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The Effect of Hyperbaric Exposure of 20 Atmospheres-Absolute ($He-O_2$) on Sphingoglycolipids of Rat Tissues[1] (38650)

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Accumulation of monoglycosyl ceramide occurs in plasma and red blood cells as well as chain elongation of fatty acids in liver glycolipids from rats subjected to a hyperbaric environment (N_2–O_2) and rapid decompression (1). Helium is more advantageous to use in diving than N_2 and has been substituted for N_2 in the breathing mixture in deep sea diving experiments. However, helium can affect cellular metabolism at both ambient and high pressures (2–7). This report presents the changes in sphingoglycolipids of liver, kidney, lung and spleen in rats exposed to a gas mixture of $He–O_2$ at 1 atmosphere-absolute (ATA) and 20 ATA.

Materials and Methods. Adult male Sprague–Dawley rats (five animals per group) with an average weight of 300 g were placed in a chamber at 5 PM. The chamber was flushed with 100% oxygen to remove all the residual nitrogen and then flushed with 100% helium until an approximate 75–25% $He–O_2$ mixture was reached. This mixture was monitored by gas liquid chromatography. The pressure was increased with 100% helium at a rate of 0.5 ATA per min to 20 ATA. Animals were held at 20 ATA pressure until 7 AM the following morning. The rats underwent decompression using the following schedule: 1 ATA per 5 min with 35 min stops at 15 ATA and 10 ATA, a 95 min stop at 5 ATA and a 155 min stop at 2.5 ATA. After reaching 1 ATA, the animals were equilibrated for 155 min before dissection. A partial pressure of oxygen was kept at a level between 150–250 mmHg throughout the experiment. The chamber temperature was 88 F at 20 ATA and 15 ATA, 86°F at 10 ATA and 5 ATA, 84°F at 2.5 ATA and 1 ATA. Two control groups of five rats each were held in the same type of chambers and exposed to ambient air and 1 ATA of 80:20 $He:O_2$ for the same period of time as the stress group (2).

After decompression, the rats were lightly anesthetized with ether and bled from the aorta. The kidney, spleen, liver and lung were removed immediately, weighed, placed in containers, frozen in dry ice:acetone mixture and stored at −20° until extraction. The lipids were extracted from the organs and analyzed as described elsewhere (1, 8, 9). The data obtained from the lipid analysis was derived from five animals per group with variations of less than ±10% of the mean values shown in Tables I–IV.

Results. Total lipids. The total lipid and glycolipids were determined in liver, kidney, lung and spleen excised from rats held in ambient air, helium:oxygen mixture at 1 ATA and 20 ATA. No differences were found in the total lipids in the organs from control and experimental groups (Table I). The amount of total glycolipids in the spleen was different in stress groups of rats, but similar in the two control groups. More pronounced glycolipid differences were observed in the liver and kidney when the two control groups were compared (Table I) than in the helium control and stress groups

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TABLE I. CONCENTRATION OF GLYCOSYL CERAMIDES IN RAT ORGANS FROM NORMAL AND STRESSED ANIMALS.

	Total lipid	Total glycolipid	Monoglycosyl ceramide	Diglycosyl ceramide	Tetraglycosyl ceramide
Liver A-C[c]	39.0[a]	31[b]	27[b]		4[b]
H-C[d]	39.8	47	42		5
S	40.7	36	30		6
Kidney A-C	37.8	114	70	21[b]	23
H-C	37.3	88	58	15	15
S[e]	39.9	105	66	17	22
Lung A-C	38.5	73	49	16	8
H-C	39.6	59	30	13	16
S	36.9	62	25	17	20
Spleen A-C	20.3	107	67	21	19
H-C	19.9	108	70	23	15
S	21.1	63	48	10	5

[a] mg/g wet tissue.
[b] μg/g wet tissue.
[c] A-C ambient air control.
[d] H-C He–O_2 control, 1 ATA.
[e] S He–O_2, 20 ATA.

of rats. There was marked decrease in the amount of mono-, di- and tetraglycosyl ceramides of the spleen under stress conditions compared to the helium control group (Table I). The amount of monoglycosyl ceramides of the lung appears to be decreased in the helium control and the stress group compared to the air control group of rats, whereas the tetraglycosyl ceramide was increased in the same groups of animals.

Fatty acid profiles of glycosyl ceramides. The fatty acid profiles of monoglycosyl ceramides of various rat organs are shown in Table II. A consistent reduction in the relative percentage of the 14:0 fatty acid was observed in the liver, kidney and lung when the air control groups were compared to the helium control and stress groups.

The diglycosyl ceramides showed a tendency toward an increase of the long-chain fatty acids in the kidneys of animals that were stressed or in the helium control compared to animals held in a normal air atmosphere (Table III). In the lung, there was a decrease of the long-chain fatty acids in helium control and stressed animals compared to the control group held at ambient air.

