated according to standard formulae (27). Δ9-Desaturation index (Δ9-DI), the ratio of the most important potentially endogenous Δ9-monounsaturated FAs (MUFAs) to the corresponding saturated FAs (SFAs), was calculated as [(mol% 14:1n-5) + (mol% 16:1n-7) + (mol% 16:1n-9) + (mol% 18:1n-9) + 18:1n-7)] ÷ [(mol% 14:0) + (mol% 16:0) + (mol% 18:0)]. Fractional mobilization (FM) was calculated by the formula: [mol% in the fed animals − mol% in the fasted animals] ÷ [mol % in the fed animals].

Multiple comparisons were performed with the one-way analysis of variance (ANOVA) followed by the *post hoc* Duncan test. Comparisons between the two study groups were performed with the Student *t*-test or the Mann-Whitney *U* test for parametric and nonparametric data. Correlations were calculated with the Spearman correlation coefficient (r_s). *P* values < 0.05 were considered statistically significant.

Results

The fasted raccoon dogs lost 31% of BM during the 8-week fast and had lower absolute liver, kidney, and ovary weights but higher relative adrenal weights (Table 2). The mean corpuscular volume (MCV), red cell distribution width (RDW), and granulocyte count were lower in the fasted raccoon dogs (Table 3). The total fat mass was 43% lower than in the fed animals (Table 4). The differences in the masses of fat depots were as follows: subcutaneous fat, 44.7%; mesenteric fat, 39.6%; omental fat, 36.9%; retroperitoneal fat, 36.5%; and total iab fat, 38.1%. The lipase activities decreased due to fasting in subcutaneous rump and omental fats. The decreases also were significant for subcutaneous, omental, and retroperitoneal fats as well as for livers and kidneys when calculated as the total lipase activity in the whole tissue or organ. The BMIs correlated positively with the total fat mass ($r_s = 0.865$, $P < 0.01$) and body fat% ($r_s = 0.708$, $P < 0.05$). Higher plasma TAG, glycerol, and LDL-Chol levels were measured from the fasted raccoon dogs after 8 weeks of fasting (Table 5). The glycogen phosphorylase activities decreased due to fasting

Table 3. Effects of 56 Days of Fasting on the Complete Blood Count of the Raccoon Dogs (Mean ± SE)[a]

	Fed	Fasted
WBC, 10^9/liter	15.4 ± 1.19	11.9 ± 1.03
HGB, g/liter	139 ± 5	133 ± 3
HCT, %	40.4 ± 1.33	32.6 ± 5.84
PLT, 10^9/liter	346 ± 21	360 ± 28
MCV, fl	58.2 ± 0.65[b]	56.3 ± 0.42[b]
MCH, pg	20.0 ± 0.32	19.6 ± 0.19
MCHC, g/dl	34.3 ± 0.20	34.8 ± 0.12
RDW, %	16.7 ± 0.19[b]	16.0 ± 0.22[b]
MPV, fl	9.8 ± 0.13	10.1 ± 0.33
LYM, 10^9/liter	1.8 ± 0.14	2.1 ± 0.26
MON, 10^9/liter	0.58 ± 0.017	0.55 ± 0.043
GRA, 10^9/liter	13.0 ± 1.05[b]	9.3 ± 0.87[b]

[a] WBC, white blood cell count; HGB, haemoglobin; HCT, hematocrit; PLT, platelet count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; MPV, mean platelet volume; LYM, lymphocyte count; MON, monocyte count; GRA, granulocyte count.
[b] Significant difference between the fed raccoon dogs and the animals fasted between December 3, 2003, and January 27, 2004 (*t*-test, Mann-Whitney *U* test, $P < 0.05$).

in the liver and muscle samples (Table 6). The decreases also were significant for the liver and kidney G6Pase activity levels when calculated as the total G6Pase activity in the whole organ. The plasma urea and total protein levels and ratios of urea to creatinine were lower in the fasted raccoon dogs after 1 and 2 months without food (Table 5). The liver protein contents decreased due to fasting when calculated as the total amount of protein in the whole liver (Table 6).

Fasting induced numerous changes in the FA composition of WATs, liver, and plasma (reported in detail in online supplemental material). In SFAs and MUFAs, fasting increased the proportion of long-chain FAs and decreased the proportion of short-chain FAs. The proportion of n-6 PUFAs increased at the expense of n-3 PUFAs. DBI and Δ9-DI did not differ between the groups, but TACL increased. The hepatic and plasma total lipids deviated from WAT by containing higher proportions of 16:0 and lower proportions of 18:0 in the fasted compared with the fed animals. The proportion of plasma 24:1n-9 increased several-fold due to fasting. In the liver lipids the n-3/n-6 PUFA ratio decreased.

