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The Effect of Cold on the Composition of the Phospholipids of the Blood Plasma of Healthy Athletes[1] (37750)

B. D. POLIS, E. POLIS, H. P. SCHWARZ, AND L. DREISBACH

Biochemistry Laboratory, U. S. Naval Air Development Center, Warminster, Pennsylvania 18974; and Department of Biochemistry, Philadelphia General Hospital, Philadelphia, Pennsylvania 19104

Previous studies from these laboratories have shown that various types of physical and mental stress cause a significant elevation of plasma phospholipid (1, 2). It appeared thereby that certain individual phosphatides, particularly phosphatidylglycerol (GPG) were more affected than others. Since these findings suggest a specific role of such individual phospholipids in stress it was decided to examine the effect of cold on the plasma lipid composition of healthy volunteers. Examination of this type of stress on phospholipid metabolism seemed to be particularly feasible because the general reactions to cold stress have been well established.

Experiment. A group of nine young athletes training for various sports such as swimming, or rugby and running, or weight lifting were selected by David Henderson from the Department of Physical Education of Temple University. Samples of heparinized blood for the phospholipid studies were drawn from the cubital vein 2 days before and immediately preceding the experiments. The experiments were carried out 4 hr after the last meal and a 15 min rest period. Individual athletes were placed in a water tank of 2° for a period of 3 min and samples of heparinized blood were taken immediately after leaving the tank and 7 min later.

Analysis of the phospholipid composition of the blood plasma. The blood samples were centrifuged immediately and the plasma was frozen and stored. No significant changes in phospholipids were observed during freezing and storage. The samples then were extracted with 20 vol of chloroform–methanol (2:1, v/v) for 24 hr at room temperature. The protein precipitate was filtered off on a sintered glass funnel and the solution was evaporated to dryness in a nitrogen stream. The residue was dissolved in chloroform–methanol–water (60:30:45). This solution was passed through a glass column packed with 2 g of Sephadex G-25 for removal of nonlipid impurities (3). The eluate was again evaporated to dryness with a stream of nitrogen and the residue was dissolved in 25 ml of chloroform. The separation of the phospholipid fractions and analyses of the individual phospholipids were carried out in duplicate as described elsewhere (4, 5) so that minor details of the method may be omitted. The purified lipid extract was passed through a column of 6 g of silicic acid for removal of the neutral lipids and aliquots of the phospholipids eluted with methanol were subjected to mild alkaline hydrolysis with 0.03 *N* NaOH in ethanol for 20 min at 37°. The hydrolyzate was neutralized with ethyl formate and again evaporated in a nitrogen stream. The residue was distributed between 2 vol of isobutanol–chloroform (1:2, v/v) and 1 vol of water and the two phases were separated by centrifugation. An aliquot of the water phase containing the deacylated (alkali labile) phospholipids was spotted on Whatman 3MM paper and

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the glycerol phosphate components were separated first by descending chromatography in phenol saturated with water–acetic acid–ethanol for 16 hr. The solvents then were removed and separation in the second dimension was performed with high voltage ionophoresis in pyridine–acetic acid–water buffer at pH 3.6 using a current of 100–125 mA at 2000 V for 1.5 hr with a Savant instrument. The chromatograms were dryed and then sprayed with Ninhydrin reagent to locate amino lipids and afterwards with acid molybdate reagent (4) to determine the phosphorus compounds. Analysis of the alkali stabile frations (plasmalogen, sphingomyelin and alkyl ethers) contained in the chloroform phase was carried out as described by Dawson *et al.* (4). The individual chromatographic spots were identified by R_f values found with deacylated authentic standards. In the case of GPG, pure phosphatidylglycerol isolated from human plasma (6) or ^{14}C labeled GPG obtained from Scenedesmus (7) have been checked. Quantitation of the individual phospholipids was carried out by digestion of the stained spots with 72% perchloric acid and subsequent determination of inorganic phosphorus (8, 9).

Results and Discussion. The results summarized in Table I demonstrate that the plasma GPG levels of the subjects were significantly higher ($P < 0.1\%$) immediately after they left the cold-water tank and that this increase still remained unchanged for 7 min afterwards. Only one other phospholipid, *e.g.*, phosphatidic acid showed a lesser increase ($P < 5\%$) after the subjects left the cold-water tank, while other phosphatides and total lipid phosphorus were not affected by cold stress. There were small differences in the degree of the increase of GPG in individual subjects, but these variations could not be attributed to the physical conditions of these healthy men. One of these athletes, a swimmer training for Olympic competitions, was so greatly affected by the cold water that he had to leave the tank after 0.5 min. In this subject, GPG of the plasma was 33% higher than before the experiment and remained at about that elevated level 7 min later.

The described results further emphasize the importance of increase of GPG in stress found previously (1, 2). It appears to be biologically significant that among a total number of 19 individual phospholipids examined one single component only would be affected in this manner.