The fatty acids of tetraglycosyl ceramides in the four rat tissues are summarized in Table IV. The ratio of 24:0/24:1 in liver and lung of air control animals was smaller than the ratio in animals exposed to He–O_2 mixture at 1 ATA and 20 ATA. There were no differences in the fatty acid composition between the two helium:oxygen animal groups. Chain elongation was noted in the kidney of stressed animals. The amount of 24:1 was decreased in the spleen of stressed animals. Hydroxy fatty acids were found in monoglycosyl ceramide of liver, kidney, lung and spleen, and a sulfatide in the kidney. No differences were observed in the hydroxy fatty acids between control and experimental animals.

Discussion. There were no changes in the total lipids of liver, kidney, lung and spleen of rats held at ambient air pressure or with helium:oxygen mixture, at 1 ATA and 20 ATA. In our previous work, no differences were found in total lipid of liver, plasma and red blood cells of rats subjected to a 9.6 ATA (N_2–O_2) exposure and fast decompression (1).

Previous work indicates that monoglycosyl ceramides were elevated in plasma and red blood cells in rats exposed to a 9.6 ATA of N_2–O_2 and fast decompression. The amounts of three glycolipids in the spleen and the monoglycosyl ceramide in the lung were decreased in stressed animals. This change could be associated with an increase of activity of glycolipid catabolic enzymes in spleen and lung. Another possibility is that there was a suppression of the anabolic enzyme activity in stressed animals.

Changes were found in the fatty acid composition of monoglycosyl ceramide in liver, kidney and lung (Table II), of diglycosyl ceramide in kidney and lung (Table III), and of tetraglycosyl ceramide in liver and lung (Table IV) when the two control groups were compared. More changes were observed in the amount of glycolipid of liver and kidney in the two control animals than in the two helium groups of animals. Ritter *et al.* (3) found a slight but significant increase of lactic acid dehydrogenase and

TABLE II. EFFECT OF HYPERBARIC HELIUM-OXYGEN ON FATTY ACIDS OF MONOGLYCOSYL CERAMIDES OF VARIOUS RAT ORGANS.

	Liver			Kidney			Lung			Spleen		
	A—C[c]	H—C[d]	S[e]	A—C	H—C	S	A—C	H—C	S	A—C	H—C	S
14:0[a]	12.0[b]	1.9	1.4	10.7	0.3	0.9	11.1	5.9	1.4	2.6	1.4	4.2
15:0				0.4	2.6	2.7	1.1	1.0		0.1	0.2	0.4
16:0	16.9	23.5	20.7	4.9	10.0	8.0	16.0	14.2	11.0	12.5	9.5	10.1
17:0				1.9	4.3	3.0	0.3	0.3	0.7	0.9	0.8	0.9
18:0	11.2	8.8	7.8	3.5	5.3	3.5	4.8	4.9	8.0	7.8	6.5	7.4
18:1	0.9	0.7	0.7	0.2	8.2		0.4	2.5				
19:0										0.8	0.7	0.3
20:0	1.6	2.3	1.3	8.5	11.9	8.8	7.0	7.7	11.8	7.2	6.7	8.0
22:0	10.6	9.8	13.0	23.3	23.7	24.1	14.3	16.7	19.7	14.4	13.2	13.8
23:0	9.4	7.7	8.4	8.5	8.2	8.7	3.1	2.8	4.1	9.6	8.9	10.8
23:1										2.4	2.2	2.2
24:0	31.4	31.1	37.2	38.3	25.1	40.0	37.3	38.9	41.6	38.2	44.6	36.7
24:1	5.9	14.3	9.3				4.8	5.0	1.7	2.9	4.8	3.6

[a] Number of carbon atoms in acid:number of double bonds.
[b] Percentage of total fatty acid.
[c] A-C Ambient air control.
[d] H-C He–O_2 control, 1 ATA.
[e] S He–O_2, 20 ATA.

TABLE III. EFFECT OF HYPERBARIC HELIUM-OXYGEN ON FATTY ACIDS OF DIGLYCOSYL CERAMIDES OF VARIOUS RAT ORGANS.

	Kidney			Lung			Spleen		
	A—C[c]	H—C[d]	S[e]	A—C	H—C	S	A—C	H—C	S
14:0[a]	0.3[b]	0.5	0.2				0.3	0.6	0.9
15:0	8.2	6.7	6.8				5.3	5.7	4.5
16:0	9.8	9.3	7.4	17.7	19.7	21.7	10.5	14.7	11.3
17:0	8.9	5.5	6.4	0.2	0.9	0.5	10.9	7.5	5.6
18:0	10.5	7.1	6.9	8.7	16.5	18.1	7.5	12.2	7.2
18:1	1.2	1.1	0.8	1.7	4.9	2.7	1.1	1.6	0.6
18:2	1.2	0.5	0.6	1.3	1.8	1.8	1.0	1.1	0.7
19:0							4.8	6.0	2.8
20:0	3.5	4.7	4.8	6.8	5.2	5.3	6.7	5.3	6.9
22:0	13.3	19.7	16.4	14.8	11.2	11.7	10.7	9.1	11.0
23:0	9.6	5.8	11.3	1.8	5.3	4.5	6.8	6.2	7.2
24:0	30.7	34.8	35.2	30.6	24.0	23.9	28.0	21.2	32.8
24:1	3.5	3.8	2.8	16.3	10.5	9.6	6.1	8.7	8.3

