

Fatty Acid Composition and Development of Hepatic Lipidosis During Food Deprivation—Mustelids as a Potential Animal Model for Liver Steatosis

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Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome characterized by asymptomatic hepatic steatosis. It is present in most cases of human obesity but also caused e.g., by rapid weight loss. The patients have decreased n-3 polyunsaturated fatty acid (PUFA) proportions with decreased percentages of 18:3(n-3), 20:5(n-3) and 22:6(n-3) and an increased n-6/n-3 PUFA ratio in liver and/or white adipose tissue (WAT). The present study examined a new experimental model to study liver steatosis with possible future applications to NAFLD. Ten European polecats (*Mustela putorius*), the wild form of the domestic ferret, were food-deprived for 5 days with 10 fed animals as controls. The food-deprived animals showed micro- and macrovesicular hepatic steatosis, decreased proportions of 20:5(n-3), 22:6(n-3) and total n-3 PUFA and increased n-6/n-3 PUFA ratios in liver and WAT. At the same time, the product/precursor ratios decreased in liver. The observed effects can be due to selective fatty acid mobilization preferring n-3 PUFA over n-6 PUFA, decreased $\Delta 5$ and $\Delta 6$ desaturase activities, oxidative stress, decreased arginine availability and activation of the endocannabinoid system. Hepatic lipidosis induced by food deprivation was manifested in the fatty acid composition of the polecat with similarities to human NAFLD despite the different principal etiologies. Exp Biol Med 234:278–286, 2009

Key words: fatty acids; hepatic lipidosis; *Mustela putorius*; NAFLD; PUFA

Introduction

The liver has a central role in lipid metabolism by importing serum free fatty acids (FA) and synthesizing and exporting lipids and lipoproteins (1). Hepatic lipid accumulation can be caused e.g., by increased FA influx, increased lipogenesis, impaired β -oxidation and reduced lipid export. Non-alcoholic fatty liver disease (NAFLD) is a manifestation of the metabolic syndrome. Its prevalence is 60% for non-alcohol-drinking obese adults in Japan (2) and 77% for obese children in China (3). The total incidence of hepatic steatosis, mostly non-alcoholic, is 31% in adults in the USA (4) and it is emerging as the most common liver abnormality (1). NAFLD is usually asymptomatic (1) and can be accompanied by normal liver transaminase values (5–6), whereas non-alcoholic steatohepatitis (NASH) may develop in more than one third of obese NAFLD patients (7).

NAFLD is characterized by depletion of n-3 long-chain polyunsaturated FA (LCPUFA; carbon chain length ≥ 20 C) from liver, an increase in the n-6/n-3 PUFA ratio in liver and white adipose tissue (WAT) and insulin resistance (8). The proportion of 18:2(n-6) increases in WAT, while the percentage of 18:3(n-3) decreases. The diet of NASH patients can contain more n-6 PUFA and have a higher n-6/n-3 PUFA ratio than the diet of controls, while some studies do not totally comply (9–10). Still, NAFLD can be ameliorated with n-3 PUFA supplementation (11). n-3 PUFA are efficient ligand activators of the peroxisome proliferator-activated receptors (PPAR), and n-3 PUFA depletion can favor FA and triacylglycerol (TAG) synthesis over FA oxidation and suppress very-low-density lipoprotein (VLDL) secretion (12).

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Table 1. The Proportions of the Most Abundant Fatty Acids in the Diet of the Polecats (Mean $\pm$ SE)^a

Fatty acid	Mol % in diet
14:0	2.82 $\pm$ 0.06
16:0	23.32 $\pm$ 0.15
16:1(n-7)	2.80 $\pm$ 0.03
18:0	13.68 $\pm$ 0.12
18:1(n-9)	31.28 $\pm$ 0.09
18:1(n-7)	2.35 $\pm$ 0.03
18:2(n-6)	11.21 $\pm$ 0.06
18:3(n-3)	2.91 $\pm$ 0.03
20:4(n-6)	0.25 $\pm$ 0.09
20:5(n-3)	0.54 $\pm$ 0.02
22:4(n-6)	0.17 $\pm$ 0.02
22:5(n-6)	0.03 $\pm$ <0.01
22:5(n-3)	0.20 $\pm$ 0.07
22:6(n-3)	0.82 $\pm$ 0.02
SFA	42.84 $\pm$ 0.11
MUFA	39.98 $\pm$ 0.08
PUFA	17.18 $\pm$ 0.17
n-6 PUFA	11.82 $\pm$ 0.12
n-3 PUFA	4.77 $\pm$ 0.06
n-6/n-3 PUFA	2.48 $\pm$ 0.03
UFA/SFA	1.33 $\pm$ 0.01
DBI	0.84 $\pm$ 0.01
Δ 9-DI	0.94 $\pm$ <0.01
TACL	17.42 $\pm$ 0.01

^a SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; DBI, double bond index; Δ 9-DI, Δ 9-desaturation index; TACL, total average chain length. Also minor fatty acids not listed in the table are included in the sums.

