White Adipose Tissue Fatty Acids of Alpine Marmots During Their Yearly Cycle

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ABSTRACT: Alpine marmots (*Marmota marmota*) were maintained on a laboratory diet, and the fatty acid composition of gonadal and subcutaneous white adipose tissues (WAT) was studied during a yearly cycle. Fatty acids (FA) released from isolated adipocytes were also identified after stimulation of *in vitro* lipolysis. Analysis of the FA composition of WAT depots showed that marmot WAT mainly contained monounsaturated FA (65%, mostly oleic acid, 18:1n-9) although laboratory food contained 45% of linoleic acid (18:2n-6) and only 21% of 18:1n-9. During stimulated lipolysis, saturated FA were preferentially released from isolated adipocytes whereas unsaturated FAs were retained. Despite this selective release of FA from isolated WAT cells *in vitro,* and despite the FA composition of the food, marmots maintained a constant FA composition in both WAT depots throughout the year. Six months of hibernation and fasting as well as an intense feeding period did not affect this composition. The potential adaptive benefit of such regulation of WAT composition, based on a high level of monounsaturated FA, might be to maintain fat with appropriate physical properties allowing animals to accommodate to and survive the wide range of body temperatures experienced during hibernation.

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Alpine marmots (*Marmota marmota*) exhibit a marked yearly rhythm of food intake, with a feeding period during summer and a fasting period during hibernation, which can last 5 to 6 mon depending on geographic and climatic conditions (1,2). During hibernation, marmots alternate long torpor bouts [lasting 10–15 d with a body temperature (Tb) regulated a few degrees above 0°C] with short euthermic phases [lasting 1–2 d with a Tb of 37°C (3)]. Rewarming during arousal mainly depends on brown and white fat lipid oxidation (4–6). Large hibernating mammals such as marmots therefore rely mainly on their large fat deposits, which can reach 50% of their body mass prior to hibernation (reviewed in Ref. 7). Nearly 80% of this stored fat can be used during hibernation (8).

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Abbreviations: FA, fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; Ta, ambient temperature; Tb, body temperature; WAT, white adipose tissue.

In small rodents, such drastic depletion in fat mass may affect the lipid composition of adipose tissue because of the known differential mobilization of fatty acids (FA) during fasting (9). Therefore, after several days of starvation in the rat, the relative distribution of the remaining FA in white adipose tissue (WAT) is profoundly altered (9). If such change in lipid composition occurs in long-term fasting hibernators, it may in turn have marked effects on hibernation. Indeed, it was shown that diet-induced changes in lipid composition of WAT affect hibernation characteristics. Deficiency in essential FA (linoleic acid, 18:2n-6; and α-linolenic acid,18:3n-3) led to marked changes in the hibernation pattern of marmots (10,11). Similarly, feeding a high polyunsaturated acid diet to chipmunks (*Tamias amoenus)* altered their hibernation bout lengths and the minimum Tb reached during deep hibernation (12). These physiological effects may be due to changes in the FA composition of cell membranes and fat stores, which would alter physical properties such as fluidity at low Tb. Although of likely functional importance, WAT lipid composition during an annual cycle with regard to preferential mobilization of FA released by lipolysis has not been investigated in marmots.

The aim of the present study was to investigate the FA composition of two WAT depots (subcutaneous and abdominal) throughout an entire annual cycle in captive marmots maintained on identical laboratory diets. Furthermore, isolated adipocytes from these two depots were stimulated *in vitro* so that we could analyze the composition of the FA released during the annual cycle.

MATERIAL AND METHODS

Animals. Marmots were trapped in the French Alps (Bonneval-sur-Arcs, Savoie). The study was performed on 12 adults (6 males and 6 females). Marmots were cared for under the French Code of Practice for the Care and Use of Animals for Scientific Purpose, and the experimental protocols were approved by the French Ministry of Agriculture Ethics Committee (Animals). They were caged in pairs under outdoor conditions of photoperiod and ambient temperature (Ta) during the summer (Ta ranged from 15 to 25°C) and during the hibernation season in a cold room (Ta = 6 ± 1°C, light/dark 0:24). Standard food (rodent chow pellet, Genthon, Cheyssieu,

France) was given to the animals. Food and water were provided *ad libitum* after the emergence from hibernation (April) and were removed when the animals ceased to feed in late fall. The mean body mass loss during hibernation was 29.4% for males and 34.0% for females (6.8 kg *vs.* 4.8 kg and 5.8 *vs.* 3.8 kg, respectively) and was not significantly different between genders.

