PLASMA CATECHOLAMINES, SEROTONIN AND THEIR METABOLITES AND β-ENDORPHIN OF WINTER SWIMMERS DURING ONE WINTER.
Possible correlations to psychological traits.

ABSTRACT

Objectives. The study was a follow-up one, in which blood pressure and hormonal changes were investigated during one winter swimming season in winter swimmers (WSs) and non-swimmer controls on three occasions (autumn, winter and spring). Humoral results were compared to psychological traits recorded at the time of the three blood samplings.

Results. Mean systolic blood pressure of the WSs fell from 134 ± 12 mmHg to 128 ± 12 mmHg (p < 0.05) during the winter, and a slight but non-significant drop was also seen in the controls. Mean plasma noradrenaline concentrations diminished significantly from autumn to spring, and more so in the WS-group, but no statistically significant difference was observed between the groups. Adrenaline levels also showed a decreasing trend, and the change was significant when calculated by using the combined means of both groups. Plasma homovanillic acid and β-endorphin values were on the same level in all seasonal samples in both groups. Plasma serotonin levels decreased in both groups by about 50 per cent by spring, but 5-HIAA did not change significantly. HVA showed correlation with blood pressure and anxiety in the autumn (r = 0.367). In the winter measurement endorphin and hysteria had a negative correlation (r = 0.370). In the spring 5-HIAA and obsessionality had a positive correlation (r = 0.351).

Discussions. In summary, blood pressure and plasma catecholamine levels decreased during winter swimming practice over one winter, but these changes were also observed in the control persons. Plasma serotonin was lower in the spring in both groups. The changes in the humoral status speak for adaptation to the research situation, or reflect seasonal variation from autumn to spring. No clear effect of winter swimming as such was detected.

Key words. winter swimming, catecholamines, serotonin, endorphins
Winter swimming or swimming in very cold water (below 8°C) has become quite a popular hobby in Northern countries, since the swimmers say that even a short dip in the ice cold water has a clearly refreshing effect abolishing general tiredness, improving mood, helping to withstand pain or even curing painless chronic diseases such as fibromyalgia and rheumatoid arthritis (1). Improvement of general wellbeing and alleged prevention of virus infections acquired by hardening via cold adaptation are other benefits which have been mentioned by active winter swimmers. Winter swimming can also be regarded as shortlasting and inexpensive outdoor activity, especially for those who have handicaps preventing them from practicing more strenuous sports.

Acute exposure to cold environment, either air or water, causes a stress reaction, including activation of sympathetic nervous system with increased secretion of catecholamines, mainly noradrenaline (2), as well as cortisol release (3,4). The enhanced secretion of these stress hormones is probably one factor behind the refreshing effect of winter swimming. Another factor is the increased secretion caused by repeated cold stimulus on TSH and thyroid hormones, which have a metabolism activating role in cold adaptation (4,5). According to comments by winter swimmers, adaptation to cold by repeated exposures to cold water increases the ability to withstand other kinds of stress. The phenomenon may be the result of hormonal stimulation and changes in the functions of both autonomic and central nervous system, where e.g. changes in neuronal transmitters such as noradrenaline, serotonin, dopamine and endorphins, may have a role in regulation of mood and pain threshold.

There are some studies dealing with the possible mechanisms of pain relieving, other than better central pain withstanding, in fibromyalgia and chronic arthritis or arthrosis by regular winter swimming. Higher level of cortisol in blood is probably one factor diminishing the inflammation and concomitant pain which are the symptoms of rheumatic diseases. Regular winter swimming increases the cytokines and leucocytes in blood, which can affect the rheumatic inflammation and might thus have a curative effect on these diseases (6). The short term exposure to cold water causes oxidative stress, but the amount of reduced glutathione and the activities of erythrocytic superoxide dismutase and catalase enzymes were higher in winter swimmers than in controls, speaking for adaptation to
oxidative stress (7). Thus, a physical factor such as cold exposure seems to affect biochemical enzymatic processes in the organism.

The increase of stress hormone levels in blood at acute cold water exposure has been shown in many experiments, but their role in cold adaptation and mental functions in winter swimmers has been rarely investigated. The authors are unaware of studies on winter swimmers where their hormonal status has been followed over one winter.

Therefore the aim of the present study was to follow possible changes in the levels of serum catecholamines, serotonin and their metabolites as well as β-endorphin during a winter swimming season both in swimmers and non-swimmers. The hormone levels were also compared with psychometric scores obtained with questionnaires and published previously (1).

