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*Original Contribution*

# URIC ACID AND GLUTATHIONE LEVELS DURING SHORT-TERM WHOLE BODY COLD EXPOSURE

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**Abstract**—Ten healthy subjects who swim regularly in ice-cold water during the winter (winter swimming), were evaluated before and after this short-term whole body exposure. A drastic decrease in plasma uric acid concentration was observed during and following the exposure to the cold stimulus. We hypothesize that the uric acid decrease can be caused by its consumption after formation of oxygen radicals. In addition, the erythrocytic level of oxidized glutathione and the ratio of oxidized glutathione/total glutathione also increased following cold exposure, which supports this hypothesis. Furthermore, the baseline concentration of reduced glutathione was increased and the concentration of oxidized glutathione was decreased in the erythrocytes of winter swimmers as compared to those of nonwinter swimmers. This can be viewed as an adaptation to repeated oxidative stress, and is postulated as mechanism for body hardening. Hardening is the exposure to a natural, e.g., thermal stimulus, resulting in an increased tolerance to stress, e.g., diseases. Exposure to repeated intensive short-term cold stimuli is often applied in hydrotherapy, which is used in physical medicine for hardening.

**Keywords**—Glutathione, Uric acid, Erythrocytes, Hypothermia, Hydrotherapy, Oxygen radicals, Free radicals

## INTRODUCTION

Life in a modern industrial society is characterized by a growing deficiency of natural stimulating factors like physical exercise. Furthermore, dynamic muscle work has been substituted more and more by static muscle work. There is also lack of thermal and climatical influences from the environment. This may contribute to increased morbidity, e.g., in degenerative diseases of the skeletal and muscle systems, heart diseases, and infectious diseases, but also to lability of the central nervous system.[1] The negative results of natural stimulator deficiency can be partly prevented by exercise and "hardening," which play an important role in physiotherapy. The exposure to an intensive short-term cold stimulus like swimming in cold water of lakes or rivers, especially during winter, has been used as one form of body hardening for many years.[2–4] Hardening means exposure against a natural, e.g., thermic stimulus, resulting in an increased resistance to diseases, especially acute respiratory diseases.[5] As of now the molecular mechanisms leading to "hardening" are not known. Because of the high frequency of acute respiratory diseases and because of the medical importance of body hardening, studies on biochemical mechanisms connected with a short-term whole body cold stimulus are interesting.

To explore the possibility of oxygen radical generation during and following a short-term whole body cold stimulus, the concentrations of uric acid, reduced glutathione (GSH), and oxidized glutathione (GSSG) in red blood cells of winter swimmers were measured before and after cold stimulus.

## MATERIALS AND METHODS

In Berlin, one winter swimmer club has been flourishing for several years. The members are not selected in any way and all swim regularly outdoors in ice-cold water. Their activities start in the fall, and members swim at least once per week for about 5 min in ice-cold water. Sometimes, ice on the lake has to be removed to allow them to swim.

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Urine and blood samples were taken from 10 healthy subjects accustomed to winter swimming. The subjects had been winter swimming for several (1–11) years. The frequency of the winter swimming was once per week for about 5 min. The age of the subjects in this group was 40.5 ± 12.3 years. The control group consisted of ten healthy control persons who never participated in winter swimming. Their age was 35.6 ± 8.8 years.

Venous blood samples of the winter swimmers were taken about 1 h before, 1 h after, and for the uric acid determinations, also 24 h after the winter swimming. Between the first and the second blood sampling (1 h before, 1 h after swimming), the subjects did not eat or drink. The swimming was carried out in the morning for about 5 min following a short running exercise. The measurements were carried out on two different days. The water temperature was 2°C on the first day, and 3°C on the second day. The air temperature was 1°C and 5°C, respectively.