[a] Number of carbon atoms in acid:number of double bonds.
[b] Percentage of total fatty acid.
[c] A-C Ambient air control.
[d] H-C He–O_2 control, 1 ATA.
[e] S He–O_2, 20 ATA.

glutamic–oxaloacetic transaminase in serum of mice exposed to a He–O_2 mixture at 1 ATA. Wang *et al.* (4) reported that a significant increase in gamma aminobutyric acid was found in the brains of mice exposed to He–O_2 at 1 ATA. Bitter and Nielsen (2) found that statistically significant increases in the excretory rates of corticosterone in He–O_2 control samples compared to samples collected in N_2–O_2. The ob-

TABLE IV. EFFECT OF HYPERBARIC HELIUM-OXYGEN ON FATTY ACIDS OF TETRAGLYCOSYL CERAMIDES OF VARIOUS RAT ORGANS.

	Liver			Kidney			Lung			Spleen		
	A—C[c]	H—C[d]	S[e]	A—C	H—C	S	A—C	H—C	S	A—C	H—C	S
15:0[a]				3.0	2.4	2.9				0.2	1.3	0.7
16:0	8.3[b]	6.1	6.5	12.8	8.5	6.4	7.6	9.9	9.7	4.9	5.9	5.8
16:1										0.8	0.4	0.6
17:0	0.6	1.0	0.9	1.9	3.3	1.9	0.5	1.9	0.3	0.1	0.6	0.1
18:0	8.6	8.8	7.2	7.3	6.4	5.6	7.1	6.5	8.3	4.6	4.8	9.5
18:1	0.8	1.1	0.6	5.0	1.3	0.7	2.7	2.6	1.4	0.4	0.9	2.9
18:2	0.3	2.2	0.8				0.6	0.2	0.3			
19:0										0.4	0.2	2.4
20:0	5.4	5.0	4.8	4.6	6.8	6.3	7.4	9.8	10.2	7.1	6.7	8.3
22:0	13.5	13.3	13.1	15.6	18.0	15.9	18.5	20.1	23.3	15.6	13.7	13.4
22:1	0.7	1.7	2.2									
23:0	6.3	7.1	11.3	6.8	6.6	6.3	3.2	1.5	3.1	10.0	9.4	10.1
23:1	2.1	2.1	3.6							3.8	3.6	2.0
24:0	30.1	33.9	33.2	35.7	37.9	46.7	25.6	31.3	28.0	46.6	43.6	42.7
24:1	23.2	17.6	15.7	7.2	8.6	7.4	26.2	16.0	15.6	5.0	8.0	1.5

[a] Number of carbon atoms in acid:number of double bonds.
[b] Percentage of total fatty acid.
[c] A-C Ambient air control.
[d] H-C He-O_2 control, 1 ATA.
[e] S He-O_2, 20 ATA.

servation that glycolipids are also affected, as demonstrated in these studies, adds additional evidence that helium can affect the cellular metabolism at ambient pressure.

The fatty acid profiles of monoglycosyl ceramide in liver and kidney, of diglycosyl ceramide in spleen and of tetraglycosyl ceramide in kidney of He–O_2 stressed animals showed that chain elongation took place. These results agree with our earlier report (1) that chain elongation of fatty acids of the monoglycosyl ceramides was observed in the livers of rats exposed to 9.6 ATA (N_2–O_2) and fast decompression. These studies show that glycolipids are affected in animals exposed to helium and hyperbaric exposures and can serve as a biochemical marker to show physiological changes under stress conditions.

Summary. The effect of hyperbaric exposure of helium–oxygen at 20 atmospheres-absolute (ATA) on sphingoglycolipids of rat liver, kidney, lung and spleen was studied. No changes were found in the total lipids of the tissues of rats held in ambient air, helium–oxygen mixtures at 1 ATA and 20 ATA. The amounts of three glycolipids in the spleen and the monoglycosyl ceramide in the lung were decreased in stressed animals. There were changes in the fatty acid profiles of glycolipids between groups of animals held in ambient air and He–O_2 at 1 ATA. Greater changes were observed in the amount of glycolipids from liver and kidney of animals held at 1 ATA in air and helium–oxygen than in animals held in helium–oxygen at 1 ATA and 20 ATA, providing additional evidence that helium can affect cellular metabolism at ambient pressure. Chain elongation of fatty acids was observed in glycolipids of liver, kidney and spleen of rats exposed to helium–oxygen at 20 ATA compared to animals held in helium–oxygen at 1 ATA.

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