Effect of Cold Environment on Hepatic Microsomal Δ6 and Δ9 Desaturase Activity of Male Rats

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Male rats maintained at 24 C and then shifted to 5 C for 5 days increased food intake and decreased in growth rate and food conversion. No modification was observed in Δ6 desaturase activity, while Δ9 desaturase activity decreased after this period of time. These results were confirmed by liver microsomal and mitochondrial fatty acid composition. The phospholipid composition of liver microsomes was unaltered, whereas in mitochondria, phosphatidylcholine and sphingomyelin decreased and phosphatidylethanolamine increased due to the cold environment. The influence of food intake and weight changes on fatty acid metabolism was studied using (i) rats maintained at 5 C with restricted food intake to match the food intake of those kept at 24 C with food ad libitum and (ii) rats maintained at 24 C whose food intake was also restricted so that their growth rate would be the same as that of rats maintained at 5 C with food ad libitum, respectively. These results indicate that the negative metabolic balance state of these cold conditions is not an active factor modifier of Δ6 desaturase activity, whereas it decreases Δ9 desaturase activity, reflecting the lipogenic characteristics of the latter enzyme. *Lipids 22,* 583–588 (1987).

Different types of organisms from microorganisms to higher plants and poikilotherm animals respond to alterations in environmental temperature by modifying fatty acid composition and, thus, the physicochemical properties of their membranes. In some microorganisms, such as *Candida utilis* (1,2), *Bacillus megaterium* (3) and *Tetrahymena* (4), and in higher plants (5), polyunsaturated fatty acid synthesis is increased in response to a decrease in the environmental temperature.

It has also been demonstrated that the Δ6 desaturase activity of fish *(Pimelodus maculatus)* liver microsomes is greater in animals adapted to lower temperature (6). The effect of temperature on membrane fluidity is not as obvious in homeothermic animals as it is in poikilotherms.

Previous laboratory studies (7) in which female rats were adapted to warm temperature (30–32 C) for 20–25 days and then shifted to cooler temperature (13–15 C) for different periods of time showed that Δ9 desaturase activity decreased after 24 hr of cold exposure, while Δ6 desaturase activity was increased after this period of time. Male rats adapted to the same conditions did not show significant changes either in Δ6 or Δ9 desaturase.

Taking into account these results, it was thought that differences in Δ6 and Δ9 desaturase activity might appear in male rats by means of a change in the environment at temperatures lower than 12 C. In addition, we attempted to determine if the food intake and/or decreased weight gain of the animals kept in the cold environment might contribute to the changes in lipid desaturation and composition. For this purpose, male rats were transferred from 24 C to 5 C, and Δ6 and Δ9 desaturase activities and lipid composition of microsomes and mitochondria were determined.

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MATERIALS AND METHODS

Materials. [1-^{14}C]Palmitic acid (53.8 mCi/mmol, 99% radiochemically pure), [1-^{14}C]stearic acid (56.0 mCi/mmol, 99% radiochemically pure) and [1-^{14}C]linoleic acid (54.7 mCi/mmol, 98.5% radiochemically pure, 1% *trans* isomer) were purchased from New England Nuclear (Boston, MA). Cofactors used for the enzymatic reactions were provided by Sigma Chemical Co. (St. Louis, MO), and all unlabeled fatty acids were from Nu-Chek-Prep Inc. (Elysian, MN). Silica Gel H (Kiesegel 60H) was purchased from Merck (Darmstadt, FRG). All chemicals and solvents were analytical grade.

Animal treatment. Male Wistar rats of 60–70 days of age weighing 180–200 g were maintained on a commercial standard pellet diet (Nutrimento rat chow 3, Escobar, Argentina) and tap water ad libitum and were housed in individual cages unless indicated otherwise. The fatty acid composition of the diet was as follows: 16.7% 16:0 (palmitic acid), 0.8% 16:1 (palmitoleic acid), 4.9% 18:0 (stearic acid), 21.8% 18:1 (oleic acid), 52.4% 18:2n-6 (linoleic acid) and 4.3% 18:3n-3 (linolenic acid).