Sodium citrate was added to the blood samples to prevent coagulation. For analysis of uric acid, plasma was separated from blood cells by centrifugation. Plasma was frozen at −17°C until analyzed. Uric acid was determined as described by Bergmeyer.[6] For measurements of GSH and GSSG concentrations, samples were drawn with ice-cold metaphosphoric acid and kept at 4°C. Samples were centrifuged for 10 min at 1200 g. Supernatants were collected and separated into two aliquots for the GSH and GSSG measurements. GSH was assayed by means of Ellman's reaction according to Beutler et al.[7] GSSG was determined fluorimetrically according to Hissin and Hilf.[8] The total concentration of glutathione, the sum of GSH and GSSG, was calculated based on the number of sulfhydryl units. The GSH autoxidation was prevented by addition of 50 mM *N*-ethylmaleimide (NEM). 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased from Sigma Chemical Co. (St. Louis, MO). NEM and o-phthaldialdehyde were from Calbiochem (San Diego, CA), and GSH and GSSG from Boehringer (Mannheim, Germany).

Uric acid additionally was measured in the urine of winter swimmers. The urinary bladder was evacuated 2 h before the whole body cold exposure. Immediately before the cold exposure the urine was collected, and volume and uric acid concentration were measured. From urine volume and uric acid concentration in urine, the renal excretion rate was calculated in mmol/h. Two h after winter swimming, the urine was collected again for volume and uric acid concentration measurements, to establish the renal excretion of uric acid during and immediately following the cold exposure.

## RESULTS

Uric acid levels measured 1 h before the winter swimming (initial) were 310 ± 126 μM, which is in the range of normal uric acid concentrations, 140–340 μM for women and 200–420 μM for men.[9] One h following the whole body cold stimulus, the uric acid levels were found to be only about the half of the initial values, 141 ± 69 μM. However, 24 h after the winter swimming, the plasma uric acid levels were back to the initial values.

Figure 1 shows that the GSH concentration in the red blood cells of the control subjects (2.08 ± 0.49 mM) was in the range of normal values reported in the literature.[7,10–12] However, the initial GSH concentration of red blood cells of subjects accustomed to hypothermic exposures by winter swimming was 2.81 ± 0.44 mM, which is significantly higher as compared to the control group. Furthermore, GSH levels tended to decrease from 2.81 to 2.61 mM, 1 h after the exposure to hypothermia. Figure 1 also illustrates the differences in the total concentration of glutathione (open columns). The level of total glutathione was higher in the erythrocytes of winter swimmers (3.12 ± 0.47 mM) as compared with the total glutathione in the erythrocytes of controls (2.42 ± 0.52 mM). This increase is almost entirely due to the increase in GSH.

Figure 2 demonstrates the GSSG concentrations of erythrocytes. Surprisingly, a comparison of the initial GSSG values of winter swimmers with controls showed decreased values in the erythrocytes of the winter swimmers. This is contrary to the increase in GSH in erythrocytes of winter swimmers. The initial GSSG values in both experimental groups are within the range of values reported in the literature.[13] However, some studies have reported lower GSSG values.[14] The intraerythrocytic GSSG concentration was markedly increased 1 h after the whole body cold stimulus. Furthermore, Figure 2 shows the behavior of the GSSG:total glutathione ratio. Because of the diverging changes in the erythrocytic GSH (decreasing, Fig. 1) and GSSG (increasing, Fig. 2) concentrations of winter swimmers and controls, the most distinct differences in the glutathione metabolism between the two groups were found for this ratio. Figure 3 shows the individual changes of the GSSG levels. The concentration of GSSG increased in all subjects.

The urine volume of winter swimmers before cold exposure was 135 ± 122 ml, and after cold exposure it was 174 ± 116 ml. The uric acid concentrations were 2.148 ± 0.415 and 1.736 ± 0.379 mmol/l, respectively. Based on these findings the uric acid excretion was 0.145 ± 0.028 mmol/h before cold exposure and 0.151 ± 0.033 mmol/h after cold exposure.