Mustelids (*Mustelidae*) are carnivores susceptible to the fatty liver syndrome after a short period of food deprivation (13–15) and when fed a high-fat diet (16). Hepatic lipidosis is a common pathological finding in the lactating American mink (*Neovison vison*) characterized by insulin resistance and n-3 PUFA deficiency (17). In many carnivores, n-3 PUFA are preferentially mobilized over n-6 PUFA (18–19), which could alter the liver lipid metabolism and play a pivotal role in the development of hepatic lipidosis. The European polecat (*Mustela putorius*)—the wild form of the domestic ferret (*M. p. furo*)—has a body mass (BM) of 0.4–1.4 kg and a mostly carnivorous diet (20). The species is adapted to food rich in protein and fat with little fiber (21). The ferret is widely used in biomedical research including respiratory physiology, gastrointestinal diseases such as peptic ulcer, carotenoid metabolism, myocardial infarct models and pharmaceutical drug development due to similarities with human physiology (22).

The principal aims of this experiment were to study: 1) if the polecat FA profile is comparable to humans, 2) how its regional WAT depots differ in FA mobilization, 3) how n-3 and n-6 PUFA respond to food deprivation, 4) if liver lipidosis with potential applications to NAFLD can be induced to the species by food deprivation, 5) if the presence of hepatic lipidosis can be verified by histological

and biochemical methods and 6) if the pathological endpoint, fasting-induced lipidosis has similarities to the steatosis in NAFLD and if these similarities thus warrant the use of moderate food deprivation as an alternative method to study the biochemistry of NAFLD.

Materials and Methods

Twenty farm-bred European polecats (12 males, 8 females, born in spring 2005) were randomly assigned into two groups. The animals were housed in pairs in a commercial fur farm (62.559812° N, 29.126052° E) in cages in a shed at natural temperature and photoperiod and fed with a commercial diet (Super-Fox Kasvu rae, metabolizable energy 16 MJ kg dry weight⁻¹, of which protein 33%, fat 24%, carbohydrates 27–30%; Suomen Rehu Oy, Espoo, Finland) since weaning supplemented with moose (*Alces alces*) carcass offal and liver (20%) from late October 2005 onwards. The FA composition of the diet including the supplementary items is depicted in Table 1. In the diet the energy % derived from fat was slightly lower than measured in the diets of NAFLD patients (24% vs. 31%; (9)) and the ratio of unsaturated FA (UFA)/saturated FA (SFA) was approximately 1.3 compared to the human dietary values of 1.6 and the ratio of 18:2(n-6)/18:3(n-3) was lower (approximately 3.9) compared to 10.3 of human diets.

The experiment was approved by the Animal Care and Use Committee of the University of Joensuu. On January 27, 2006, the food-deprived group ($n = 10$) was subjected to a 5-day fast but water or ice was available *ad lib*. BM were recorded on the first day of the experiment and at sampling and body lengths at sampling. Body mass indices (BMI) indicating body adiposity of mustelids (19) were calculated with the formula: BMI = BM (kg)/[body length³ (m)]. On February 1, the animals were anaesthetized with intramuscular ketamine (20 mg kg⁻¹) and xylazine (2 mg kg⁻¹). The fed group was food-deprived overnight to avoid lipemic plasma. The blood samples were obtained using cardiac punctures and the animals were euthanized with intracardial T 61 (Intervet International B.V., Boxmeer, the Netherlands). The blood samples were placed into test tubes containing EDTA, centrifuged at 4000 $\times$ g for 15 mins and the plasma was removed. Liver samples were dissected and stored at -80°C . WAT samples were collected from subcutaneous (sc) interscapular, sc rump, sc ventral, intra-abdominal (iab) omental (om), iab mesenteric (mes), iab retroperitoneal (rp), iab diaphragmatic (dia), intermuscular (im) and pericardial fat tissues and from peripheral body parts (tail base, tail tip, front paw, rear paw and nose; these tissues consist not only of fat but also e.g., of connective tissue) and stored at -80°C .

A part of each fresh liver was stored in formalin fixative, dehydrated and embedded in paraffin, cut into sections and attached to glass slides for staining with hematoxylin-eosin. The slides were examined three times at