When the influence of body weight changes and food intake on fatty acid metabolism was studied, experiments were carried out using four groups of four rats each monitoring daily weights and food intake. Group I comprised rats maintained at 22–24 C fed ad libitum (control ad libitum); group II was rats maintained in a cold temperature-controlled room at 5–6 C fed ad libitum (cold ad libitum); group III was rats maintained at 5–6 C whose food intake was restricted to match the food intake of group I (cold pair-fed to group I); and group IV was rats maintained at 24 C whose food intake was restricted so that their growth rate would match the growth rate of group II (control pair-weighed to group II). Food-restricted rats maintained at 5–6 C in group III were studied to determine whether changes in desaturation or lipid composition in rats maintained at the cold temperature might be due to increased food intake.

Since rats maintained in a cold environment have relative hyperphagia but gain less weight than those at 24 C, the food-restricted rats at 24 C (group IV) were studied to determine if changes in desaturation or lipid composition in rats maintained at cold temperatures might be due to their decreased growth rate and relatively increased fat mobilization. All animals were subjected to a daily photoperiod of 12 hr light and 12 hr darkness (midnight being the midpoint of the dark period). After five days under these conditions, animals were killed at 8 a.m. to equalize circadian effects (8), and liver microsomes and mitochondria were isolated.

Fractionating procedures: preparation of microsomes. Microsomes were prepared from rats killed by decapitation. Livers were rapidly removed and homogenized in a cold solution (3:1, v/w) containing 0.25 M sucrose, 0.15 M KCl, 62 mM potassium phosphate buffer (pH 7.4), 5 mM $MgCl_2$ and 1.4 mM N-acetyl-L-cysteine. The homogenate was centrifuged at 10,000 × g for 20 min; the pellet was discarded and the resulting supernatant was centrifuged at 100,000 × g for 60 min to obtain the microsomal pellet. The microsomal fraction was resuspended in the same homogenizing solution to an appropriate protein concentration. In some instances, pellets were frozen and stored at −80 C for several weeks before use. All microsomal preparations were carried out at 0–4 C. When frozen in concentrated form (15 mg of microsomal protein/ml of homogenizing solution), enzyme activities were stable for up to a month. All subsequent assays and composition analyses used these microsomal membrane fractions.

Preparation of mitochondria. A portion of the liver was chilled immediately after removal by immersion in 0.25 M sucrose and 10 mM potassium phosphate buffer (pH 7.4) and was homogenized at 0–4 C (9:1, v/w). Liver mitochondria were prepared by differential centrifugation according to the method of Hogeboom (9).

Enzymatic assays. The Δ9 (16:0 → 16:1 and 18:0 → 18:1) and Δ6 (18:2n-6 → 18:3n-6) desaturase enzyme assays were carried out by measuring the conversion rate of the [1-^{14}C]labeled fatty acid substrate into the corresponding [1-^{14}C]labeled fatty acid product. Only the exogenous substrate added to the incubation mixture was considered for calculation of the enzymatic activity, since the amount of endogenous substrate (<7 nmol; only 1.5% [w/w] of microsomal lipids in all groups was free fatty acids) was negligible compared to the 50 nmol of exogenous substrate. Reactions were initiated by adding microsomal protein to preincubated flasks containing 0.25 M sucrose, 0.15 M KCl, 0.04 M potassium phosphate buffer (pH 7.2), 1.41 mM N-acetyl cysteine, 0.04 M KF, 1.3 mM ATP, 0.06 mM CoA, 0.87 mM NADH, 5 mM $MgCl_2$, 5 nmol of [1-^{14}C]labeled and 45 nmol of unlabeled acid in a final volume of 1.6 ml. For Δ9 desaturase assay, palmitic and stearic acids were used as substrates with 1.5 and 2 mg of microsomal protein. The substrate for Δ6 desaturase assays was linoleic acid and 3 mg of microsomal protein was used. The protein was determined by the Lowry procedure using crystalline bovine serum albumin as standard (10). The reaction mixture was incubated with constant shaking at 36 C for 15 min. The desaturation reaction was stopped by adding 2 ml of 10% KOH in ethanol. The fatty acids were recovered by saponification of the incubation mixture (45 min at 82 C), acidification and extraction with petroleum ether (bp 30–40 C). The fatty acids were esterified with methanolic 3 M HCl (1 hr at 64 C). The analyses were carried out by gas liquid radiochromatography in a Packard apparatus, Model 893, equipped with a proportional counter using 6P 10% SP-2330 on Chromosorb WAW (100–120 mesh) (11). A control assay without the addition of microsomes was done and no desaturation was observed.