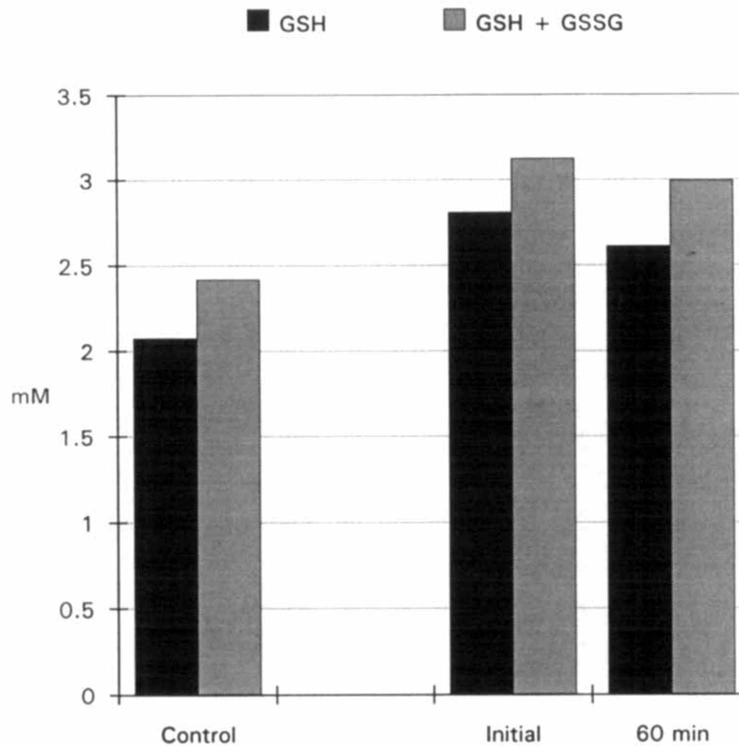


Fig. 1. Erythrocytic concentrations of reduced glutathione and total glutathione in controls and in winter swimmers before and after cold stimulus ($n$ = 10). Significant differences in GSH level exist between control and the group of winter swimmers. This is also seen for total glutathione.

The hematocrit values did not show significant differences between controls (40.8 ± 2.7%) and winter swimmers (41.9 ± 4.5%). It should be mentioned that there were no clinical or clinical–chemical indices for injuries to skeletal muscles from winter swimmers.

## DISCUSSION

The hypothesis of increased formation of reactive oxygen species following winter swimming is supported by the finding of a drastic decrease of plasma uric acid levels under these circumstances. The uric acid level decreased within 1 h after cold exposure from 0.31 to 0.14 mmol/l. Because the total plasma volume is about 3 L, the total loss of uric acid would be 0.5 mmol/h. The total loss of uric acid in the extracellular fluid, which is about 15 L and includes plasma, is 15 × 0.17 mmol, which equals 2.5 mmol/h. Because nucleosides and nucleobases are transported rapidly through biological membranes,[15–17] it is likely that each change in uric acid concentration in plasma can lead to a net transport from the other fluid compartments of the organism into the blood plasma.[18–20] Therefore, the total uric acid loss during and following the winter swimming is probably closer to 2.5 mmol/h than to 0.5 mmol/h, because of the high transport rates of urates. The uric acid pool of the male adult is about 5.0–9.5 mmoles, and of the female adult 3.5–4.5 mmoles. The daily turnover is estimated to be about 5.0 mmoles. In purine-free nutrition, a daily purine excretion of 2–3 mmoles was measured.[20] A uric acid excretion of 0.12–0.18 mmol/h was reported for normal metabolic conditions predominantly via the kidneys,[21–23] which was also found in winter swimmers before cold exposure. An increase by a factor of 4 to 20 would be necessary to explain the drastic uric acid loss that we observed, by a change in excretion alone. Because we found no significant change in uric acid excretion directly following cold exposure, it is unlikely that the drop in plasma uric acid we observed is caused by excretion alone.

A possibility for the decrease of uric acid is its consumption while scavenging radicals. The capability of uric acid to scavenge oxygen free radicals was first described by Ames et al.[24] in 1981, and confirmed in recent years by several other studies.[25–33] The contributions of the chain-breaking antioxidants in human blood plasma were estimated by Wayner et al.[28] They estimated that the uric acid contributes to 35–65% of the total plasma antioxidant capacity.[28]

Loss of urate into extracellular stores, or general loss from the blood stream into other compartments,