Sequencing of the cycle. The marmot cycle was studied for 15 mon, beginning in October and finishing in late December of the following season (see Fig. 1). In the first part of the cycle, the period of hibernation occurred within the fasting period. During the second part of the study, marmots reduced their food consumption in mid-September, somewhat earlier than in the previous year. This drop in food consumption coincided with a sudden drop in the outdoor temperature, which was a few degrees above 0°C during the night. During the study, animals were kept alive and sampled at least twice during the experimental cycle (except for marmot #4 which was sampled once, see Fig. 1). The sampling frequency was approximately one animal every 15 d. Because marmots did not start fasting at the same time prior to the first and second hibernation season, we decided to consider only the periods in which all the animals were both hibernating and fasting (Fasting period) or both active and eating (Food Intake period). Among the 12 marmots we used in this study, 7 were sampled once at both periods (marmots #1, 5, 6, 7, 8, 9, 11) and then data on these 7 were used to perform the statistical analysis.

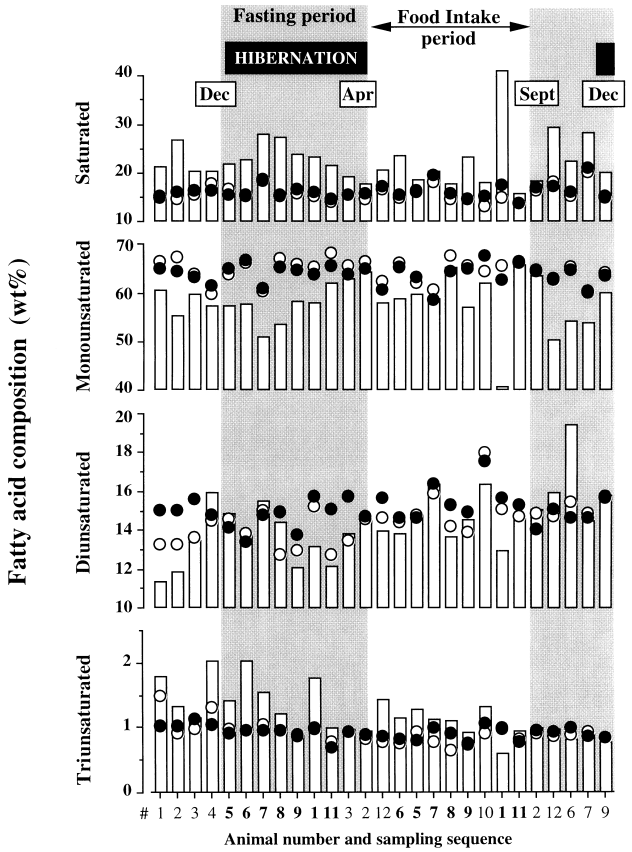

FIG. 1. Fatty acid composition of gonadal (open circles) and subcutaneous white adipose tissue (closed circles) compared to the composition of the fatty acids released *in vitro* (open bars) from gonadal isolated adipocytes over the annual cycle of marmots. The sampling sequence was approximately one animal every 15 d. The identification number of the marmots is reported on the abscissa and the numbers in bold indicate marmots used for statistics; for comments see the paragraph on "Sequencing of the cycle" in the Materials and Methods section.

Adipose tissue samplings. Gonadal and inguinal subcutaneous WAT was surgically removed from marmots during the active and hibernating periods. In summer, nonhibernating marmots were fasted overnight before being anesthetized the next morning with zoletil 100 (Virbac, Carros, France; 100 mg/kg) and a fat biopsy performed (4–5 g). In winter, fasting hibernating marmots were removed from the cold room 24 h before surgery and allowed to arouse at room temperature (Ta = 21°C) without any food being available. Marmots were then anesthetized and biopsies were performed as described above. Fat samples were either immediately frozen at –30°C until lipid extraction and analysis or else minced in a collagenase buffer and incubated at 37°C for adipocyte isolation. Rodent pellet samples were collected four times over the food intake period to analyze the lipid composition of the diet.