Microsomal and mitochondrial lipid composition. Total microsomal and mitochondrial lipids were extracted using the method of Folch et al. (12), and methyl esters were prepared and analyzed by gas liquid chromatography (GLC) as previously described (13) in a Hewlett-Packard model 5840-A chromatograph, equipped with the 5840-A 6C terminal and using a 6-ft column filled with 10% SP 2330 on 100–200 mesh Chromosorb WAW. Peaks were identified by comparison of retention times with standards.

Phospholipids were separated by two-step thin layer chromatography (TLC) procedure using Silica Gel H plates (14). After the plate was exposed to iodine vapors, the phospholipid fractions were scraped off for quantification by colorimetric determination of phosphorus (15).

Statistical analysis. All results were tested for statistical significance by Student's t-test for paired comparison with control.

RESULTS

Food intake, growth rate, food conversion and liver weight in animals in a cold environment. Table 1 summarizes the daily food intake, growth rate and liver weight in the four groups. Although the rats kept at 5 C with food ad libitum (group II) showed an increase of 25% in food intake, they had a decrease in growth rate and food conversion of 74% and 86%, respectively, as a consequence of an increase in catabolism. In the cold environment, food calories are preferentially used maintaining body temperature.

Group III, animals maintained at 5 C with the same food intake as animals at 24 C, showed a decrease in the growth rate, food conversion and liver weight of 142%, 162% and 32%, respectively. The negative metabolic balance due to cold was even greater in this group as a consequence of the decrease in calories provided by the restricted food intake.

Rats of group IV, animals at 24 C and with food intake restricted to match the group-I growth rate, showed a decrease in food conversion and liver weight of 126% and 30%, respectively, since the restriction in food intake provoked an increase in the catabolism of endogenous substrates. The increased catabolism of groups II, III and IV must have been accompanied by increased lipolysis.

Influence of cold, temperature, food intake and growth rate on desaturation. The effect of cold and food restriction on the fatty acid desaturase activities is shown in Figure 1.

The enzymatic activity of Δ6 desaturase was not modified in the group at 5 C with food ad libitum, while it increased when food intake was restricted either at 5 C (group III) or at 24 C (group IV). Animals on the diet given ad libitum at 5 C (group II) showed a marked decrease not only in food conversion but also in the enzymatic activity of Δ9 desaturase.

It is hardly probable that the increase in food intake of rats kept at low temperatures should cause the changes observed in the enzymatic activity of Δ9 desaturase, since the restriction in food intake at 5 C (group III) emphasized rather than annulled the changes. On the other hand, the decrease in the growth rate of rats in the cold environment would be an important factor, since in rats kept at 24 C with a similar growth rate (group IV), a decrease in Δ9 desaturase enzymatic activity was also observed.

Groups showing a marked decrease in food conversion (II, III and IV) also had decreased Δ9 desaturase activity.

When rats were kept at 5 C for 15 days instead of five with food ad libitum, a behavior similar to that shown

TABLE 1

Effect of Temperature and Food Restriction on Food Intake, Growth Rate, Food Conversion and Liver Weight

	Group I, control ad libitum (n = 10)	Group II, cold ad libitum (n = 10)	Group III, cold pair-fed to group I (n = 4)	Group IV, control pair-weighed to group II (n = 4)
Starting weight (g)	197.0 ± 15.1	174.8 ± 14.4	192.5 ± 13.1	176.8 ± 16.0
Food intake (g/g)	0.108 ± 0.003	0.135 ± 0.004*	0.100 ± 0.004	0.064 ± 0.004*
Growth rate (g/day)	6.38 ± 0.36	1.68 ± 0.51*	−2.70 ± 0.30*	0.95 ± 0.70*
Food conversion	54.02 ± 5.03	7.48 ± 4.58*	−32.4 ± 4.4*	−13.7 ± 8.0*
Liver weight (g)	9.92 ± 0.80	8.44 ± 0.90	6.70 ± 0.35**	6.90 ± 0.42**

Food intake (daily food intake/body weight); growth rate (daily increase in body weight); food conversion (growth rate/food intake × 100) and liver weight. Animal treatments are described in Materials and Methods. Results are the mean ± S.E. n, represents the number of animals for each group. Significant differences from control: *, $p < 0.001$; **, $p < 0.01$.