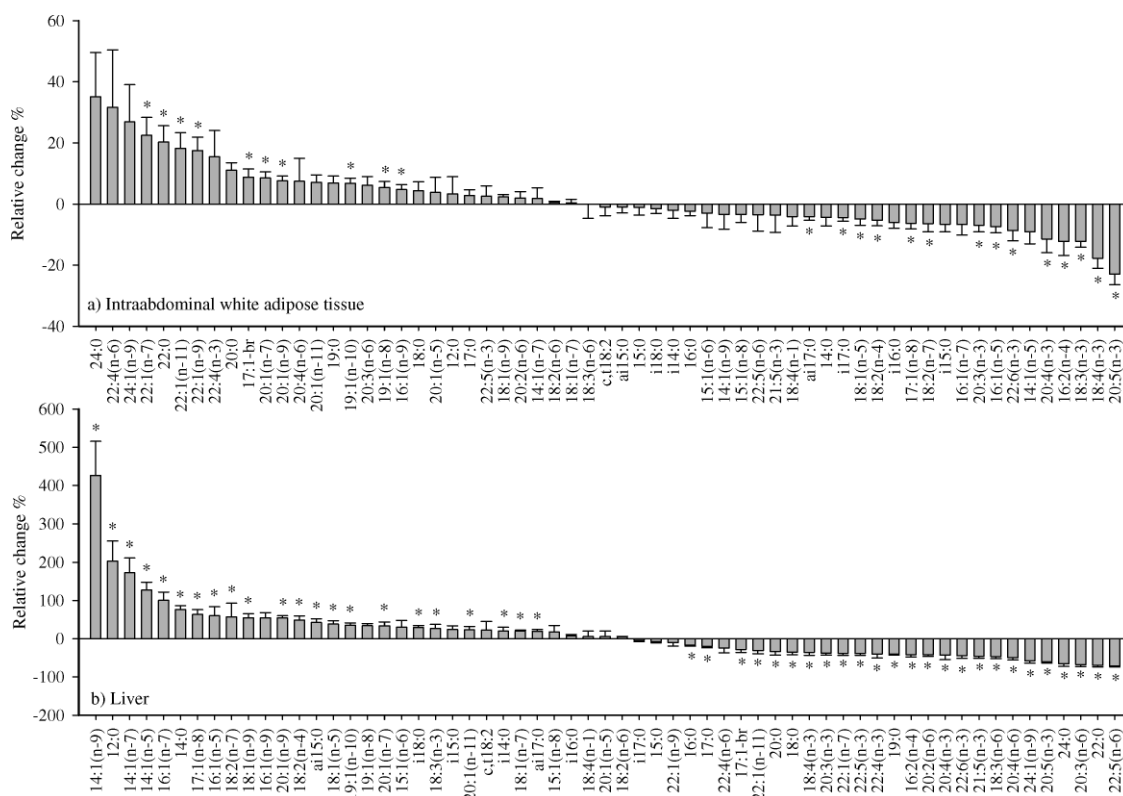


Figure 1. *In vivo* relative change (%) in the proportions of the most abundant fatty acids (FA) in intraabdominal (iab) white adipose tissue (a) and liver (b) of the polecats food-deprived for 5 days, all iab adipose tissues analyzed as a whole (mean + SE). Relative change is the percentage of the initial mol % of a FA that was lost from different adipose tissues during the fast. Positive (+) values indicate that the proportion of a FA has increased in the food-deprived animals compared to the fed control group, negative (–) values signify that the proportion of a FA has decreased compared to the fed group. The asterisk (*) indicates that the proportion (mol %) of a FA in the food-deprived group is significantly different from the corresponding value of the fed animals. Note that the scales of the Y-axes and the positions of the individual FA on the X-axes differ between adipose tissue and liver.

×400 magnification under a light microscope (Leica DM LB, Heerburg, Switzerland) by a consultant pathologist (V. Kärjä) in a randomized double-blind manner. The histological appearance was evaluated as described by Kleiner *et al.* (23). The samples were examined for micro- and macrovesicular steatosis. The extent of macrovesicular steatosis was graded based on the percentage of cells with this type of steatosis (<5, 5–33, 33–66 and >66%). Inflammatory changes were examined by observing the presence of lobular inflammation, portal inflammation, microgranulomas, lipogranulomas and fibrosis. Liver cell injury was examined by observing ballooning and the presence of acidophil bodies, pigmented macrophages and megamitochondria. The samples were also examined for Mallory's hyaline and glycogenated nuclei and evaluated for the presence of borderline or definite steatohepatitis.

The FA compositions of WAT, plasma and liver were determined from total lipids (8) using methods described previously (18–19). The comprehensive list of FA analyzed can be found in Figure 1. The double bond index (DBI) and the total average chain length (TACL) were calculated as described previously (18, 24). The $\Delta 9$ -desaturation index ($\Delta 9$ -DI), the ratio of the most important potentially endogenous $\Delta 9$ -monounsaturated FA (MUFA) to the

corresponding SFA, was calculated according to Käkälä and Hyvärinen (25). The product/precursor ratios (8–9, 26) were calculated for the principal products of the essential PUFA as follows: [mol % 20:4(n-6)]:[mol % 18:2(n-6)] and [mol % 20:5(n-3) + mol % 22:6(n-3)]:[mol % 18:3(n-3)]. The fractional mobilization (FM) was calculated by the formula: (mol % food-deprived animals – mol % fed animals): (mol % fed animals). The FA composition and FM were also determined for the pooled sc (scapular, rump and ventral combined) and iab (om, mes, rp and dia combined) fat depots. The plasma glucose, total cholesterol (Chol), low-density lipoprotein (LDL) Chol, high-density lipoprotein (HDL) Chol, TAG, free FA, arginine (Arg), insulin and cortisol concentrations, the plasma alanine (ALT) and aspartate aminotransferase (AST) activities and the liver Chol and TAG concentrations were determined according to Mustonen *et al.* (14, 27–28).