The FM of the most abundant FAs in different adipose tissues of the trunk is represented in Figure 1. The FM of SFAs correlated inversely with the chain length in all WATs ($r_s = -0.389$ to -0.709, $P < 0.01$; Fig. 2). The same was observed in 1n-5, 1n-7, and 1n-9 MUFAs ($r_s = -0.330$ to -0.403, $P < 0.01$; Fig. 3a) but not in 1n-11 MUFAs. The influence of chain length on the FM of PUFAs was investigated by comparing PUFAs that had the same degree of unsaturation and position of the first double bond from the methyl end but a different chain length. The difference was statistically significant in five of eight pairs in that the PUFA with the longer chain length had a lower mobilization rate (Fig. 4a). When SFAs and corresponding MUFAs were compared, FM increased with Δ9-desaturation in one of five cases. The influence of unsaturation on the FM of PUFAs was investigated by comparing PUFAs that had the same chain length and position of the first double bond but a different double-bond number. The difference was significant in five of nine pairs in that the PUFA with the higher number of double bonds had also higher FM (Fig. 4b). The influence of positional isomerism on the FM of MUFAs was investigated by comparing MUFAs that had the same chain length but a different position of the double bond. The FMs of 14:1n-, 16:1n-, and 18:1n-MUFAs correlated inversely with the position of the double bond ($r_s = -0.211$ to -0.663, $P < 0.05$; Fig. 3b), but this was not observed for 20:1n- and

Table 4. Effects of 56 Days of Fasting on the Fat Masses and Tissue Lipase Activities of the Raccoon Dogs (Mean ± SE)

	Fed	Fasted
Subcutaneous fat, g	1559 ± 241[a]	861 ± 77[a]
Omental fat, g	138 ± 18[a]	87 ± 10[a]
Retroperitoneal fat, g	153 ± 15[a]	97 ± 13[a]
Mesenteric fat, g	157 ± 13[a]	95 ± 10[a]
Total intra-abdominal fat, g	456 ± 39[a]	283 ± 33[a]
Total fat, g	2015 ± 252[a]	1144 ± 109[a]
Fat, % BM	21.0 ± 1.97[a]	15.9 ± 1.12[a]
Subcutaneous lipase, g 2-naphthol whole subcutaneous fat^{-1} hr^{-1}	3.4 ± 0.55[a]	1.6 ± 0.28[a]
Omental lipase, mg 2-naphthol whole omental fat^{-1} hr^{-1}	199 ± 25[a]	80 ± 13[a]
Mesenteric lipase, mg 2-naphthol whole mesenteric fat^{-1} hr^{-1}	257 ± 82	130 ± 27
Retroperitoneal lipase, mg 2-naphthol whole retroperitoneal fat^{-1} hr^{-1}	248 ± 27[a]	143 ± 20[a]
Liver lipase, g 2-naphthol whole liver^{-1} hr^{-1}	5.7 ± 0.48[a]	4.0 ± 0.32[a]
Kidney lipase, mg 2-naphthol whole kidneys^{-1} hr^{-1}	727 ± 90[a]	470 ± 35[a]
Muscle lipase, μg 2-naphthol mg^{-1} hr^{-1}	3.3 ± 0.38	3.4 ± 0.20

[a] Significant difference between the fed raccoon dogs and the animals fasted between December 3, 2003, and Jan 27, 2004 (*t*-test, Mann-Whitney *U* test, $P < 0.05$).

Table 5. Effects of Fasting on the Plasma Biochemical Variables of the Raccoon Dogs (Mean ± SE)

	Fed			Fasted		
	November 26	December 30	January 27	November 26 Before fasting	December 30 After 4-week fasting	January 27 After 8-week fasting
Glucose, m*M*	6.7 ± 0.37	5.6 ± 0.32	6.0 ± 0.22	5.9 ± 0.50	4.7 ± 0.37	6.6 ± 0.44
Triacylglycerols, m*M*	0.76 ± 0.23	0.73 ± 0.13	0.54 ± 0.04[a]	0.63 ± 0.08	0.89 ± 0.08	0.74 ± 0.07[a]
Glycerol, μ*M*	233 ± 90	223 ± 63	88 ± 22[a]	222 ± 34	110 ± 19	179 ± 24[a]
Cholesterol, m*M*	3.8 ± 0.46[a]	4.4 ± 0.30[a]	3.1 ± 0.29[a]	5.7 ± 0.46[a]	3.2 ± 0.25[a]	3.9 ± 0.26[a]
Urea, m*M*	4.0 ± 0.12	6.7 ± 0.13[a]	5.4 ± 0.47[a]	4.6 ± 0.59	2.6 ± 0.50[a]	3.2 ± 0.37[a]
Ammonia, μ*M*	170 ± 9	175 ± 5	153 ± 5	157 ± 2	158 ± 3	150 ± 3
Uric acid, μ*M*	75.6 ± 15.63[a]	111.4 ± 24.73	24.9 ± 2.00	34.9 ± 1.87[a]	59.5 ± 21.19	24.8 ± 2.02
Total protein, g/liter	64.1 ± 3.44	69.6 ± 2.61[a]	55.3 ± 1.12[a]	57.5 ± 3.12	60.7 ± 2.70[a]	51.1 ± 1.55[a]
Bilirubin, μ*M*	29.2 ± 8.83[a]	42.9 ± 12.11	2.8 ± 0.47	5.0 ± 0.95[a]	23.3 ± 11.11	2.4 ± 0.23
ALT, U/liter	60 ± 21	53 ± 6	63 ± 13	50 ± 8	50 ± 5	37 ± 6
AST, U/liter	73 ± 11	95 ± 21	68 ± 10	46 ± 4	73 ± 9	51 ± 2
Creatine kinase, U/liter	296 ± 40	870 ± 349	407 ± 40	291 ± 42	481 ± 173	337 ± 70
Creatinine, μ*M*	117 ± 4	124 ± 9	125 ± 5	110 ± 6	131 ± 15	139 ± 8

[a] Significant difference between the fed raccoon dogs and the animals fasted between December 3, 2003, and January 27, 2004 (*t*-test, Mann-Whitney *U* test, $P < 0.05$).