To investigate whether the diet could similarly influence the WAT composition in another species, we also analyzed the FA composition of gonadal WAT from 6-mon-old rats fed the same diet. Gonadal WAT samples from rats were treated as described above for marmot WAT samples.

Composition of fatty acids released after in vitro *stimulated lipolysis.* At different times during the yearly cycle, the FA composition found in the fat depots was compared with the composition of the FA released *in vitro* after stimulated lipolysis of gonadal and subcutaneous WAT (feeding period only). This was achieved by preparing a suspension of 30–35 mg of isolated adipose cells per milliliter of buffer as described previously (13) with slight modifications. The pieces of gonadal and subcutaneous WAT were subjected to collagenase P digestion (0.3 mg/mL, Boehringer-Mannheim Chemicals, Meylan, France) in a Krebs-Hepes buffer (10 mM, pH = 7.4) containing 5% (wt/vol) of FA free bovine serum albumin and 6 mM glucose. An aliquot of the cell suspension was incubated at 37°C (shaking water bath) in polypropylene microcentrifuge tubes, and lipolysis was stimulated with adrenocorticotropic hormone (ACTH, from Sigma Chemicals, Saint Quentin Fallavier, France). ACTH was as effective as norepinephrine in stimulating FA release from isolated marmot adipocytes as noted in rats (14) and in rabbits (15,16). After 1 h incubation, samples were centrifuged and cells were removed by vacuum suction. Fatty acids in the medium were recovered with chloro-form/methanol (2:1, vol/vol) containing 50 mg/L of butylated hydroxytoluene (BHT) and stored immediately at –30°C under nitrogen prior to lipid extraction and analysis.

FA analysis. WAT samples were homogenized in chloroform/methanol (2:1, vol/vol) containing 50 mg/L of BHT, extracted, and purified according to the procedure of Folch *et al.* (17). Lipids were evaporated to dryness under a stream of

nitrogen, taken up in an appropriate volume of chloroform/methanol, and stored in glass tubes at –30°C under nitrogen. The methylation was performed according to Slover and Lanza (18) with minor modifications. A known amount of heptadecanoic acid (17:0) was added as an internal standard, transmethylation was achieved by heating at 80°C for 150 min in methanol/sulfuric acid (98:2, vol/vol). After neutralization with a 5% K_2CO_3 solution, methyl esters were extracted in hexane and dried on anhydrous sodium sulfate. Samples were analyzed by gas–liquid chromatography at constant oven temperature (180°C) over the whole run, using a Chrompack CP-9001 (Chrompack, Middelburg, The Netherlands) equipped with a silica 50 m × 0.25 mm i.d. capillary column coated with CP SIL88, using nitrogen as the carrier gas. FA standards were run periodically to determine correct retention times for the FA identification. Peaks were measured using Maestro Chromatography data system software (Chrompack).

Statistics. The composition of FA from both depots (gonadal and subcutaneous) and of FA released from gonadal WAT were compared over the same period and between food intake and fasting periods using a one repeated factor two-way-analysis of variance with Bonferoni/Dunnett *post-hoc* tests ($P < 0.03$). Before calculations, percentage values were transformed into angular values [arcsin root of (value/100)].

RESULTS

Marmot gonadal and subcutaneous WAT composition did not change significantly over the year (Fig. 1). It is clear from this figure that no profound variation in the saturated FA (SFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA) composition occurred in either of the depots in either the feeding or fasting period. In addition, no significant difference between depots was observed between the food intake/fasting periods (Table 1). Within depots, small though significant differences in MUFA and PUFA (mainly diunsaturated and triunsaturated) composition were observed between the food intake and fasting periods. The unsaturation index was similar in both depots and did not change during the year.

Marmot WAT FA composition differed markedly from that

TABLE 1
Major Fatty Acid Species Identified in Gonadal and Subcutaneous WAT of The Alpine Marmot.

