EFFECT OF LIPID ADDITIVES ON OXYGEN SUPPLY OF SKELETAL MUSCLE

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UDC 612.744:612.262].06:613.288

Key words: diet; oxygen; permeability; fatty acids.

The introduction of lipid additives to food changes the fatty-acid composition of the lipids of biological membranes [5, 8, 15] and the viscosity and flowability of their matrix [11]. Functional and structural features of the biomembranes affect the rate of diffusion of substances in the membrane [4], including oxygen [1]. Since the transfer of oxygen from blood to tissue takes place through the blood—parenchymatous barrier (BPB), the physicochemical properties of which can affect the rate of oxygen transport, it was interesting to study the oxygen supply of skeletal muscle during modification of the state and structure of the BPB.

The aim of this investigation was to study the effect of modification of BPB caused by lipid additives to the standard daily diet on the oxygen supply to skeletal muscle.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male chinchilla rabbits weighing 2.0-2.7 kg and 20 male rats weighing 180-240 g. The rabbits of group 1 (10 animals) received the standard general animal house diet. For 4-5 weeks before the beginning of the experiment, rabbits of group 2 (10 animals) received the general animal house diet with the addition of vegetable oil in a dose of 0.2 g/100 g body weight, whereas the rabbits of group 3 (10 animals) received butter in the same dose as an addition to their diet. Rats of group 1 (10 animals) received the general animal house diet. Rats of group 2 (10 animals) received the general animals house diet for 10 days with 50% replacement of its fats by a phospholipid concentrate (PLC) (0.6 g PLC/ 100 g body weight). The PLC contained a large quantity of unsaturated fatty acids (UFA), consisting mainly of $C_{18:1}$, $C_{18:2}$, and $C_{20:4}$. The ratio of saturated (SFA) to unsaturated (UFA) was 0.37.

To assess the oxygen supply of the tissues direct polarographic measurements were made of the partial pressure of oxygen in the gastrocnemius muscle (p_mO_2) and in the arterial blood flowing into it (p_aO_2) and the venous blood flowing out of this muscle (p_vO_2). p_mO_2 Was measured by means of an LP-7 polarograph and the pH of the blood by means of a biomicroanalyzer ("Radel-RIS"). The hemoglobin concentration (C_{Hb}) was measured by the hemoglobin—cyanide method, and the velocity of the blood flow in the muscle tissue ($\dot{Q}_m$) by the hydrogen-clearance method. Blood samples were taken from the femoral artery and vein under local anesthesia with 2% procaine solution. The oxygen consumption of the gastrocnemius muscle ($\dot{V}_mO_2$) was calculated from the blood flow rate and the arteriovenous difference in oxygen concentration. The permeability of the BPB for oxygen was calculated as in [7]. Plasma membranes were isolated by the method in [6] and phospholipids were extracted from the plasma membranes of the gastrocnemius muscle by Folch's method. The

R. E. Kavetskii Institute for Problems in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev. Research Institute of Food Hygiene, Republican Scientific Hygienic Center, Ministry of Health of the Ukrainian SSSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 8, pp. 137-139, August, 1991. Original article submitted November 16, 1990.

 0007-4888/91/0008-1086$12.50

TABLE 1. Parameters of Oxygen Transport by the Blood and Oxygen Supply of the Gastrocnemius Muscle in Rabbits Kept on Diets Differing in their Fat Content (M ± m)