22:1n-MUFAs. When comparing PUFAs that had the same chain length and unsaturation but a different position of the first double bond, the difference was statistically significant in two of seven pairs in that the PUFA with the first double bond closer to the methyl end of the molecule had a lower or a higher mobilization rate.

Discussion

Although weight cycling can induce adverse health effects in humans (1), the BM changes of wild animals are not pathologic, but rather a natural part of their circannual rhythms regulated by factors such as photoperiod (20). After the 8-week fasting period, the raccoon dogs were in phase II of fasting with stimulated fat oxidation and conservation of body proteins. Fat hydrolysis was indicated by the 43% decrease in body fat stores. While WAT can account for 30% of BM of the species in late autumn (12), fat reserves are utilized economically in winter. Energy can be saved by hypothermic bouts in the morning (–0.2 to 0.8°C for 4–5 hrs; Ref. 28) and downregulation of metabolism (–25 %; Ref. 29). In addition to fat hydrolysis, some body proteins must have been utilized for gluconeogenesis during fasting. However, the low rate of weight loss (0.56% BM per day), stable tissue protein and plasma ammonia and uric acid concentrations, and decreased plasma urea levels and ratios of urea to creatinine indicate that protein catabolism was limited. Bears (*Ursus* sp.) have the capacity to reutilize >99% of urea produced during winter sleep, leading to efficient conservation of skeletal muscle protein (30). It is

Table 6. Effects of 56 Days of Fasting on the Tissue Glycogen and Protein Concentrations and Enzyme Activities of the Raccoon Dogs (Mean ± SE)

	Fed	Fasted
Liver		
Glycogen, g whole liver^{-1}	2.4 ± 0.29	2.1 ± 0.42
Phosphorylase, g P whole liver^{-1} hr^{-1}	12.2 ± 1.24[a]	7.0 ± 1.15[a]
Glucose-6-phosphatase, g P whole liver^{-1} hr^{-1}	7.2 ± 0.68[a]	5.4 ± 0.43[a]
Alkaline phosphatase, mol *p*-nitrophenol whole liver^{-1} hr^{-1}	2.1 ± 0.27[a]	4.2 ± 0.85[a]
Protein, g whole liver^{-1}	12.0 ± 0.90[a]	7.8 ± 0.63[a]
Kidney		
Glycogen, mg whole kidneys^{-1}	32.1 ± 3.14	25.9 ± 1.80
Phosphorylase, mg P whole kidneys^{-1} hr^{-1}	270 ± 38	203 ± 15
Glucose-6-phosphatase, mg P whole kidneys^{-1} hr^{-1}	781 ± 66[a]	508 ± 49[a]
Muscle		
Glycogen, μg mg^{-1}	2.0 ± 0.38	1.4 ± 0.11
Phosphorylase, μg P mg^{-1} hr^{-1}	233 ± 14[a]	165 ± 14[a]
Protein, μg mg^{-1}	54.8 ± 2.60	60.9 ± 2.98

[a] Significant difference between the fed raccoon dogs and the animals fasted between December 3, 2003, and January 27, 2004 (*t*-test, Mann-Whitney *U* test, $P < 0.05$).