	Food Intake period		Fasting period and hibernation	
	Gonadal	Subcutaneous	Gonadal	Subcutaneous
Saturated (wt%)				
14:0	1.10 ± 0.1	1.32 ± 0.1	1.29 ± 0.04	1.28 ± 0.07
16:0	12.31 ± 0.3	12.93 ± 0.5	12.91 ± 0.4	13.07 ± 0.3
18:0	1.59 ± 0.2	1.70 ± 0.2	1.45 ± 0.1	1.38 ± 0.1
20:0	0.25 ± 0.04	0.29 ± 0.03	0.20 ± 0.01	0.23 ± 0.02
22:0	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Total	15.30 ± 0.6	16.12 ± 0.7	15.85 ± 0.6	15.96 ± 0.5
Monounsaturated (wt%)				
14:1n-7	0.52 ± 0.1	0.51 ± 0.05	0.61 ± 0.06	0.58 ± 0.06
16:1n-9	0.79 ± 0.1	0.90 ± 0.1	0.98 ± 0.05	0.98 ± 0.06
16:1n-7	4.25 ± 0.2	4.30 ± 0.2	5.80 ± 0.2[b]	5.18 ± 0.2
18:1n-9	56.85 ± 0.8	55.16 ± 0.9	55.18 ± 0.9	55.49 ± 0.8
18:1n-7	1.93 ± 0.1	2.03 ± 0.1	2.12 ± 0.1[b]	1.99 ± 0.09
20:1n-9	0.58 ± 0.1	0.66 ± 0.04	0.51 ± 0.02	0.57 ± 0.02
22:1n-9	0.05 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
24:1n-9	0.09 ± 0.02	0.11 ± 0.01	0.13 ± 0.03	0.11 ± 0.01
Total	65.01 ± 0.9	63.67 ± 0.9	65.33 ± 1.0	64.61 ± 0.7
Diunsaturated (wt%)				
18:2n-6	14.48 ± 0.3	15.04 ± 0.2	13.68 ± 0.4	14.32 ± 0.3
20:2n-6	0.27 ± 0.01	0.28 ± 0.02	0.23 ± 0.02	0.25 ± 0.02[b]
Total	14.75 ± 0.2	15.31 ± 0.2	13.91 ± 0.4	14.57 ± 0.3
Triunsaturated (wt%):				
18:3n-6	0.02 ± 0.01	0.02 ± 0.00	0.05 ± 0.01	0.04 ± 0.01
18:3n-3	0.80 ± 0.04	0.83 ± 0.04	0.84 ± 0.04	0.84 ± 0.03
20:3n-6	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.01
Total	0.82 ± 0.05	0.87 ± 0.04	0.95 ± 0.03	0.91 ± 0.04[b]
Tetra- and hexaunsaturated (wt%)				
20:4n-6	0.16 ± 0.01	0.19 ± 0.01	0.17 ± 0.02	0.16 ± 0.01
22:6n-3	0.45 ± 0.03	0.52 ± 0.03	0.46 ± 0.06	0.44 ± 0.04
Total	0.61 ± 0.04	0.71 ± 0.03	0.63 ± 0.06	0.60 ± 0.05
Others	3.51 ± 0.4	3.32 ± 0.3	3.33 ± 0.2	3.35 ± 0.2
UI	1.01 ± 0.01	1.01 ± 0.01	1.00 ± 0.01	1.01 ± 0.01

[a]Fatty acids (mean ± SEM; gonadal, $n = 7$; subcutaneous, $n = 7$) are expressed in % of the total fatty acids identified in each tissues. [b]Significant difference between Food intake and Fasting periods within the same depot ($P < 0.05$). No difference was found between the gonadal and the subcutaneous depots. UI, unsaturation index.