GPG was discovered by Maruo and Benson (10) who established it to be a quantitatively important and highly dynamic phospholipid of chloroplasts. GPG subsequently was found to be abundant in various species of bacteria (11–13) and was detected in animal tissues, *e.g.*, rat liver mitochondria (14, 15) and also in rat liver microsomal and lysosomal fractions (1). GPG was synthesized enzymatically from L-α-glycerophosphate in systems containing liver (16) or brain homogenates (17). Catecholamines such as L-norepinephrine were found to stimulate the incorporation of ^{32}P into GPG four- to sixfold *in vitro* in pineal cultures (18). Studies on the effect of acceleration carried out in these laboratories showed that the GPG elevation found after that stress failed to occur in hypophysectomized animals, but that an increase of GPG content of the brain appeared instead (2). These experiments suggest that the described changes of GPG in stress are somehow related to the pituitary–adrenal system and the brain, the importance of which for the adaptation to stress appeared to be established. The exact mechanism of this function of GPG can only be surmised at present. Macfarlane (19) and others (20) have given convincing evidence that a number of bacteria produce ortho-amino acid esters of GPG and phosphatidopeptides have been repeatedly isolated from liver and other tissues (21). One may be tempted to postulate a possible release of polypeptide ester of GPG from the pituitary to be involved, but such an assumption can only be realized by extensive additional investigations.

Summary. The phospholipid composition of the blood plasma of nine healthy athletes was examined before and after the subjects were placed in a tank filled with water at 2°. It was found that the phosphatidylglycerol (GPG) content of the plasma was very significantly elevated immediately after and 7 min

TABLE I. Effect of Exposure to Cold on Phospholipid Composition of the Blood Plasma in Nine Human Volunteers.

	Total lipid phosphorus	Lecithin	Phosphatidyl ethanolamine	Phosphatidyl-serine	Cardiolipin	Phosphatidic acid	Phosphatidyl-glycerol	Phosphatidyl-inositide	Inorganic phosphorus	Plasmalogens	Sphingomyelin	Alkyl ethers	Unknowns
	Mean lipid phosphorus (μmoles/liter plasma $\pm$ SE)												
Immediately before	2203 $\pm$46.6	1490 $\pm$36.0	31.54 $\pm$1.76	10.50 $\pm$0.46	21.66 $\pm$1.34	8.93 $\pm$0.79	25.82 $\pm$0.81	75.95 $\pm$3.07	10.45 $\pm$0.91	70.84 $\pm$2.43	341.17 $\pm$7.46	44.44 $\pm$0.83	32.14 $\pm$1.68
Immediately after	2213 $\pm$49.9	1481 $\pm$38.3	30.53 $\pm$1.30	10.61 $\pm$0.41	21.52 $\pm$1.60	11.29[b] $\pm$0.63	33.24[a] $\pm$1.68	76.26 $\pm$3.13	10.69 $\pm$0.78	71.07 $\pm$1.46	343.99 $\pm$6.58	44.78 $\pm$1.42	33.13 $\pm$1.57
7 Min after	2228 $\pm$47.8	1519 $\pm$43.4	31.24 $\pm$1.92	10.93 $\pm$0.58	21.76 $\pm$1.16	10.29[b] $\pm$0.62	33.41[a] $\pm$1.83	76.06 $\pm$1.84	9.89 $\pm$0.71	67.04 $\pm$2.85	342.87 $\pm$7.70	44.78 $\pm$0.83	28.64 $\pm$2.53
	Mean distribution (%) of lipid phosphorus $\pm$ SE												
Immediately before		67.64 $\pm$0.42	1.43 $\pm$0.07	0.48 $\pm$0.02	0.98 $\pm$0.05	0.41 $\pm$0.04	1.17 $\pm$0.02	3.44 $\pm$0.11	0.47 $\pm$0.04	3.22 $\pm$0.09	15.53 $\pm$0.36	2.00 $\pm$0.03	1.46 $\pm$0.06
Immediately after		66.91 $\pm$0.37	1.38 $\pm$0.04	0.48 $\pm$0.02	0.97 $\pm$0.06	0.51[b] $\pm$0.03	1.50[a] $\pm$0.05	3.45 $\pm$0.13	0.48 $\pm$0.03	3.22 $\pm$0.07	15.60 $\pm$0.43	2.04 $\pm$0.09	1.51 $\pm$0.08
7 Min after		68.08 $\pm$0.69	1.40 $\pm$0.07	0.49 $\pm$0.02	0.97 $\pm$0.04	0.46 $\pm$0.03	1.50[a] $\pm$0.07	3.42 $\pm$0.08	0.44 $\pm$0.03	3.00 $\pm$0.09	15.45 $\pm$0.50	2.02 $\pm$0.05	1.29 $\pm$0.11

[a] $P < 0.1\%$.
[b] $P < 5\%$.

following the exposure to the cold. Only one other phospholipid, phosphatidic acid, showed much lesser elevation of GPG after the subjects left the cold-water tank. All the remaining individual phospholipids and total lipid phosphorus were not affected by the experiment. The possible significance of these changes of GPG in stress are discussed.

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