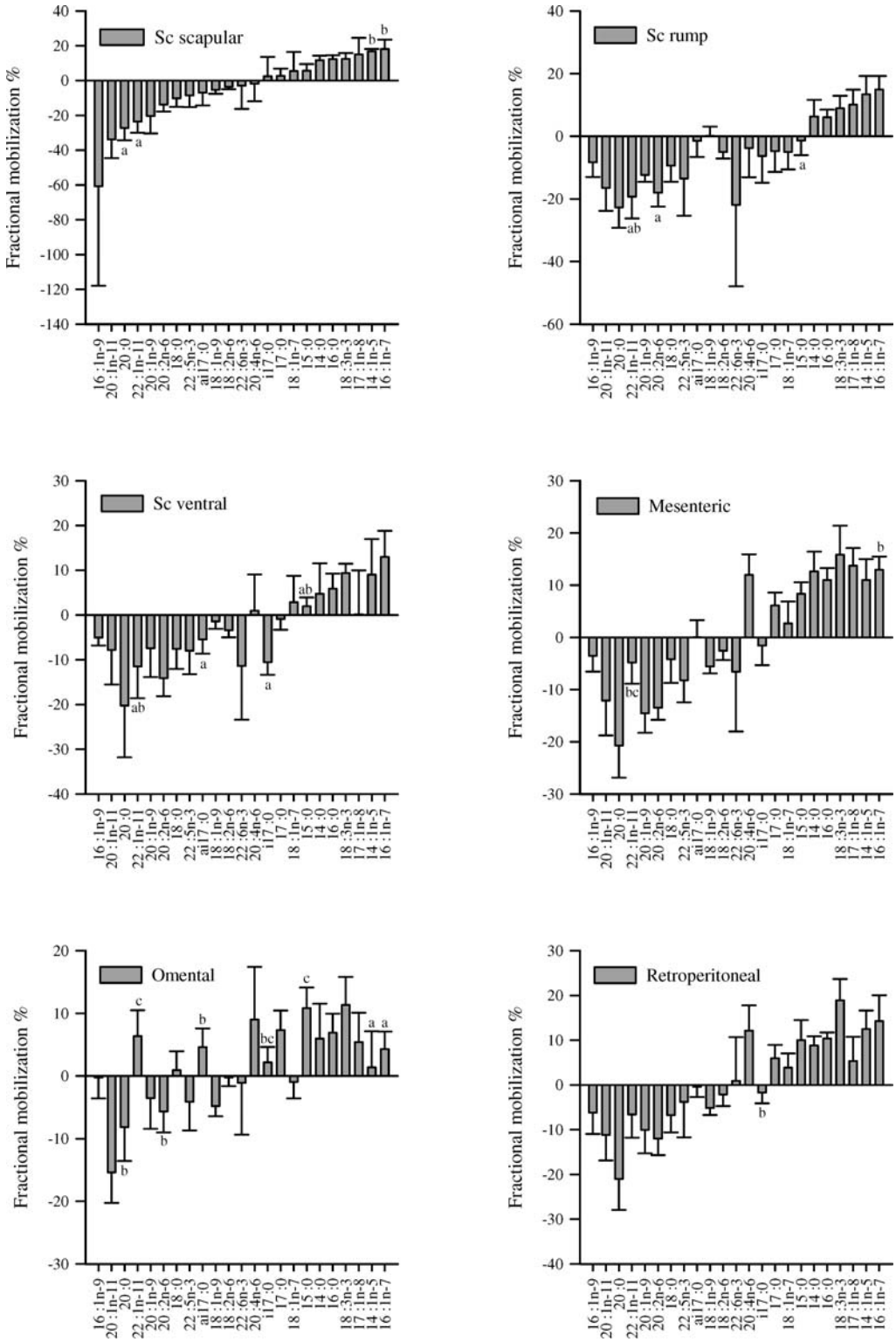

Figure 1. *In vivo* fractional mobilization (%) of the most abundant FAs from different adipose tissues of the raccoon dogs fasted for 56 days (mean + SE). Fractional mobilization was calculated by the formula: [mol% in the fed animals − mol% in the fasted animals] ÷ [mol% in the fed animals]. Positive values indicate that an FA has decreased in proportion during the fast, and negative values signify its increase in proportion compared to the fed control group. Dissimilar letters indicate significant differences between the adipose tissues (one-way ANOVA, $P < 0.05$). Note that the scales of the *y* axes differ from each other. sc, subcutaneous; iab, intra-abdominal.