TABLE 2
Fatty Acid Composition of Marmot Laboratory Diet and Epididymal WAT of 6-month-old Rats Fed with the Same Diet as Marmots[a]

	Laboratory diet (n = 4)	Rat WAT epididymal depot (n = 4)
Saturated (wt%)		
14:0	3.1 ± 0.6	1.5 ± 0.0
16:0	15.6 ± 0.3	22.1 ± 0.3
18:0	2.9 ± 0.7	3.6 ± 0.1
20:0	1.0 ± 0.1	0.8 ± 0.1
Total	23.3 ± 0.2	28.2 ± 0.0
Monounsaturated (wt%)		
16:1n-7	1.3 ± 0.3	3.3 ± 0.2
18:1n-9	21.5 ± 0.2	26.7 ± 0.1
Total	24.5 ± 0.8	32.5 ± 0.3
Diunsaturated (wt%)		
18:2n-6	45.4 ± 3.1	35.5 ± 0.5
Triunsaturated (wt%)		
18:3n-3	3.0 ± 0.2	1.7 ± 0.0
UI	1.2 ± 0.0	1.1 ± 0.0

[a]Fatty acids (mean ± SEM) are expressed in wt% of the total fatty acids identified. WAT, white adipose tissue.

of the diet (Table 2). The linoleic acid (18:2n-6) content of marmot gonadal and subcutaneous WAT depots (13–15%) was three times lower than that found in the rodent diet (45%, Tables 1 and 2), whereas oleic acid (18:1n-9) content (55–57%) was three times higher in both marmot fat depots as compared with the diet (21%, Tables 1 and 2). The total SFA content in marmot WAT was decreased by 7% in comparison with the diet, and the linolenic content by 2%.

In rats, the WAT composition reflected more closely the laboratory diet composition than in marmots. In rat WAT (Table 2), the 18:2n-6 content was 35.5% (diet was 45%). This decrease in the percentage of 18:2n-6 was balanced by high SFA and MUFA content, and the relative percentage of α-linolenic acid (18:3n-3, 1.7%) was nearly 50% of that ingested and presumably absorbed from the diet (3%).

The composition of FA released into the medium was quite different from that found in WAT, as indicated by the lowering of the UI of the total FA identified in the medium after *in vitro* lipolysis (compare Tables 1 and 3). The main differences observed were for 14:0, 16:0, and 18:0. Bars in Figure 1 also

TABLE 3
Major Fatty Acids Released *in vitro* from Gonadal WAT and Subcutaneous WAT Isolated Adipocytes[a]

	Food Intake period		Fasting period and hibernation[b]
	Gonadal	Subcutaneous	Gonadal
Saturated (wt%)			
14:0	2.64 ± 0.6[d]	5.00 ± 1.6	1.43 ± 0.05[c]
16:0	15.78 ± 1.6[d]	15.42 ± 0.5	16.89 ± 0.4[d]
18:0	4.27 ± 1.1[d]	4.64 ± 1.1	5.50 ± 0.6[d]
20:0	0.05 ± 0.0	—	0.06 ± 0.04
22:0	—[e]	—	—
Total	22.75 ± 3.2[d]	25.06 ± 2.8	23.88 ± 0.9[d]
Monounsaturated (wt%)			
14:1n-7	0.82 ± 0.1[d]	1.20 ± 0.2	0.88 ± 0.03[d]
16:1n-9	1.44 ± 0.4[d]	1.48 ± 0.3	1.62 ± 0.2[d]
16:1n-7	4.95 ± 0.5	5.28 ± 0.5	5.60 ± 0.4[c]
18:1n-9	48.28 ± 3.0[d]	45.08 ± 2.4	46.00 ± 1.0[d]
18:1n-7	2.00 ± 0.1[d]	2.08 ± 0.4	2.29 ± 0.1[c]
20:1n-9	0.37 ± 0.1[d]	0.47 ± 0.3	0.24 ± 0.07[c,d]
Total	57.86 ± 3.1[d]	55.60 ± 2.8	56.63 ± 1.3[d]
Diunsaturated (wt%):			
18:2n-6	14.11 ± 0.4	14.31 ± 0.7	13.68 ± 0.5
20:2n-6	0.26 ± 0.1[d]	—	—
Total	14.37 ± 0.4	14.31 ± 0.7	13.68 ± 0.5
Triunsaturated (wt%)			
18:3n-6	0.10 ± 0.04[d]	0.03 ± 0.01	0.26 ± 0.1
18:3n-3	0.87 ± 0.09	1.04 ± 0.06	1.05 ± 0.1
20:3n-6	0.05 ± 0.05	—	0.09 ± 0.09
Total	1.01 ± 0.08[d]	1.07 ± 0.08	1.40 ± 0.2[c,d]
Tetra- and hexaunsaturated (wt%)			
20:4n-6	0.68 ± 0.2[d]	0.83 ± 0.5	0.60 ± 0.2[d]
22:6n-3	—	—	0.05 ± 0.03[d]
Total	0.68 ± 0.2	0.83 ± 0.53	0.65 ± 0.2
Others	3.33 ± 0.4	3.13 ± 0.5	3.76 ± 0.2
UI	0.93 ± 0.03	0.92 ± 0.03	0.92 ± 0.01