Parameter	Control	Butter	Vegetable oil
PaO_2, mm Hg	87,7±1,9	86,8±1,8	88,9±1,9
kPa	11,71±0,25	11,57±0,24	11,89±0,25
P_vO_2, mm Hg	39,2±1,5	38,8±1,7	39,6±1,6
kPa	5,23±0,20	5,17±0,23	5,28±0,21
pH_a	7,41±0,015	7,39±0,014	7,41±0,011
pH_V	7,36±0,011	7,34±0,011	7,35±0,012
S_{aO_2}, %	92,2±0,89	92,0±1,1	92,3±1,0
S_{vO_2}, —»—	59,1±1,21	57,4±1,10	60,0±1,17
CaO_2, ml/liter	16,4±0,57	16,0±0,71	16,4±0,65
C_{vO_2}, —»—	10,5±0,49	10,0±0,47	10,7±0,052
C_{Hb}, g/liter	13,2±1,22	12,7±0,98	13,1±1,05
Q_m, ml/min/ 100 g	37,8±3,45	30,4±2,65*	44,9±2,61*
P_{mO_2}, mm Hg	2,2±0,12	1,9±0,11*	2,6±0,13*
100	28,9±2,1	22,4±2,3*	36,4±2,2*
P_{mO_2}, mm Hg	3,85±0,28	3,00±0,30	4,85±0,29
P_{BPB}, ml/min/mm Hg	0,083±0,006	0,059± ±0,005*	0,133± ±0,008*
kPa, ml/min	0,623±0,067	0,439±0,054	0,995±0,071

Legend. *p < 0.05 Compared with value of corresponding parameter for control.

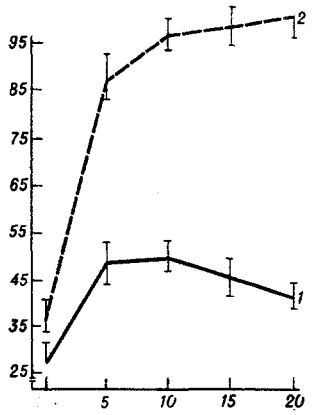

Fig. 1. Changes in partial pressure of oxygen in gastrocnemius muscle (p_mO_2) during inhalation of hyperoxic gas mixture (80% O_2) in rats receiving ordinary diet (1) and diet supplemented by fats rich in unsaturated fatty acids (2). Ordinate, p_mO_2 (in mm Hg); abscissa, time (in min).

TABLE 2. Fatty Acid Composition (in %) of Plasma Membranes of Gastrocnemius Muscle in Rabbits Receiving Diets Differing in their Fat Content (M ± m)

Parameter	Control	Butter	Vegetable oil
$C_{15:0}$	Traces	0,9±0,3***	Traces
$C_{16:0}$	15,0±1,8	19,8±1,3*	16,2±0,9
$C_{18:0}$	28,4±1,4	29,8±0,6	22,5±1,9*
$C_{18:1}$	16,3±1,9	10,8±1,1*	8,5±0,7***
$C_{18:2}$	24,9±1,3	25,4±1,3	34,2±1,7***
$C_{18:3}$	Traces	0,3±0,01*	0,5±0,07*
$C_{20:4}$	15,4±0,6	13,0±1,2	18,1±1,0*
SFA/UFA	0,77	1,02	0,63
Total cholesterol content, mg/g protein	0,072±0,010	0,098± ±0,011*	0,067±0,009

Legend. *$p < 0.05$; **$p < 0.001$ Compared with values of corresponding parameter for control.

fatty acid composition of the phospholipids and the total cholesterol (Ch) content in the plasma membranes were determined by gas–liquid chromatography. The state of lipid peroxidation (LPO) was determined from the malonic dialdehyde (MDA) level.

EXPERIMENTAL RESULTS

In the rabbits of group 2, p_mO_2 during inhalation of air was higher (by 26%, $p < 0.05$), whereas in the rabbits of group 3 it was lower (by 22%, $p < 0.05$) than in the animals of group 1. $\dot{V}_mO_2$ and $\dot{Q}_m$ in the animals of group 2 were raised, but in the rabbits of group 3 they were lowered (Table 1). The increase in $\dot{V}_mO_2$ evidently took place as a result of increased activity of respiratory chain enzymes in the presence of an increased UFA content of the phospholipids in biological membranes [12] and an increase in the oxygen supply [1]. The increase in $\dot{Q}_m$ may be connected with the decrease in viscosity of the blood and a change in its rheologic properties [11]. The possibility likewise cannot be ruled out that this takes place as a result of a change in vascular tone [10]. Similar result were obtained in the experiment on rats. The investigations showed that rats received a diet with part replaced by PLC, containing a large quantity of UFA, p_mO_2 was significantly higher (38%, $p < 0.01$) than in animals of the control group (Fig. 1). $\dot{Q}_m$ in rats of the experimental group also was higher (38.3 ± 3.01, 29.7 ± 2.48 ml/min/100 g tissue respectively, by 29%, $p < 0.05$). The differences in the values of p_mO_2 in animals breathing a hyperoxic gas mixture (80% O_2 in nitrogen) were manifested particularly clearly. In the rats of group 2, p_mO_2 in the first minutes of breathing the mixture rose more sharply than in the animals of group 1, and continued to rise during the next 30 min of respiration. This was one factor confirming the improvement in the oxygen supply of the muscles in the animals after feeding on extra UFA.