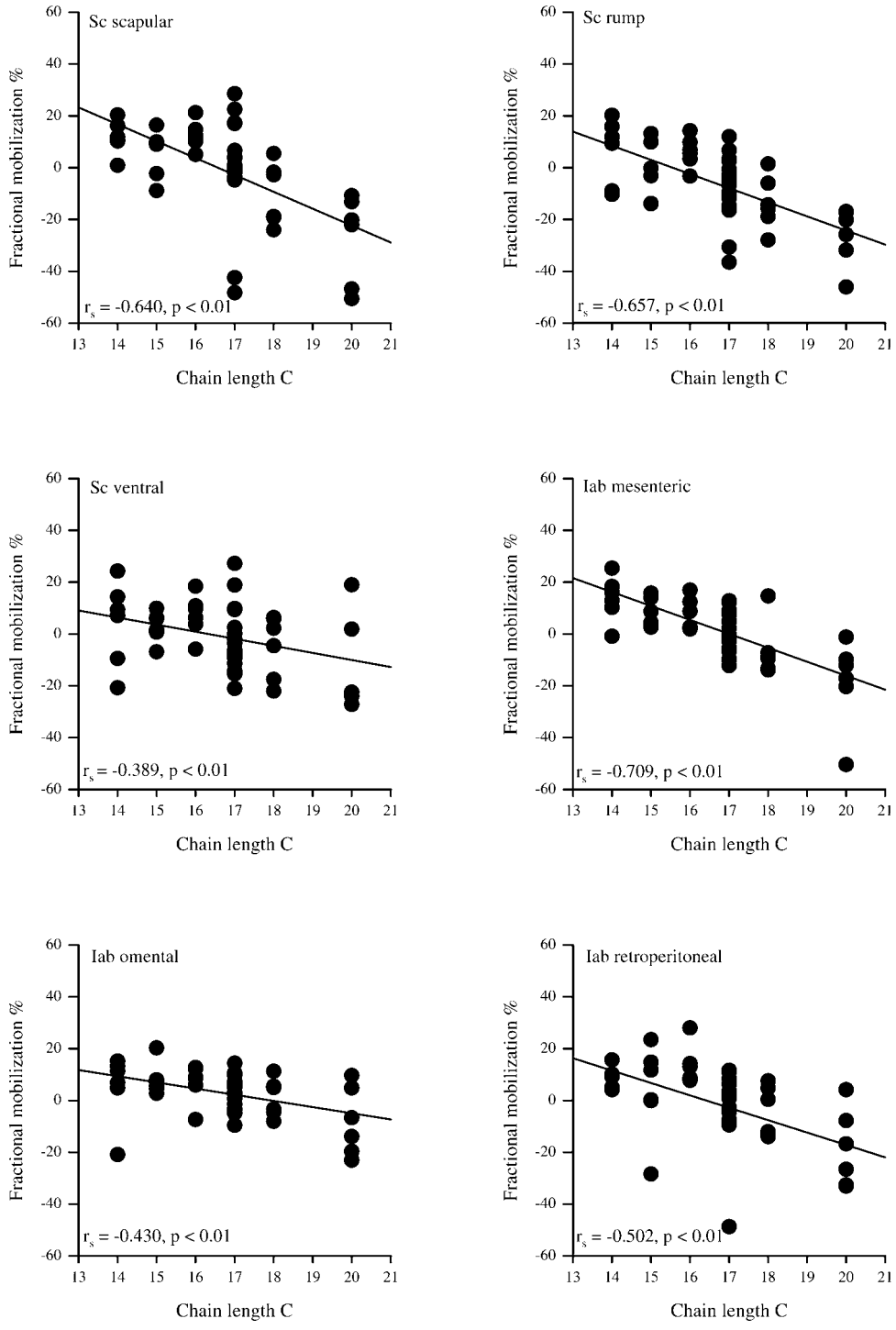

Figure 2. The correlations between fractional mobilization (%) and chain length (C) of saturated FAs in different adipose tissues of the raccoon dogs fasted for 56 days. Fractional mobilization was calculated by the formula: [mol% in the fed animals – mol% in the fasted animals] ÷ [mol% in the fed animals]. Positive values indicate that an FA has decreased in proportion during the fast, and negative values signify its increase in proportion compared with the fed control group. sc, subcutaneous; iab, intra-abdominal.

not known whether the raccoon dog can recycle urea-N (15).

It must be emphasized that also the fed raccoon dogs lost weight (–7.5% BM; $P > 0.05$). The wintertime weight loss is a part of the natural weight cycling of the species, as the appetite of the raccoon dog decreases in the winter (20). The slightly negative energy balance of the fed animals may have decreased their tissue glycogen concentrations to the levels of the fasted group. On the other hand, the American black bear *Ursus americanus* is able to maintain stable muscle glycogen concentrations during denning (31). Long-term wintertime fasting does not cause deleterious effects on