Comparisons between the two experimental groups were performed with the Student's *t* test for independent samples or, for nonparametric data, with the Mann-Whitney *U* test. Multiple comparisons between the trunk and extremity tissues were performed with the one-way analysis of variance (ANOVA) followed by the Duncan's *post hoc* test. The occurrence of steatosis in the histological samples

Table 2. General Biochemical and Endocrinological Variables of the Fed and Food-Deprived Polecats (Mean $\pm$ SE)^a

	Fed	Food-deprived
P glucose mmol l ⁻¹	10.4 $\pm$ 1.3	8.2 $\pm$ 0.6
P insulin μ U ml ⁻¹	26.3 $\pm$ 9.4	10.0 $\pm$ 1.1*
P cortisol nmol l ⁻¹	7.2 $\pm$ 2.2	10.5 $\pm$ 3.0
P arginine μ mol l ⁻¹	135 $\pm$ 8	76 $\pm$ 7*
P total cholesterol mmol l ⁻¹	3.5 $\pm$ 0.2	2.9 $\pm$ 0.1*
P LDL cholesterol mmol l ⁻¹	0.50 $\pm$ 0.06	0.38 $\pm$ 0.04
P HDL cholesterol mmol l ⁻¹	2.82 $\pm$ 0.18	2.30 $\pm$ 0.11*
P triacylglycerols mmol l ⁻¹	1.1 $\pm$ 0.2	1.1 $\pm$ 0.1
P free fatty acids mmol l ⁻¹	0.27 $\pm$ 0.04	0.54 $\pm$ 0.07*
P ALT U l ⁻¹	115 $\pm$ 18	133 $\pm$ 22
P AST U l ⁻¹	143 $\pm$ 14	146 $\pm$ 17
L total cholesterol mg g liver ⁻¹	2.6 $\pm$ 0.5	7.6 $\pm$ 2.6*
L triacylglycerols mg g liver ⁻¹	11.6 $\pm$ 1.5	28.9 $\pm$ 2.9*

^a P, plasma; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; L, liver.

* Significant difference between the fed and the food-deprived animals.

was tested with the χ^2 test. Correlations were calculated using the Spearman correlation coefficient (r_s). $P < 0.05$ was considered to be statistically significant. The results are presented as mean $\pm$ SE. To analyze the relationships in the FA composition of the anatomically different WAT, the data were also subjected to the multivariate principal component analysis (PCA) using the SIRIUS 6.5 software package [Pattern Recognition Systems AS, Bergen, Norway (29)].

Results

The initial BM (fed: ♂ 2.03 $\pm$ 0.10, ♀ 1.28 $\pm$ 0.04 kg vs. food-deprived: ♂ 2.19 $\pm$ 0.11, ♀ 1.23 $\pm$ 0.03 kg) did not differ between the groups, while the final BM (fed: ♂ 2.09 $\pm$ 0.10, ♀ 1.28 $\pm$ 0.03 kg vs. food-deprived: ♂ 1.91 $\pm$ 0.10, ♀ 1.09 $\pm$ 0.03 kg) of the food-deprived females were lower. The initial BMI did not differ between the groups, while the final BMI of the food-deprived animals were lower (♂ 29.9 $\pm$ 1.1 vs. 26.8 $\pm$ 0.8, ♀ 27.9 $\pm$ 0.7 vs. 22.8 $\pm$ 0.5 kg (m³)⁻¹). The calculated BMI correlated with the total fat mass (males: $r_s = 0.643$, females: $r_s = 0.929$, $P < 0.01$). In the males, the absolute and relative masses of the pericardial fat depot were lower due to food deprivation. In the females, the absolute masses of the rp, mes, dia, total iab and total fat depots and the relative mass of the rp depot were lower in the food-deprived group. The food-deprived animals had significantly more micro- and macrovesicular hepatic steatosis ($P < 0.001$). Of the inflammatory signs, only lobular inflammation was present in 3/10 food-deprived animals and the same number of fasted polecats had also ballooning of the hepatocytes and borderline steatohepatitis, but the occurrence of these signs did not reach statistical significance compared to the fed animals. The liver TAG and Chol concentrations and the plasma free

FA concentrations were higher in the food-deprived animals, while the plasma total Chol, HDL Chol, Arg and insulin concentrations were lower (Table 2). The plasma free FA, liver Chol and liver TAG concentrations correlated with the grade of macrovesicular steatosis ($r_s = 0.592$ – 0.815 , $P < 0.01$) and the liver TAG concentrations also with the observed liver cell injury ($r_s = 0.498$, $P < 0.05$).