MATERIAL AND METHODS

Subjects

Winter swimmers in the city of Oulu were asked to volunteer for the study. Physically healthy persons aged between 47 and 65 years were accepted to the test group. Because of difficulties in finding a control group of the same age we asked the swimmers to ask a non-swimmer of the same age and gender to join the reference group. All subjects were fully informed and gave their written consent. The study plan was accepted by the ethical committee of the Medical Faculty of the University of Oulu.

The subjects came to our laboratory to give a blood sample in October (sample 1), in January (sample 2) and in May (sample 3). The day of the sample was not connected with a single swimming time, but was adjusted to the swimming season. Blood pressure was measured and the general state of health was checked by interviewing on the three occasions. At the same time they completed a questionnaire testing psychological traits.

The test group consisted of 25 swimmers (18 females and 7 males) and the control group of 11 non-swimmers (8 females and 3 males). The average ages in the groups were 55.1 and 53.8 years, respectively.

The swimmers took the cold water dip in average 5-6 times a week. All swimmers had at least one other physical activity like jogging, cycling or skiing.
The biochemical methods
Blood samples were collected into EDTA tubes containing Na-bisulfite as antioxidant.

Noradrenaline (NA) and adrenaline (A) from plasma were extracted into Al₂O₃ using 3,4-dihydroxybenzylamid hydrobromide as an internal standard. The catechols were then eluted into acetic acid solution and assayed with high performance liquid chromatography (HPLC) using a multichannel electrochemical detector (ESAm Coul Aray Model 5600).

Homovanilic acid (HVA), the metabolite of dopamine, as well as serotonin and its metabolite 5-HIAA were also assayed with HPLC using external standards after precipitation of proteins from the plasma.

For β-endorphin measurements, 1 ml plasma samples were extracted with Sep-Pak C 18 cartridges using a Gilson ASPEC automatix sample preparation system. The Sep-Pak eluates were dried in Speed-Vac, reconstituted with radioimmuneassay buffer and measured with the radioimmune method. The detection limit of the assay is 2 pg/tube and the interassay coefficient of variation less than 15 per cent (8).

The psychometric methods
Two methods to measure the psychological traits of the swimmers and non-swimmers were used as described earlier (1). These were the Crown Crisp Experimental Inventory (CCE1) as selected for assaying psychopathology and the Toronto Alexithymia Scale (TAS-20) for alexithymia.

Alexithymia was measured with the twenty-item Toronto Alexithymia Scale (= TAS-20). Both the original scale and the Finnish version of the scale have been shown to be psychometrically correct (9). The TAS-20 total score was calculated and subjects with a score TAS-20 > 60 were defined as alexithymic. The Crown Crisp Experiential Index (CCEI, see Crown Crisp 2000), earlier also known as the Middlesex Hospital Questionnaire was used for measuring neurotic type of psychopathology. It is a self-rating scale comprising six subscales measuring free-floating anxiety, phobic anxiety, obsessionality, depression, somatic anxiety, and hysteria. The validity of the Finnish version of the instrument has been shown to be satisfactory and normal values in a Finnish student sample have been published earlier by one of the present authors (10).
Statistical methods
Repeated measures Anova method was used in the statistical calculation for the biochemical results.

For the correlation between the hormone results and psychological parameters non-parametric Spearman correlation test was used.

RESULTS
Blood pressure
In the first measurement mean systolic blood pressure was 134.2 ± 12.0 mmHg in the WSs and 130.9 ± 17.8 mmHg in the controls, and diastolic pressure 80.4 ± 7.2 mmHg and 77.7 ± 12.5 mmHg, respectively. The mean systolic pressure of the WSs decreased during the winter, being 128.5 ± 12.2 mmHg (p < 0.05) in the third measurement (spring). In the controls the mean systolic pressure decreased as well, being 124.4 ± 16.5 mmHg (nonsignificant) in the spring. The mean diastolic pressure did not change in either group during the winter.

No significant differences were seen between the groups.

| Table 1. Noradrenaline (NA) and adrenaline (A) concentration in plasma in three seasonal samples of winter swimmers (WS) and control persons (CO) and the groups combined (All). Mean and standard deviation. Both NA and A decreased significantly (p<0.001 and p<0.05 respectively) |
|---|---|---|---|
| Season | Group | n  | NA(nmol/l) | A(nmol/l) |
| October | WS  | 22 | 6.0 ± 2.5 | 0.37 ± 0.25 |
|         | CO  | 10 | 4.5 ± 1.0 | 0.25 ± 0.17 |
|         | All | 32 | 5.5 ± 2.2*** | 0.33 ± 0.23** |
| January | WS  | 22 | 4.3 ± 0.17 | 0.22 ± 0.17 |
|         | CO  | 10 | 4.2 ± 1.5 | 0.20 ± 0.25 |
|         | All | 32 | 4.2 ± 1.2 | 0.21 ± 0.19 |
| May     | WS  | 22 | 4.6 ± 1.8 | 0.21 ± 0.19 |
|         | CO  | 10 | 3.7 ± 1.1 | 0.17 ± 0.19 |
|         | All | 32 | 4.3 ± 1.6 | 0.20 ± 0.19 |
Hormones and metabolites

Since the number of the males was too small for reliable statistical calculations, results on the hormone assays are given considering the WSs and controls as one group without separating the genders.