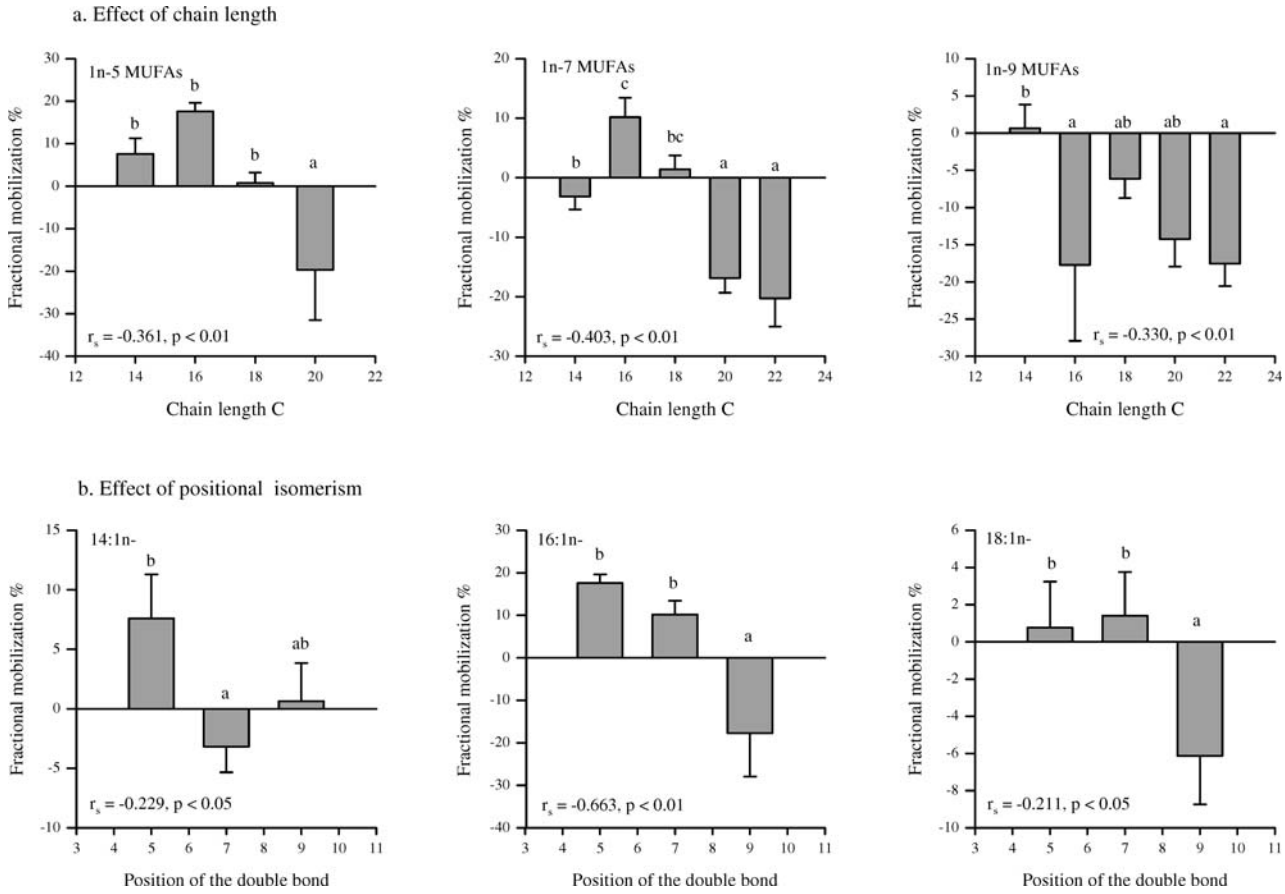

Figure 3. The effect of chain length (a) and position of the double bond (b) on fractional mobilization (%) of MUFAs (mean + SE). Fractional mobilization was calculated by the formula: [mol% in the fed animals − mol% in the fasted animals] ÷ [mol% in the fed animals]. Positive values indicate that an FA has decreased in proportion during the fast, and negative values signify its increase in proportion compared to the fed control group. The results have been pooled from six subcutaneous and intra-abdominal adipose tissues. Dissimilar letters signify statistically significant differences between the bars (one-way ANOVA, $P < 0.05$).

the health of the raccoon dog (14, 15). The increase in the relative adrenal mass presumably was not caused by stress, as the absolute adrenal weight was unaffected. Fasting did not induce liver dysfunction, unlike in carnivores without sophisticated adaptations to fasting, such as the American mink (*Mustela vison*; Ref. 32). The blood count revealed minor decreases in the granulocyte count, RDW, and MCV, the latter of which may be related to factors such as incipient iron deficiency (33).

The lipid masses of the fasted raccoon dog decreased in the following order of magnitude: subcutaneous (–45%) > mesenteric (–40%) > omental and retroperitoneal fats (–37%). Also, some actively wintering carnivores, such as red foxes *Vulpes vulpes*, utilize both subcutaneous and iab fat stores in the winter (34). Harp seal *Phoca groenlandica* pups mobilize core lipids as well as blubber during the postweaning fast (35). The thick-haired raccoon dog may be able to recruit its subcutaneous fat as an additional energy store without jeopardizing insulation. According to the demands of regional body temperature (36), the Δ9-DI and the ratio of unsaturated FAs (UFAs) to SFAs were higher in subcutaneous rump and ventral fats than in iab fats. In the rat, no clear site-specific differences in the selective FA mobilization were found *in vitro* (37), but the regional fat depots of the raccoon dog had some specializations of FA release. Omental fat diverged the most from the other depots, such as by mobilizing more 15:0, *i*17:0, *ai*17:0, and 22:1n-11 and by preserving more 14:1n-5 and 16:1n-7, whereas subcutaneous fat preserved more 22:1n-11 than iab fat. This suggests that *in vivo* the FA mobilization of the raccoon dog can depend on the location of WAT.

The FA composition of the WAT of the farmed animals was similar to that in wild raccoon dogs (16), showing that the diet had been nutritionally balanced prior to the fast. The WAT FA composition of the species corresponded quite closely to other carnivores such as the European brown bear *Ursus arctos arctos* and the gray wolf *Canis lupus* (16). As previously demonstrated in laboratory rodents (5), humans (9), and wild emperor penguins *Aptenodytes forsteri* (38), the mobilization of FAs from the WAT was selective. Fasting decreased the proportions of short-chain SFAs and MUFAs, whereas long-chain SFAs and MUFAs were enriched. In addition, particular n-6 PUFAs increased and n-3 PUFAs decreased in proportion, 22:6n-3 being the most