The most abundant FA in WAT of the polecats was 18:1(n-9) followed by 18:2(n-6), 16:0, 16:1(n-7), 18:0, 18:1(n-7), 14:0 and 18:3(n-3) (Table 3). In liver and plasma, the proportion of 18:1(n-9) was lower and the percentages of 16:0, 18:0, 18:2(n-6), 20:4(n-6), 22:5(n-3) and 22:6(n-3) were higher than in WAT. Due to food deprivation, LCPUFA [20:3(n-6), 20:4(n-6), 20:5(n-3), 21:5(n-3), 22:5(n-6), 22:5(n-3) and 22:6(n-3)] decreased in proportion in liver, the total PUFA decreased and the n-6/n-3 PUFA ratio increased. The product/precursor ratios also decreased in liver. The percentages of 22:0, 16:1(n-9), 19:1(n-10), 19:1(n-8), 20:1(n-9), 20:1(n-7), 22:1(n-11), 22:1(n-9) and 22:1(n-7) increased in the total iab fat, while the proportions of *i*17:0, *ai*17:0, 18:1(n-5), 16:2(n-4), 18:2(n-7), 18:2(n-4), 18:3(n-3), 18:4(n-1), 20:3(n-3), 20:4(n-3), 20:5(n-3) and 22:6(n-3) decreased. The total n-3 PUFA decreased and the n-6/n-3 PUFA ratio increased. The proportion of total SFA was lower and that of total PUFA higher in the extremities than in the trunk (online supplemental material). PCA revealed that the nasal samples differed from the other extremities by containing higher percentages of total SFA and PUFA, a higher n-6/n-3 PUFA ratio, DBI and TACL, a lower proportion of total MUFA and a lower $\Delta 9$ -DI. The extremity fats showed only minor differences due to fasting.

There were no significant differences in FM between the sc and iab fat depots. In the total iab fat the FA that were the most efficiently mobilized were 20:5(n-3), 18:4(n-3), 18:3(n-3), 16:2(n-4), 20:4(n-3), 14:1(n-5), 22:6(n-3), 16:1(n-5) and 20:3(n-3), while 24:0, 22:4(n-6), 24:1(n-9), 22:1(n-7), 22:0, 22:1(n-11) and 22:1(n-9) were preserved (Fig. 1). In the sc WAT most FA were preserved, while FM of the im depot were between those of the sc and iab fats. FM of SFA correlated negatively with the chain length and the same was observed in n-7, n-9 and n-11 MUFA ($r_s = -0.159$ – -0.269 , $P < 0.01$). The influence of chain length on FM of PUFA was tested by comparing PUFA with the same degree of unsaturation and position of the first double bond from the methyl end but with a different chain length. The difference was significant in 29% of the cases in that the PUFA with the longer chain length had a lower FM value. When SFA and the corresponding MUFA were compared, FM increased with $\Delta 9$ -desaturation in 2 out of 7 pairs and in 1 out of 9 pairs the PUFA with the higher number of double bonds had higher FM. The MUFA with the double bond closer to the methyl end had higher FM in 2 out of 11 pairs. In PUFA, the difference was significant in 3 out of 7 pairs in that the PUFA with the first double bond closer to the methyl end had higher FM.

Table 3. Effects of Food Deprivation on the Composition of the Most Abundant Fatty Acids (Mol %) in the Total sc and iab Fat Tissues, Plasma and Livers of the Polecats (Mean $\pm$ SE)^a