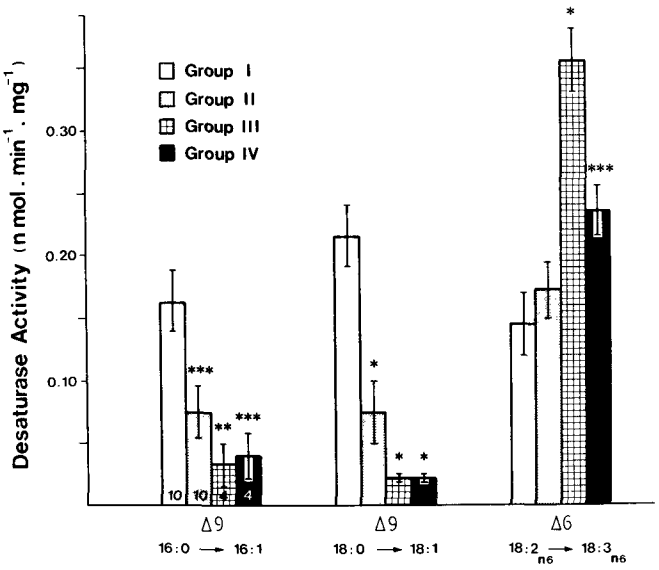

FIG. 1. Effect of environmental temperature and food restriction on hepatic microsomal fatty acid desaturase activity. Desaturase enzyme assays were carried out as described in Materials and Methods. Group I, control ad libitum; group II, cold ad libitum; group III, cold pair-fed to group I; group IV, control pair-weighed to group II. Results are shown as mean ± S.E. The number of animals in each group is shown in the first set of bars. Significantly different from controls: *, $p < 0.001$; **, $p < 0.01$; ***, $p < 0.05$.

in Figure 1 was observed in the enzymatic activity of Δ9 and Δ6 desaturases (results not shown).

Microsomal and mitochondrial lipid composition. Tables 2 and 3 show the lipid fatty acid composition of hepatic microsomes and mitochondria, respectively. The modifications observed in fatty acid composition of liver microsomes and mitochondria were generally similar.

The rats at 5 C with food ad libitum (group II) showed in both fractions increased percentage of 18:0 and decreased 18:1. Only in mitochondria did the percentage of 16:1 decrease significantly, and so did the unsaturation index in microsomes. These changes agree with the decrease found in microsomal Δ9 desaturase enzymatic activity in the same group.

In group III, the same modifications as in group II were observed in the percentages of 16:1, 18:0 and 18:1 in lipids of liver microsomes and mitochondria. As in group II, these changes are the consequences of a decrease in the enzymatic activity of Δ9 desaturase shown by group III. Only the microsomes had an increase in the percentage of 20:4n-6, which agreed with the one observed in the enzymatic activity of Δ6 desaturase (regulatory enzyme in the biosynthesis of polyunsaturated acids) (16).

Group IV presented an increased percentage of 18:0 and decreased 16:1 both in microsomes and mitochondria. The percentage of 18:1 decreased only in mitochondria. These changes are the consequences of the decrease observed in Δ9 desaturase enzymatic activity. Besides, both fractions showed an increase in the percentage of 20:4n-6 that could be attributed to an increase observed in the Δ6 desaturase enzymatic activity.

Table 4 shows the phospholipid composition of hepatic microsomes and mitochondria of rats kept at 24 C (group I) and 5 C (group II) on food ad libitum. No modification was observed in the phospholipid composition of liver microsomes, whereas in mitochondria the percentages of phosphatidylcholine (PC) and sphingomyelin (SM) decreased and that of phosphatidylethanolamine (PE) increased in rats kept in the cold environment. The changes found in liver mitochondria of rats kept at 5 C resembled those found in mitochondria of brown adipose tissue of rats kept at 4 C (17).

DISCUSSION

When a rat is exposed to a cold environment, adaptation processes are initiated characterized by shivering or nonshivering thermogenesis, depending on the conditions (18), tending to lower heat loss and to increase its production. There is an increase in thermogenesis (19), and the supply of substrates comes from an increase in fatty acid mobilization (by lipolysis of white and brown adipose tissue) (20–22) and from glucose (by liver glycogenolysis and gluconeogenesis) (23,24). In this substrate mobilization, the sympathetic nervous system takes part via catecholamines (18). Together with endogenous substrates, the exogenous ones coming from increased food intake also contribute to thermogenesis. This is the reason that when male rats are exposed to cold for five days, food intake increases and growth rate and food conversion decrease, since calories are preferentially used in heat production to keep body temperature.