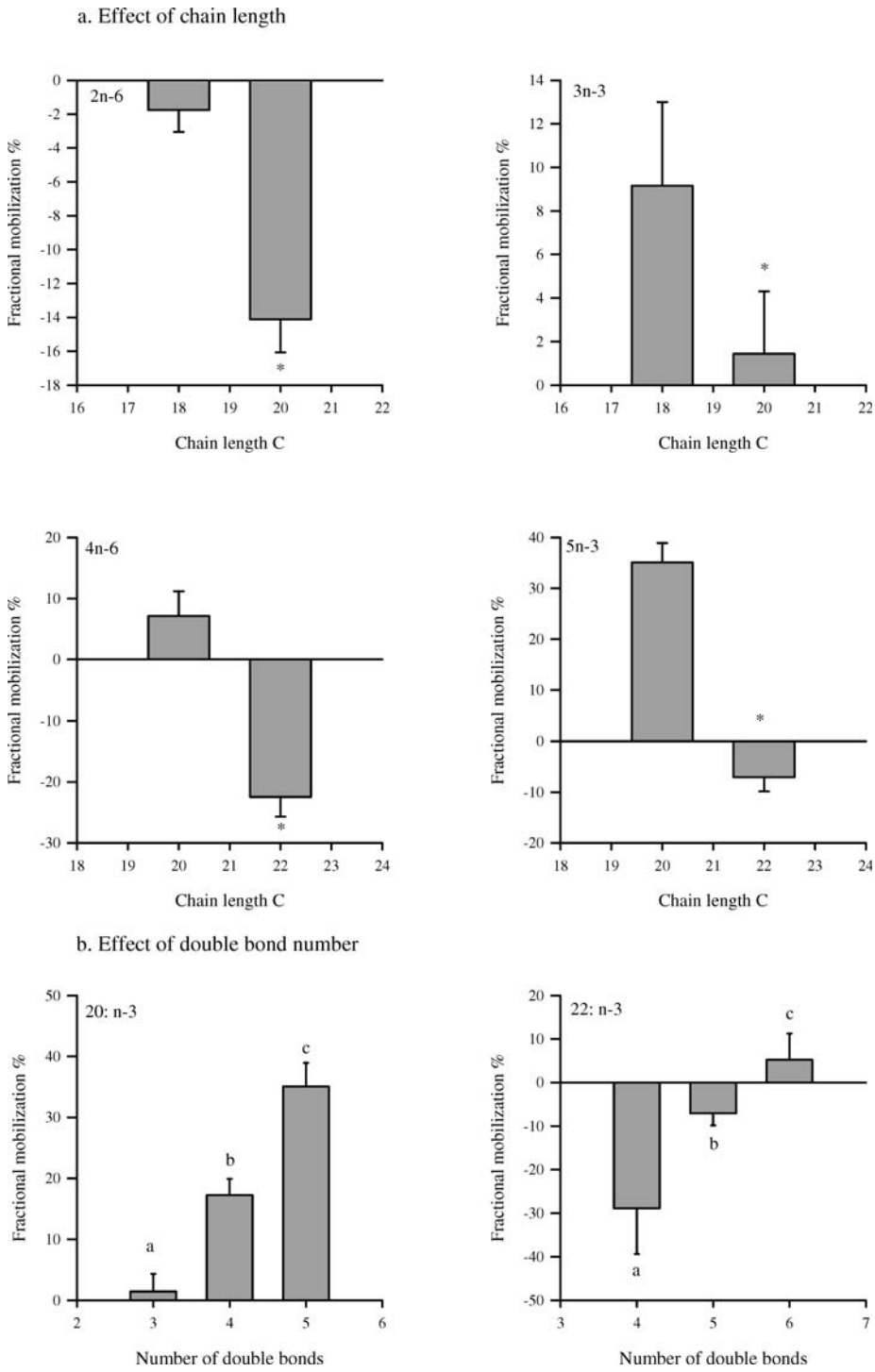

Figure 4. The effect of chain length (a) and double-bond number (b) on fractional mobilization (%) of PUFAs (mean + SE). Fractional mobilization was calculated by the formula: [mol% in the fed animals – mol% in the fasted animals] ÷ [mol% in the fed animals]. Positive values indicate that an FA has decreased in proportion during the fast, and negative values signify its increase in proportion compared to the fed control group. The results have been pooled from six subcutaneous and intra-abdominal adipose tissues. Asterisks (*t*-test, $P < 0.05$) and dissimilar letters (one-way ANOVA, $P < 0.05$) signify statistically significant differences between the bars.

important exception. Different structural preferences have been reported for FA utilization in fasting mammals. The frugivorous fat-tailed dwarf lemur *Cheirogaleus medius* (39) and the insectivorous echidna *Tachyglossus aculeatus* (40) mobilize mainly MUFAs during hibernation, whereas the herbivorous yellow-bellied marmot *Marmota flaviventris* (41) utilizes SFAs. The omnivorous European brown bear uses UFAs during winter sleep (42), whereas the

denning American black bear mobilizes SFAs, MUFAs, and PUFAs (43). The southern elephant seal *Mirounga leonina* cow, feeding mainly on cephalopods and fish, mobilizes longer-chain PUFAs while fasting and nursing (44). The differences in FA mobilization may be associated with factors such as different FA composition of diets, geographical distribution, and strategies for seasonal rest.

Like observed *in vivo* in the raccoon dog, 16:1n-7, 18:3n-3, and 20:4n-6 are more readily mobilized *in vitro* from the rat retroperitoneal fat than 16:1n-9, 18:0, 20:1n-11, 20:1n-9, 22:1n-11, and 22:5n-3 (45). Although there are also differences in FM between the raccoon dog and the rat, the longer-chain SFAs and MUFAs 20:0, 20:1n-11, 20:1n-9, and 22:1n-11 have relatively low mobilization rates in both species. Subcutaneous fat of the fasting emperor penguin also shows low mobilization values for longer-chain MUFAs (38). These preferences could be a general feature of vertebrate adipose tissue and could be related to physicochemical properties of FAs. In the present study the FA composition was determined from total lipids, also including membrane lipids. The FA composition of phospholipids (PLs) is different from that of TAGs. As membrane PLs are not mobilized, their contribution to total lipids presumably increased throughout the fast. This may have led to underestimation of FM of, for example, 18:0 and 20:4n-6 abundant in biological membranes.