Calculations showed that the permeability of the BPB for oxygen in rabbits of group 2 rose by 59% ($p < 0.05$), and in the animals of group 3 it fell by 29% ($p < 0.05$). These results are in agreement with data published previously, obtained by other methods on rats [1].

The results show that the oxygen supply of the skeletal muscle largely depends on $\dot{Q}_m$ and on the permeability of the BPB for O_2, which change when the composition and structure of the biomembranes are modified. We know that the permeability of biological membranes depends on their structural organization and the physicochemical properties of individual components. It was interesting to examine how the fatty acid composition of the plasma membranes of skeletal muscles changed in animals kept on different lipid diets.

The investigation showed that the $C_{16:0}$ content in the rabbits of group 2 was unchanged, whereas that of $C_{18:0}$ decreased. The content of $C_{18:1}$ fell significantly (by 48%), but there was a marked increase (by 37%) in the content of $C_{18:2}$, precursors of $C_{20:4}$, the content of which also rose by 18%. Under these circumstances $C_{18:3}$ appeared in small quantities (Table 2). The SFA/UFA ratio was 0.63, which is 18% lower than in the animals of group 1. Unlike the animals

of group 2, rabbits of group 3 showed a raised content of UFA $C_{16:0}$ (by 32%), but no change in the content of $C_{18:0}$. Meanwhile, a decrease in $C_{18:1}$ (by 34%) was recorded, less marked than in the animals of group 2. The content of $C_{18:2}$ and $C_{20:4}$ was unchanged compared with animals of group 1. Just as in the animals of group 2, a very small quantity of $C_{18.3}$ appeared. As a result of the changes, the SFA/UFA ratio rose by 32% (Table 2). In the animals of group 3 the total Ch content also was increased (by 36%) in the plasma membranes of the gastrocnemius muscle. In rabbits of group 2 there was a decrease in the total Ch content (by 29%), evidently due to inhibition of Ch biosynthesis in the presence of UFA [14], and an increase in the excretion of CS with the bile and with the feces [13].

LPO in the plasma membranes of the animals of group 2 rose from 2.67 ± 0.21 to 3.8 ± 0.35 mmoles/mg protein (by 44%, $p < 0.05$), and in the rabbits of group 3 it fell to 2.00 ± 0.24 mmoles/mg protein (by 25%, $p < 0.05$). The increase in LPO in the animals of group 2 can be attributed to the presence of a large quantity of UFA in phospholipids and a lower Ch content in the biological membranes [3]. Weakening of LPO in the rabbits of group 3 was associated with the presence of a large quantity of SFA and Ch, which are highly resistant to oxidation. Furthermore, during oxidation of several lipids, containing mainly SFA, substances which are antioxidants are formed [5]. In this way, secondary inhibition of oxidation may develop.

The results are evidence that an increase in the UFA content ($C_{18:2}$, $C_{18:3}$, $C_{20:4}$) accompanied by a decrease in SFA ($C_{18:0}$), a decrease in the total C_H content, and intensification of LPO in the plasma membranes of the gastrocnemius muscle are accompanied by an increase in permeability of the BPB for O_2. The decrease in permeability of BPB for O_2 is found when the content of UFA ($C_{18:1}$) was reduced and that of SFA ($C_{15:0}$, $C_{16:0}$) was increased, the Ch content was increased, and the intensity of LPO reduced. A decrease in the rate of oxygen transport in the erythrocyte when the total Ch content was stated in [9].

It can be concluded from these results that the oxygen supply of a skeletal muscle can vary depending on structural and functional modifications of BPB, caused by alimentary factors.

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