TABLE 2

Effect of Environmental Temperature and Food Restriction on Liver Microsomal Fatty Acid Composition in Male Rats

Fatty acid (%)	Group I, control ad libitum	Group II, cold ad libitum	Group III, cold pair-fed to group I	Group IV, control pair-weighed to group II
16:0	21.4 ± 0.1	20.7 ± 0.4	20.2 ± 0.2**	18.9 ± 0.4*
16:1	2.1 ± 0.09	2.2 ± 0.20	1.5 ± 0.50	0.7 ± 0.40***
18:0	20.7 ± 0.2	23.5 ± 0.3*	23.8 ± 0.4*	24.3 ± 0.5*
18:1	10.2 ± 0.2	8.3 ± 0.3**	8.2 ± 0.4**	8.2 ± 1.7
18:2n-6	15.1 ± 0.4	15.3 ± 0.3	15.2 ± 0.5	14.7 ± 0.6
20:3n-6	2.5 ± 0.20	1.8 ± 0.20***	1.4 ± 0.09**	1.7 ± 0.20***
20:4n-6	21.0 ± 0.3	21.0 ± 0.6	22.8 ± 0.2**	25.5 ± 1.2**
22:5n-3	2.5 ± 0.20	2.1 ± 0.10	1.6 ± 0.08**	1.4 ± 0.20**
22:6n-3	4.6 ± 0.2	5.2 ± 0.3	5.3 ± 0.3	4.5 ± 0.4
Unsaturation index[a]	1.62 ± 0.01	1.58 ± 0.01***	1.62 ± 0.01	1.67 ± 0.03

Fatty acid composition was determined by gas liquid chromatography as described in Materials and Methods for the four groups of animals described in the text. Only major fatty acids were considered. Results are shown as the mean of 4 rats ± S.E.

[a]Unsaturation index = Σ number unsaturated mol × number double bonds/Σ number total mol. Significant differences from control: *, $p < 0.001$; **, $p < 0.01$, ***, $p < 0.5$.

TABLE 3

Effect of Environmental Temperature and Food Restriction on Liver Mitochondrial Fatty Acid Composition in Male Rats

Fatty acid (%)	Group I, control ad libitum	Group II, cold ad libitum	Group III, cold pair-fed to group I	Group IV, control pair-weighed to group II
16:0	20.0 ± 0.2	20.3 ± 0.5	17.8 ± 0.5**	17.3 ± 0.4*
16:1	2.0 ± 0.10	1.6 ± 0.10***	1.4 ± 0.08**	1.4 ± 0.06**
18:0	18.0 ± 0.3	20.7 ± 0.3*	21.3 ± 0.3*	21.1 ± 0.2*
18:1	10.9 ± 0.06	9.0 ± 0.30*	9.1 ± 0.20*	8.5 ± 0.20*
18:2n-6	21.7 ± 0.4	22.3 ± 0.2	22.0 ± 0.3	22.7 ± 0.7
20:3n-6	1.9 ± 0.10	1.3 ± 0.09**	1.5 ± 0.30	1.2 ± 0.08**
20:4n-6	19.7 ± 0.4	18.8 ± 0.3	20.8 ± 0.3	23.2 ± 0.9***
22:5n-3	2.1 ± 0.3	1.9 ± 0.2	1.9 ± 0.3	1.0 ± 0.1**
22:6n-3	3.7 ± 0.3	4.1 ± 0.2	4.2 ± 0.3	3.6 ± 0.3
Unsaturation index[a]	1.59 ± 0.01	1.54 ± 0.02	1.66 ± 0.03	1.70 ± 0.03

Fatty acid composition was determined by gas liquid chromatography as described in Materials and Methods for the four groups of animals described in the text. Only major fatty acids were considered. Results are shown as the mean of 4 rats ± S.E.

[a]Unsaturation index is explained in Table 2. Significant differences from control: *, $p < 0.001$; **, $p < 0.01$; ***, $p < 0.05$.