Fatty acid	Total sc fat			Total iab fat			Plasma			Liver		
	Fed	Food-deprived		Fed	Food-deprived		Fed	Food-deprived		Fed	Food-deprived	
14:0	2.73 $\pm$ 0.07	2.78 $\pm$ 0.06		2.44 $\pm$ 0.07	2.30 $\pm$ 0.07		0.39 $\pm$ 0.04	0.39 $\pm$ 0.03		0.67 $\pm$ 0.05	1.14 $\pm$ 0.08*	
16:0	9.28 $\pm$ 0.15	9.31 $\pm$ 0.18		12.10 $\pm$ 0.20	11.76 $\pm$ 0.19		15.53 $\pm$ 0.29	13.90 $\pm$ 0.28*		17.11 $\pm$ 0.31	14.30 $\pm$ 0.47*	
16:1(n-7)	9.83 $\pm$ 0.36	9.80 $\pm$ 0.37		5.70 $\pm$ 0.28	5.12 $\pm$ 0.22		1.02 $\pm$ 0.07	1.30 $\pm$ 0.07*		1.99 $\pm$ 0.16	3.81 $\pm$ 0.32*	
18:0	4.79 $\pm$ 0.24	4.80 $\pm$ 0.24		8.43 $\pm$ 0.39	8.59 $\pm$ 0.35		17.95 $\pm$ 0.45	18.07 $\pm$ 0.19		17.23 $\pm$ 0.63	10.89 $\pm$ 0.96*	
18:1(n-9)	49.87 $\pm$ 0.39	49.57 $\pm$ 0.40		48.65 $\pm$ 0.42	49.75 $\pm$ 0.47		12.93 $\pm$ 0.45	16.05 $\pm$ 0.35*		23.68 $\pm$ 1.10	36.36 $\pm$ 1.61*	
18:1(n-7)	3.00 $\pm$ 0.03	2.92 $\pm$ 0.03		2.89 $\pm$ 0.04	2.89 $\pm$ 0.03		1.98 $\pm$ 0.04	2.77 $\pm$ 0.14*		2.89 $\pm$ 0.04	3.44 $\pm$ 0.08*	
18:2(n-6)	10.85 $\pm$ 0.05	10.99 $\pm$ 0.06		10.77 $\pm$ 0.04	10.81 $\pm$ 0.04		22.23 $\pm$ 0.85	16.15 $\pm$ 0.35*		14.71 $\pm$ 0.27	15.31 $\pm$ 0.33	
18:3(n-3)	1.68 $\pm$ 0.04	1.65 $\pm$ 0.04		1.60 $\pm$ 0.04	1.40 $\pm$ 0.04*		0.58 $\pm$ 0.05	0.41 $\pm$ 0.01*		1.31 $\pm$ 0.08	1.63 $\pm$ 0.12*	
20:4(n-6)	0.211 $\pm$ 0.004	0.221 $\pm$ 0.005		0.198 $\pm$ 0.005	0.210 $\pm$ 0.015		10.675 $\pm$ 0.673	13.555 $\pm$ 0.307*		5.511 $\pm$ 0.310	2.732 $\pm$ 0.370*	
20:5(n-3)	0.176 $\pm$ 0.008	0.175 $\pm$ 0.011		0.144 $\pm$ 0.008	0.109 $\pm$ 0.007*		2.986 $\pm$ 0.253	1.602 $\pm$ 0.088*		1.385 $\pm$ 0.129	0.496 $\pm$ 0.038*	
22:4(n-6)	0.049 $\pm$ 0.002	0.050 $\pm$ <0.001		0.046 $\pm$ 0.001	0.060 $\pm$ 0.009		0.331 $\pm$ 0.020	0.342 $\pm$ 0.022		0.249 $\pm$ 0.011	0.188 $\pm$ 0.033	
22:5(n-6)	0.016 $\pm$ <0.001	0.017 $\pm$ 0.001		0.013 $\pm$ <0.001	0.013 $\pm$ <0.001		0.175 $\pm$ 0.010	0.143 $\pm$ 0.013		0.111 $\pm$ 0.008	0.031 $\pm$ 0.003*	
22:5(n-3)	0.266 $\pm$ 0.007	0.268 $\pm$ 0.005		0.261 $\pm$ 0.006	0.264 $\pm$ 0.009		1.583 $\pm$ 0.102	2.173 $\pm$ 0.066*		1.632 $\pm$ 0.073	0.989 $\pm$ 0.076*	
22:6(n-3)	0.488 $\pm$ 0.018	0.513 $\pm$ 0.023		0.437 $\pm$ 0.017	0.393 $\pm$ 0.017*		4.947 $\pm$ 0.411	7.281 $\pm$ 0.293*		6.015 $\pm$ 0.273	3.274 $\pm$ 0.422*	
SFA	18.32 $\pm$ 0.42	18.47 $\pm$ 0.41		24.75 $\pm$ 0.52	24.41 $\pm$ 0.50		36.29 $\pm$ 0.37	34.36 $\pm$ 0.21*		36.85 $\pm$ 0.77	27.92 $\pm$ 1.26*	
MUFA	66.85 $\pm$ 0.42	66.53 $\pm$ 0.41		60.87 $\pm$ 0.53	61.46 $\pm$ 0.52		18.13 $\pm$ 0.56	22.44 $\pm$ 0.47*		30.57 $\pm$ 1.30	46.41 $\pm$ 1.91*	
PUFA	14.83 $\pm$ 0.10	15.00 $\pm$ 0.12		14.38 $\pm$ 0.09	14.13 $\pm$ 0.09		45.58 $\pm$ 0.31	43.21 $\pm$ 0.33*		32.59 $\pm$ 0.62	25.68 $\pm$ 0.70*	
n-3 PUFA	11.35 $\pm$ 0.05	11.50 $\pm$ 0.07		11.24 $\pm$ 0.04	11.31 $\pm$ 0.05		34.65 $\pm$ 0.29	30.96 $\pm$ 0.44*		21.58 $\pm$ 0.35	18.69 $\pm$ 0.33*	
n-6 PUFA	2.80 $\pm$ 0.07	2.80 $\pm$ 0.09		2.59 $\pm$ 0.07	2.30 $\pm$ 0.07*		10.53 $\pm$ 0.26	11.80 $\pm$ 0.33*		10.64 $\pm$ 0.32	6.55 $\pm$ 0.40*	
n-6/n-3 PUFA	4.13 $\pm$ 0.11	4.23 $\pm$ 0.14		4.44 $\pm$ 0.11	5.11 $\pm$ 0.15*		3.91 $\pm$ 0.08	4.15 $\pm$ 0.09*		2.04 $\pm$ 0.04	2.91 $\pm$ 0.12*	
UFA/SFA	4.54 $\pm$ 0.13	4.49 $\pm$ 0.12		3.12 $\pm$ 0.09	3.17 $\pm$ 0.09		1.76 $\pm$ 0.03	1.91 $\pm$ 0.02*		1.72 $\pm$ 0.06	2.64 $\pm$ 0.16*	
TACL	17.53 $\pm$ 0.01	17.53 $\pm$ 0.01		17.58 $\pm$ 0.01	17.61 $\pm$ 0.01*		18.29 $\pm$ 0.04	18.44 $\pm$ 0.01*		18.07 $\pm$ 0.03	17.82 $\pm$ 0.04*	
Pro/pre n-6 PUFA	0.019 $\pm$ <0.001	0.020 $\pm$ <0.001		0.018 $\pm$ <0.001	0.019 $\pm$ 0.001		0.498 $\pm$ 0.052	0.842 $\pm$ 0.024*		0.377 $\pm$ 0.025	0.182 $\pm$ 0.030*	
Pro/pre n-3 PUFA	0.390 $\pm$ 0.007	0.411 $\pm$ 0.010		0.358 $\pm$ 0.007	0.354 $\pm$ 0.009		15.300 $\pm$ 2.163	21.983 $\pm$ 0.863*		5.889 $\pm$ 0.471	2.757 $\pm$ 0.794*	