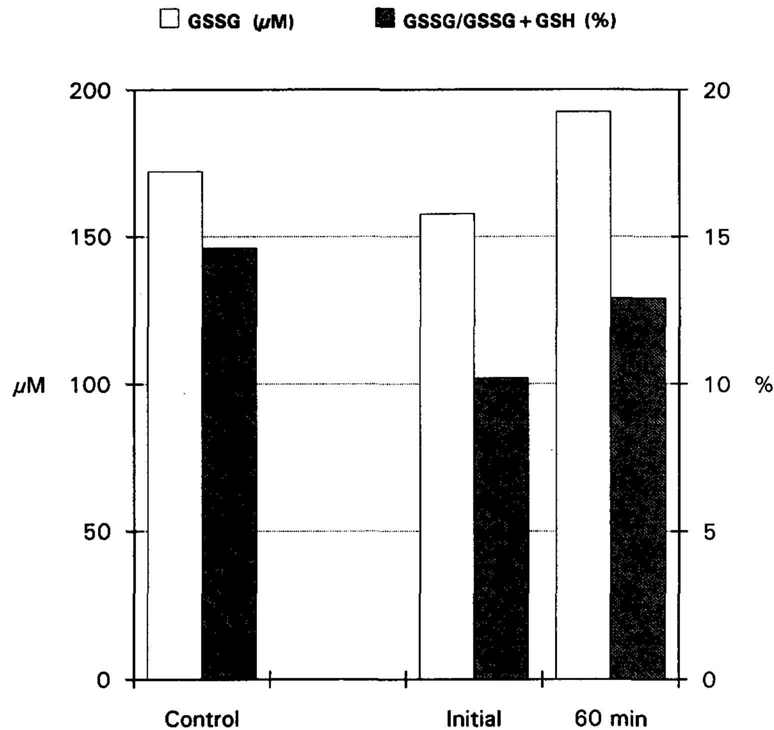


Fig. 2. Concentration of GSSG and GSSG:total glutathione ratio of circulating erythrocytes in controls and winter swimmers before and after hypothermic exposure ($n$ = 10). Significant differences both in GSSG and in GSSG:total glutathione ratio exist between control and initial values of winter swimmers, and also between initial and 1 h values of winter swimmers.

can be excluded as a source for urate loss, because of the extremely rapid exchange of uric acid between intra- and extracellular space. It is known that purine bases and purine nucleosides pass the cell membrane by facilitated diffusion. For purine bases, the existence of two different carrier proteins have been described.[34] It was found that purine bases and nucleosides are transported equally well through the membrane in either direction.[35] A special urate transporter was characterized in studies using renal brush border membranes.[36,37] These studies clearly demonstrate that it is not possible to loose purine bases and nucleosides into the extracellular stores or into other compartments of the organism. It would be possible to loose them by metabolic (enzymatic) conversion in different cell types.[34–37] However, metabolic conversion of uric acid in human cells can be excluded because of the absence of urate oxidase in human cells.

If the suggestion of a high oxygen radical formation during whole body hypothermia is true, one should expect changes in other components of the antioxidative system, in blood plasma as well as in tissues. The red blood cells are known as useful indicators of oxidative stress.[13,38,39] One of the most sensitive antioxidative criteria is the glutathione system. Changes of the erythrocytic concentrations of GSH and GSSG have also been used as an indicator of increased free radical formation in the liver[13] and the lung.[38,39] The changes in glutathione metabolism and plasma uric acid concentrations support the hypothesis of oxygen radical formation during whole-body hypothermia. The increased erythrocytic GSH and lowered GSSG values of winter swimmers, in comparison with those of a control population, speak in favor of an adaptation to repeated oxidative stress. Increased erythrocytic GSH levels were also observed in smokers who are chronically exposed to oxidative stress.[38]

Sudden changes in metabolic activity from cold exposure could disturb energy metabolism, which could affect glutathione and uric acid. There are several reasons why the changes in glutathione and uric acid levels we observe are not caused by changes in energy metabolism. First, the temperature of the body nucleus, thus of all inner organs and circulating blood, is reduced by 0.5–1 °C during winter swimming, which causes only minimal changes of metabolic flux rates. Second, the glutathione synthesis, which is adenosine triphosphate (ATP) dependent, is a very slow process in red blood cells. Under normal conditions, the half-life time of GSH is 4 days, which means that each hour only 0.5% of the GSH pool is replaced by synthesis. Since erythrocyte ATP levels do not change follow-