TABLE 4

Effect of Cold Environment on Phospholipid Composition of Microsomal and Mitochondrial Fractions % Phospholipid (μg/mg Total Lipid)

	Microsomes		Mitochondria	
	Group I, control ad libitum	Group II, cold ad libitum	Group I, control ad libitum	Group II, cold ad libitum
PC	59.3 ± 7.3	66.4 ± 3.1	52.1 ± 0.1	43.9 ± 0.5*
PE	21.2 ± 1.3	16.8 ± 0.9	22.5 ± 0.8	36.4 ± 1.1*
CL	—	—	15.2 ± 1.1	13.5 ± 0.8
PI	13.8 ± 4.0	11.6 ± 2.2	6.5 ± 0.5	5.4 ± 0.9
SM	3.1 ± 1.8	2.8 ± 1.1	1.8 ± 0.1	0.7 ± 0.2**
PS	2.6 ± 0.2	2.5 ± 1.3	1.9 ± 1.1	0.10 ± 0.02

PC, phosphatidylcholine; PE, phosphatidylethanolamine; CL, cardiolipin; PI, phosphatidylinositol; SM, sphingomyelin; PS, phosphatidylserine. Phospholipids were separated as described in Materials and Methods. Results are expressed as the mean ± S.E. The number of samples analyzed is 3 for microsomal and 5 for mitochondrial fraction. Significant differences from control: *, $p < 0.001$; **, $p < 0.01$.

Δ6 Desaturase activity. Concerning the effect of environmental temperature on desaturating activity, no modification due to cold was observed either in Δ6 desaturase activity or in the percentage of linoleic acid in liver microsomal and mitochondrial lipids of male rats fed ad libitum. Hughes and York also observed no changes in the Δ6 desaturase enzymatic activity due to temperature in the lean mouse (25). These results also agree with those found by González et al. (7) when male rats were shifted from 30-32 C (adapted to that temperature for 20 days) to 12 C for five days, since in this case no modifications in the Δ6 desaturase were observed either. These results indicate that metabolic changes induced by an environmental temperature decrease expressed by a thermogenesis increase are not active factor modifiers of Δ6 desaturase activity. However, in female rats adapted to the same conditions, an increase in the enzymatic activity of Δ6 desaturase due to cold was observed and, in this change, estradiol levels would be involved (26). On the other hand, when the enzymatic activity in male rats was depressed by a hyperglycidic diet and animals were shifted from 24 C to 12 C, an increase was observed in Δ6 desaturase enzymatic activity due to cold environment (27).

The increase in Δ6 desaturase enzymatic activity observed in animals on a restricted diet, both at 5 C and at 24 C, was unexpected, and it was difficult to elucidate since fasting inhibits enzymatic activity (28,29). These results, as has been pointed out by Faas and Carter, emphasize the importance of food intake as another factor to be taken into account for the complex control of the desaturating system (30,31).

Δ9 Desaturase activity. González et al. found that Δ9 desaturase enzymatic activity was not modified when male rats were shifted to 13-15 C after being previously adapted to 30-32 C, whereas in female rats adapted to the same conditions, they observed a decrease in that desaturating activity (7). We have observed, however, that when male rats were kept at 5 C on food ad libitum (group II), the Δ9 desaturase activity decreased. This lowering was confirmed by a decrease in the percentages of 16:1 and 18:1 and an increase in 18:0 in the fatty acid composition of both liver microsomes and mitochondria. In rats kept at 5 C on a restricted diet (group III), the decrease in the Δ9 desaturase enzymatic activity was even more marked than at 5 C on food ad libitum. Moreover, a decrease in Δ9 desaturase activity was also detected in animals on a restricted food intake at 24 C (group IV).

In group II, there was a negative metabolic balance (decreased food conversion) and an increase in food intake to provide substrates for the thermogenesis process, which was increased by the cold temperature. In group III, the negative metabolic balance was even greater than in group II, since there was increased catabolism of endogenous substrates in the thermogenesis process due to the restriction imposed on food intake. Finally, group IV rats also had a negative metabolic balance to counterbalance food intake restriction enhancing the use of endogenous substrates.

In groups II, III and IV, characterized by a negative metabolic balance, the Δ9 desaturase enzymatic activity was diminished. It is known that Δ9 desaturase is very sensitive to nutritional (29,32-36) and hormonal (30,31,37,38) factors. Moreover, it is considered a lipogenic enzyme, since during fasting, when there is a negative metabolic balance and lipolysis is increased, its enzyme activity is decreased (29). In addition, lipogenic diets are the most efficient ones in increasing the above-mentioned activity (29,36). Lipogenesis and Δ9 enzymatic activity respond in the same way to regulatory factors (39).

Therefore, it can be considered that the decrease observed in the Δ9 desaturase enzymatic activity in male rats at 5 C is the consequence of the negative metabolic balance state set off by these conditions.

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