[a]Fatty acids (mean ± SEM; gonadal, n = 7; subcutaneous, n = 4) are expressed in wt% of the total.
[b]Release of fatty acids from subcutaneous WAT isolated adipocytes taken from animals during the fasting period was not determined.
[c]Significant difference between Food Intake and Fasting periods within a same depot ($P < 0.05$).
[d]Significant difference between gonadal WAT composition and fatty acids released from gonadal WAT after an *in vitro* lipolysis.
[e]—, below the detection limit. For abbreviations see Tables 1 and 2.

show the clear difference between the FA released from gonadal adipocytes *in vitro* and gonadal WAT composition. This suggests a preferential release of SFA throughout the whole cycle whereas MUFA appeared to be retained. However, we do not know how the absolute concentration of each FA changes but only how each FA changes as a percentage of the total. It follows that the increased release of one FA species will result in an apparent decreased release of other FA species.

In the case of fasting, the same general results were observed in gonadal WAT as seen during the feeding period (Tables 1 and 3), although slight differences emerged between the relative mobilization rates of FA in gonadal WAT for the two periods.

DISCUSSION

The main finding of the present study is that, despite fasting and refeeding periods and a differential mobilization of FA during *in vitro* lipolysis, alpine marmots maintain a surprisingly stable WAT FA composition throughout the yearly cycle.

Numerous studies have reported that the FA composition of WAT is largely influenced by the dietary FA intake, both in nonhibernating mammals (19,20) and hibernators (10,21–24). However, the FA profile of captive alpine marmot WAT (Table 1) exhibited greater differences from the diet than that of the rat (Table 2), indicating that the pattern of the FA storage may be species-specific. Present results indicate that, in marmots, a preferential accumulation of MUFA occurs at the expense of PUFA, although the latter are known to have beneficial effects on hibernation (10–12,25). Surprisingly, this FA composition is maintained throughout the annual cycle, during the intense feeding period, when marmots rapidly reconstitute their fat stores, as well as during the fasting period, when marmots metabolize most of these fat stores. In the rat, by contrast, several days of starvation profoundly alter the distribution of the FA remaining in WAT (9).

As previously observed in rats (9,26,27), the composition of the FA mobilized from marmot white adipocytes (bars in Fig. 1) did not exactly reflect the composition of WAT. However, the pattern appeared to differ somewhat between rats and marmots. The conclusions drawn from rat studies are that short-chain and unsaturated FA, with a double bond close to the methyl end of the chain, are preferentially mobilized during *in vitro* lipolysis. In marmots, however, FA mobilization seems directed toward a preferential release of SFA rather than PUFA, whereas MUFA are retained within the tissue (Fig. 1 and Table 3). Previous results with marmots *in vivo* correlate well with the present *in vitro* data, *i.e.*, SFA amount to 60% of the FA found in plasma during deep hibernation, rewarming, and arousals (28). Moreover, contrary to rats, this preferential mobilization of FA does not lead to changes in WAT composition after several months of fasting.