^a sc, subcutaneous; iab, intraabdominal; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; TACL, total average chain length; pro/pre, product-precursor ratio. The fatty acids were determined from total lipids. Also minor fatty acids not listed in the table are included in the sums.

* Significant difference between the fed and the food-deprived animals (Student's *t* test, Mann-Whitney *U* test, *P* < 0.05).

Table 4. Average Liver and WAT FA Profiles of Humans and Fed Polecats (Wt % or Mol %), Manifestations of NAFLD in Liver, WAT and Plasma of NAFLD Patients, Signs of Food Deprivation in Liver, WAT and Plasma of Polecats Food-Deprived for 5 Days^a

	Human liver (8)	Fed polecat liver	Human WAT (8, 30)	Fed polecat WAT
Total SFA	37	37	27–28 (30)	18–25
Total MUFA	29	31	52 (30)	61–67
Total PUFA	33	33	19 (30)	14–15
n-6 PUFA	26	22	9 (8)	11
n-3 PUFA	7	11	2 (8)	3
n-6/n-3 PUFA ratio	4	2	4 (8)	4
	NAFLD liver (8)	Food-deprived polecat liver	NAFLD WAT (8)	Food-deprived polecat iab WAT
Liver histology	Steatosis	Steatosis		
Liver TAG	↑	↑		
18:2(n-6)	↓	NS	↑	NS
18:3(n-3)	NS	↑	↓	↓
20:4(n-6)	↓	↓	NS	NS
20:5(n-3)	↓	↓	↑	↓
22:6(n-3)	↓	↓	↓	↓
n-3 PUFA	↓	↓	↓	↓
n-6/n-3 PUFA ratio	↑	↑	↑	↑
20:4(n-6)/18:2(n-6)	↓	↓	ND	NS
(20:5(n-3) + 22:6(n-3))/18:3(n-3)	↓	↓	ND	NS
	NAFLD plasma		Food-deprived polecat plasma	
FFA (1)	↑		↑	
ALT and AST (8)	NS		NS	

^a WAT, white adipose tissue; FA, fatty acid; NAFLD, non-alcoholic fatty liver disease; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; iab, intraabdominal; TAG, triacylglycerols; NS, nonsignificant; ND, not determined; FFA, free fatty acids; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ↑, increase; ↓, decrease. The fatty acids were determined from total lipids.

Discussion

According to literature (8–9, 30), the proportions of total SFA, MUFA, PUFA and individual FA in human liver and WAT show great variability. Compared to previous data, the FA compositions in the tissues of the fed polecats in the current study shared many similarities with humans (Table 4). Exceptions to this were, for instance, the high amount of 18:1(n-9) but low levels of 16:0 in the polecat WAT compared to humans and the higher total MUFA % of the polecats. The proportions of total n-3 and n-6 PUFA were fairly similar in the polecats and humans. Fatty liver with numerous similarities to NAFLD was induced in the polecats after 5 days of food deprivation. It should be emphasized that this fasting-induced fatty liver must be considered a pathological condition also for mustelids as observed e.g., in the American mink with nursing sickness (17) and in the ferret when fed a high-fat diet (16). Although the etiology of the polecat lipodosis and the most common cause of NAFLD are different, it is known that rapid weight loss and starvation are possible causes of NAFLD (1). In the present study it could be observed that despite the different etiologies, the endpoint—liver steatosis—shared many characteristics between the polecats and NAFLD patients.

The most important biochemical manifestations shared by the polecats in the current study and humans with NAFLD (8) were a decrease in the total n-3 PUFA % and an

increase in the n-6/n-3 PUFA ratio in liver and WAT (Table 4). In common with many NAFLD patients (5–6, 8), the plasma ALT and AST activities were unaffected. The depletion of n-3 PUFA during food deprivation could be partly due to the mechanisms of selective FA mobilization. The location of the first double bond from the methyl end affected FM of PUFA, as mobilization was higher for n-3 than n-6 PUFA. The effect of positional isomerism on FA mobilization has been documented in rodents (31) and several species of carnivores (18–19, 32). This principle may be partly responsible for the loss of n-3 PUFA in NAFLD cases associated with weight loss or weight cycling and contribute to the development of hepatic steatosis. The relative depletion of n-3 PUFA may also be due to the principle of competitive inhibition, where n-3 substrates are preferred over n-6 ones in FA desaturation reactions (33). In addition, shorter-chain SFA and MUFA were more readily mobilized than longer-chain FA in the polecats (18–19, 32, 34–35).