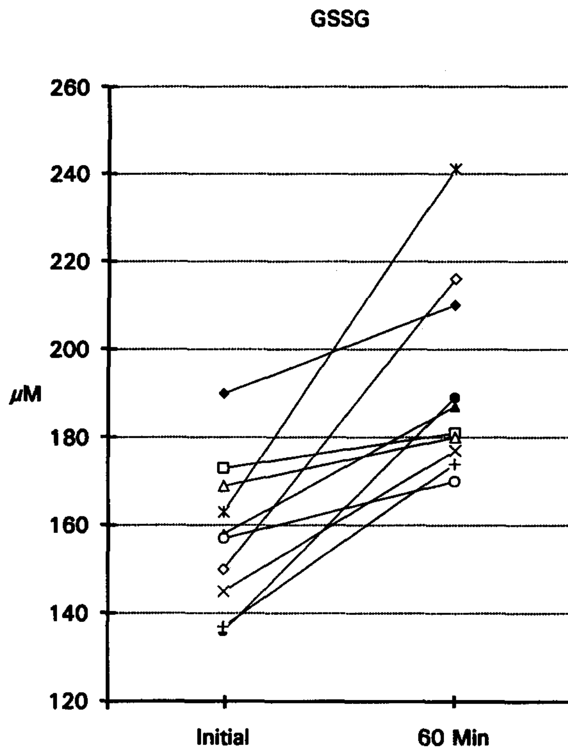


Fig. 3. Individual GSSG concentrations of circulating erythrocytes of winter swimmers before and 60 min after winter swimming. Each subject is characterized by a different symbol.

ing winter swimming, the GSH decrease cannot be explained by changes in energy metabolism. Third, a disturbance of energy metabolism during hypothermia could only cause an increase in uric acid levels, because of accelerated nucleotide degradation, due to ATP loss and adenosine monophosphate (AMP) accumulation.

Oxygen radical generation during and following whole-body hypothermia could be the result of muscle shivering during exposure to cold. There are reports indicating that there is an increased free radical generation during skeletal muscle exercise.[40–48] The source for the increased free radical formation could be the mitochondria of the muscle cells, in connection with a partial uncoupling of the respiratory chain. Extreme exercise and muscle shivering is connected with hyperthermia of the skeletal muscle even when the organism is exposed to a cold stimulus such as winter swimming. It was reported that muscle mitochondria undergo a partial uncoupling during temperature increases from 37 to 45°C, which represents the physiological temperature range during exercise of various intensities.[48,49] It was reported by Chance et al.[50] that mitochondria are one of the main intracellular sources of superoxide and hydrogen peroxide under physiological conditions, and that uncoupled mitochondria generate superoxide and hydrogen peroxide at much higher rates that do well-coupled mitochondria.

Another source of the generation of activated oxygen species during conditions of winter swimming, could be the increased autoxidation of catecholamines. This source of radical generation could play an important role both during exposure against the cold stimulus and during exercise/muscle shivering. Under such conditions the catecholamine concentrations in blood plasma can tremendously increase.[51–53]

The finding of urate loss during short-term cold exposure is indirect evidence for increased free radical formation. More direct evidence could be obtained by the measurement of direct criteria of free radical formation or lipid peroxidation in further studies.

It should be mentioned that a decrease of the plasma uric acid levels following a whole-body cold stimulus was also found during cold chamber therapy for treatment of rheumatological diseases.[54] In that study, only a smaller decrease of 5–10% of the uric acid concentration was measured, which was interpreted as a change in the flux rates of purine degradation.[54] However, our findings indicate that the decrease in uric acid could also be due to formation of oxygen radicals, using the model of body hardening through hypothermic exposures. Knowledge of such relationships could open a new approach to the explanation of mechanisms of hardening as a result of repeated short-term whole-body cold stimulus.

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## ABBREVIATIONS

AMP—adenosine monophosphate
ATP—adenosine triphosphate
DTNB—5,5′-dithiobis-(2-nitrobenzoic acid)
GSH—reduced glutathione
GSSG—oxidized glutathione
NEM—*N*-ethylmaleimide