The mechanism(s) underlying this process may involve hormone-sensitive lipase specificity and/or differential reesterification by analogy with the situation in rats. In mammals, hormone-sensitive lipase preferentially hydrolyses the first and the third ester bonds of triacylglycerols (29) where SFA are frequently found. However, in spite of this specificity, lipolysis in the rat (26,30) is directed toward the preferential release of PUFA. Wilson *et al.* (31) reported the presence of high amounts of messenger RNA for hormone-sensitive lipase in marmot WAT during fall and winter, but the activity and the specificity of this enzyme on triacylglycerols were not determined. Another mechanism could be FA reesterification which has been shown to occur *in vitro* in rats, even under stimulated lipolysis (32,33), and could explain the unchanged FA composition in marmot in spite of a nonramdom FA mobilization. However, during hibernation of *M. flaviventris*, under nonstimulated conditions the venous blood of gonadal fat shows a molar ratio FA/glycerol of 3:1 (10), which is not in favor of reesterification within WAT. Whatever the mechanism, it appears that, owing to a marked repletion/depletion of fat stores during the yearly cycle of marmots and because of the preferential release of FA by adipose tissue (as shown by *in vitro* lipolysis), the FA composition should markedly change throughout the year. Because we did not observe such changes, it is likely that the composition of fat depots is actively regulated in marmots. Such regulation has never been described before in either hibernators or nonhibernators.

Apart from the unresolved problem of the mechanisms allowing the constancy of FA composition throughout the yearly cycle of marmots, the question arises as to what could be the role of such regulation of FA composition. It is tempting to suggest that a particular FA composition may play a major role during hibernation and particularly during periodic arousals. When body temperature has to change rapidly within a 35°C range, i.e., from a few degrees above zero to +37°C and back in a period of few days, some physical properties of fat may be necessary to maintain the optimal functions of the tissue. Because such dramatic change in tissue temperature is likely to affect fluidity of membranes and triglyceride stores, a given FA composition may ensure a low WAT melting point. Supporting this interpretation, WAT melting temperature is −4.3°C in the adult California ground squirrel (*Spermophilus beldingi*) before hibernation (34). Similarly, differential fluidity of outer and inner sides of subcutaneous fat depots in cattle (19) and polar bear (35) is also achieved through regulation of FA composition.

In this way, a high UI, an estimate of the amount of unsaturated FA in a tissue (36), is usually associated with high fluidity at low temperature. Present results in marmots suggest that the apparent regulation of the FA composition of WAT, and possibly fluidity, is primarily achieved through the regulation of the MUFA content. This is in accord with the well-established fact that the introduction of the first double bond in SFA chains has the maximal effect on the decrease in melting point and thus fluidity of WAT, whereas the introduction of further double bonds affects physical properties less (36–38). In agreement with this point is the observation that the decrease in WAT melting point in the ground squirrel (*S. beldingi*) before hibernation is more highly correlated with the content of MUFA, which increases from 25 to 44%, than with the content of PUFA, which decreases from 54 to 38%

(34). Similarly, physiological differences were reported in chipmunks fed with a diet supplemented in either 18:0, 18:1, or 18:2 (22). Geiser *et al*. (22) demonstrated that the addition of 18:1 to the food caused the greatest change in the pattern of torpor (minimum Tb reached during torpor and duration of torpor bouts), and these observations were highly correlated with the increase of MUFA in the WAT of chipmunks (22). In captive yellow-bellied marmots, it was also observed that, relative to free-ranging animals, the drastic lowering of 18:3n-3 was compensated by an increase in 18:1n-9 rather than 18:2n-6, despite the high availability of the latter in the lab food (10,11).

It therefore appears that the preferential storage of MUFA may be a general feature of hibernating animals, enabling the constitution of fat with appropriate physical properties for deep hibernation and periodic arousals. The synthesis of MUFA, which does not depend on the diet, may be an efficient and simple adaptive mechanism allowing marmots and possibly other hibernators to survive rapid and frequent large changes in body temperature without further FA chain restructuration. In captive marmots (present study) fed a laboratory diet low in n-3 PUFA, it appears that the regulation of WAT optimal fluidity leads to a steady FA composition during the yearly cycle. The importance of MUFA in hibernating marmots may be in relation with the preferential release of SFA by isolated adipocytes (present study), contrary to what is observed in nonhibernating rodents such as the rat (26,30). The precise physical characteristics and thus biological activity which are to be preserved in hibernating animals by such precise regulation now needs to be determined.

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