n-3 LCPUFA may have been lost also due to the depletion of 18:3(n-3), the precursor of 20:5(n-3) and 22:6(n-3), from WAT. 18:3(n-3) could have been mobilized for β -oxidation (36) or it could have been converted via 20:5(n-3) into 3-series prostaglandins (33). Moreover, 18:3(n-3) may have been modified to its longer-chain and more unsaturated derivatives incorporated into membrane

phospholipids (33). The increased presence of 18:3(n-3) in liver failed to keep the proportions of the important n-3 LCPUFA stable, as evidenced by the decreased product/precursor ratio. This may be associated with down-regulation of the key enzymes of this metabolic pathway, $\Delta 5$ and $\Delta 6$ desaturases, of which the activity of $\Delta 5$ desaturase was documented to decrease in obesity (37).

Oxidative stress may be yet another factor in the depletion of n-3 PUFA. Serum free FA derived from WAT contribute to hepatic TAG accumulation of NAFLD patients (38). Free FA can also increase the production of reactive oxygen species and be directly hepatotoxic (1). n-3 LCPUFA [20:5(n-3) and 22:6(n-3)] are susceptible to oxidative stress and could therefore be more easily depleted from liver (39–40). In steatotic livers of patients with chronic hepatitis C, peroxidation products of n-3 PUFA can be detected in excess over patients without steatosis (41). The relative decrease of LCPUFA could contribute to the development of hepatic steatosis in several ways. Increased TAG accumulation in hepatocytes is an expected outcome of the depletion of n-3 PUFA, as they contribute to the regulation of lipid metabolism (42–46). LCPUFA inhibit the transcription of lipogenic and glycolytic genes but induce genes encoding FA oxidation through PPAR- α . As n-3 PUFA activate PPAR- α more effectively than n-6 PUFA, a decrease in hepatic lipid oxidation may ensue during n-3 PUFA depletion. In fact, PPAR- α -null mice develop fatty livers rapidly during food deprivation (45).

The altered n-6/n-3 PUFA ratio may be important also in the activation of the endocannabinoid system, which is upregulated in human obesity (47). Activation of the cannabinoid receptor 1 in liver is proposed to play a key role in increased FA synthesis (48). Endocannabinoids increase in the brain after dietary 20:4(n-6) supplementation (49) and exogenous glucocorticoids (50). The brain levels of 2-arachidonoylglycerol increase in n-3 PUFA deficiency, while dietary 22:6(n-3) supplementation has reducing effects (51). It is therefore plausible that the overrepresentation of n-6 PUFA in relation to n-3 PUFA as observed also in the increase of the tissue n-6/n-3 PUFA ratios of the food-deprived polecats of the present experiment could trigger the activation of endocannabinoid pathways and be responsible for many of the key features observed in the metabolic syndrome including hepatic steatosis.

An additional explanation to the development of hepatic lipidosis during food deprivation could be protein catabolism. Although the polecats were in phase II of fasting with stimulated fat mobilization, some body proteins must still have been utilized for gluconeogenesis. The breakdown of muscle proteins during food deprivation appears to be related to Arg deficiency (52). Arg is essential for the functioning of the urea cycle for the detoxification of ammonia. In strict carnivores, elevated urinary orotic acid is an indicator of Arg deficiency (53–55). The increased concentrations of orotic acid can interfere with hepatic LDL and VLDL assembly and secretion causing hepatic lipid

accumulation (52, 56). The rapid development of hepatic lipidosis in strict carnivores during fasting could thus be related to Arg depletion and the loss of muscle mass due to the reliance on gluconeogenesis for blood sugar provision during food deprivation (27).

Animal experiments to study human pathology are approximations and the present application of food deprivation-induced lipidosis also has its limitations. The etiology is different from the majority of NAFLD cases and the plasma endocrinology does not suggest insulin resistance during fasting. However, the many similarities in the FA manifestations can have applications in further studies. Another promising model is the methionine choline-deficient (MCD) diet, which induces hepatic fat accumulation and histological similarities to NASH, while it is also limited by its relatively weak association with the metabolic syndrome (57–58). A potential benefit of the present carnivore model could be the easily and quickly inducible steatosis compared to the MCD diet. The mechanisms of lipid accumulation in the MCD model are the increased FA uptake and decreased VLDL secretion (58). In the future, these crucial steps of hepatic lipid metabolism can be examined in fasted carnivores to further assess the suitability of food deprivation experiments in hepatology research.

Conclusions

1) The tissue FA compositions of the fed polecats have similarities to those of human subjects. 2) FA are mobilized more efficiently from the visceral fat depots of the polecats. 3) n-3 PUFA are mobilized more effectively than n-6 PUFA probably predisposing the animals to the development of fatty liver. 4) Hepatic lipidosis with many similarities in the FA profiles to human NAFLD can be induced to mustelids by food deprivation. 5) The TAG concentration of liver increases due to food deprivation and the histological characteristics of lipidosis in the polecat resemble the microscopic appearance of NAFLD. 6) Mustelids could be used as a model to study the FA manifestations of fasting-induced liver steatosis with potential applications to NAFLD.

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