The mechanisms that stimulate or prevent the loss of particular FAs from adipose tissue include selective hydrolysis of TAGs by hormone-sensitive lipases, selectivities of phospholipases and acyltransferases for individual FAs, and selective transport of FAs to the extracellular compartment (46, 47). Selective uptake of FAs from plasma and their esterification in WAT also could affect tissue FA composition. In rats, FAs in white and brown adipose tissues (BAT) are more readily mobilized during fasting when they are (i) short, (ii) unsaturated, and (ii) have double bonds located close to the terminal methyl group of the chain (5, 46). As elongation, desaturation (48), and *de novo* synthesis of FAs (49) are probably downregulated during fasting, the differences in the mobilization rates at least partly originate from the hydrolysis of TAG stores. Raclot and Groscolas (45) have suggested that TAGs are distributed in the lipid droplet according to their polarity. As the shorter and more unsaturated FAs are more polar, they are located at the periphery of the lipid droplet and are more accessible to hormone-sensitive lipases, resulting in the preservation of the longer-chain FAs. Selectivity also may arise from the fact that the positional distribution of different FAs in the glycerol backbone of lipids is different (50, 51), and the function of lipases could be position specific. Similar to the rat (5, 46), the number of double bonds had some effect on the FM of PUFAs in the raccoon dog, but the influence of unsaturation was less clear than that of the chain length.

The mobilization of shorter-chain FAs and the accumulation of longer-chain FAs would lead to solidification of the fat depots by increasing the melting point. As the *de novo* synthesis of shorter-chain FAs cannot be stimulated during fasting, the raccoon dog WAT may utilize peroxisomal chain shortening and convert longer-chain FAs into shorter molecules. Since the first double bond is almost exclusively introduced to the $\Delta 9$ position in mammalian cells (48), the double-bond position of a MUFA is an indicator of its metabolic pathway. For instance, the efficiently utilized pool of 16:1n-7 may have been partly replaced by the elevated peroxisomal chain shortening of 18:1n-9, producing more 16:1n-9 (Fig. 1). However, even if present, the mechanism was not able to fully counteract the preferable mobilization of short-chain FAs, and the average chain length of the acyl residues increased.

In general, n-6 PUFAs increased and n-3 PUFAs decreased in proportion due to fasting. This is in accordance with the principle of competitive inhibition in PUFA metabolism of mammalian cells, which states that in the rate-limiting steps of FA desaturations n-3 substrates are favored over n-6 ones (2). Also, the fasting rat has been noted to enrich n-6 PUFAs in BAT TAGs and PLs (46). In the scarcity of n-3 PUFAs, the shorter-chain n-3 PUFAs could be metabolized to produce the biologically most important member of the n-3 family, 22:6n-3, the percentages of which were well preserved. The long-chain PUFAs are important for the physical properties and functions of biologic membranes (2, 52). As a net effect of peroxisomal chain shortening and preferential utilization of n-3 PUFAs, the proportions of the FAs with double bonds located near the methyl end tended to decrease during fasting. In addition, many other FAs with double bonds near the methyl end are PUFAs that originate from the diet, are very hydrophilic, and decreased in proportion during fasting. Thus, we suggest that the double-bond position *per se* as an underlying structural property may not be the causative factor affecting the FM of FAs.

The proportion of n-3 PUFAs and the ratio of n-3 to n-6 PUFAs decreased in the livers, and the percentage of n-6 PUFAs increased in plasma and subcutaneous fats of the fasting raccoon dogs. A change in the ratio of n-3 to n-6 PUFAs in mammalian diets and tissue lipids affects the quality of eicosanoids produced (2). When the one-, two- and four-series prostaglandins (derived from cyclooxygenated n-6 PUFAs) increase their proportion at the expense of three-series prostaglandins (derived from cyclooxygenated n-3 PUFAs), and comparable cascades occur in other eicosanoids as well, platelet aggregation, vascular tonus, and blood pressure change in an undesirable direction. Therefore, fasting or weight cycling may cause adverse health effects through altered eicosanoid production. The nutritional status of an animal affects the FA composition of its WAT, which should be taken into account when interpreting FA profiles of wild animals with unknown nutritional history. For example, starvation and diseases can affect the FA pool, and thus great care should be taken when using tissue FA signatures of dead animals as indicators of their diet. When monitoring the dietary supply of essential

PUFAs in humans by utilizing tissue FA composition, the possible effects of weight cycling on, for instance, the ratio of n-3 to n-6 PUFAs should be recalled.

The effects of fasting on plasma FA composition have been investigated previously in the European brown bear, with increased proportions of 16:0, 18:2n-6, and 22:6n-3, and decreased percentages of 17:0 and 20:5n-3 (42). These responses may be common to passively wintering carnivores, as similar changes also were noted in the plasma of the raccoon dog. Levels of 18:2n-6 also are increased in the plasma, liver (53), and BAT (46) of fasted rats and in the depot fat of hibernating echidnas (40). In addition to the raccoon dog, a decrease in the ratio of n-3 to n-6 PUFAs has been found in the hepatic lipids of the fasted American mink (54). The observed changes in the ratio of n-3 to n-6 PUFAs are seemingly not hazardous to the raccoon dog. Understanding the mechanisms behind the adaptations of wild mammals may in the future offer new strategies to counteract the harmful effects of increased n-6 PUFA levels in humans.

In conclusion, the *in vivo* FA mobilization from the different WATs of the fasting raccoon dog was a selective process, and the molecular structure of FAs affected its efficiency. Fasting stimulated the mobilization of shorter-chain SFAs, MUFAs, and PUFAs, whereas unsaturation and the position of the first double bond had a smaller effect on the mobilization rate. The effect of double-bond position probably is due to chain shortening of long-chain MUFAs and preferential utilization of n-3 PUFAs over n-6 PUFAs. There were site-specific differences in FM, the omental adipose tissue being the most divergent.

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