During the winter the mean NA concentration in plasma dropped both in WS and control groups, more clearly so in the WS group, but there was no statistically significant difference between the groups. The autumn values were significantly ($p<0.001$) higher than in the other samples when the combined mean values were compared (Table 1).

The mean A concentration dropped during the winter similarly to NA, and no significant differences were observed between the groups, but the difference between the combined means of autumn and spring samples was significant ($p<0.05$) (Table 1).

The relative drop of NA from autumn to spring was 23.3 per cent and that of A 43 per cent in WSs. In the controls the relative drops were 17.7 per cent (NA) and 32 per cent (A).

In the measurements dopamine was found in only three plasma samples. Its metabolite homovanillic acid (HVA) did not vary during the winter and was at the same level in swimmers and controls (Table 2).

The $\beta$-endorphin plasma concentrations stayed on the same level throughout the season and did not show any differences between the groups (Table 2).

| Season | Group | n  | HVA (ng/ml) | $\beta$-endorphin (ng/ml) |
|--------|-------|----|-------------|--------------------------|
| October| WS    | 21 | 13.6 ± 8.7  | 27.2 ± 2.9               |
|        | CO    | 10 | 9.1 ± 2.4   | 27.8 ± 8.8               |
|        | All   | 31 | 12.1 ± 7.5  | 27.4 ± 5.3               |
| January| WS    | 21 | 11.8 ± 5.6  | 28.2 ± 4.4               |
|        | CO    | 10 | 12.1 ± 5.0  | 26.8 ± 7.8               |
|        | All   | 31 | 11.9 ± 5.6  | 27.7 ± 5.6               |
| May    | WS    | 21 | 10.8 ± 3.5  | 26.9 ± 4.9               |
|        | CO    | 10 | 12.2 ± 5.5  | 25.9 ± 3.6               |
|        | All   | 31 | 11.3 ± 4.3  | 26.6 ± 4.5               |
Plasma serotonin (5-HT) values were higher in the WSs than in the controls in all three samples, but the differences were not statistically significant. The 5-HT content decreased in both groups during the winter by about 50 per cent, the decrease already becoming apparent in the second or winter sample. The 5-HIAA values showed no significant changes from autumn to spring (Table 3).

Table 3. Serotonin (5-HT) and the metabolite 5-HIAA in plasma in the three seasonal samples of winter swimmers (WS) and control persons (CO) and groups together (All). Mean and standard deviation. 5-HT decreased significantly (p<0.001).

| Season | Group | n  | 5-HT (ng/ml) | 5-HIAA (ng/ml) |
|--------|-------|----|--------------|----------------|
| October | WS    | 21 | 318.9 ± 179.6 | 5.4 ± 2.9 |
|        | CO    | 10 | 219.3 ± 93.4  | 6.4 ± 6.8 |
|        | All   | 31 | 286.9 ± 162.3*** | 5.7 ± 4.4 |
| January | WS    | 21 | 173.8 ± 103.4  | 5.0 ± 3.5 |
|        | CO    | 10 | 127.5 ± 76.6   | 4.2 ± 1.8 |
|        | All   | 31 | 158.9 ± 96.8   | 4.7 ± 3.0 |
| May    | WS    | 21 | 149.8 ± 77.5   | 6.1 ± 5.1 |
|        | CO    | 10 | 117.1 ± 73.3   | 4.6 ± 0.9 |
|        | All   | 31 | 139.2 ± 76.6   | 5.6 ± 4.3 |

Correlation between hormone values and psychological scores
Because there were no significant differences between the groups in the hormone and metabolite values, both groups were pooled. The 6 biochemical parameters and the scores of psychological parameters, free floating anxiety, phobic anxiety, obsessionality, somatic anxiety, depression, hysteria and alexithymia, were compiled for every individual and the correlations were calculated.

A few correlations between the biochemical and psychological parameters appeared.

In the autumn measurement HVA correlated with anxiety (r= 0.360, p < 0.05), which also showed a correlation with diastolic blood pressure (r = 0.393, p < 0.05). 5-HIAA correlated with obsessionality and blood pressure in the spring measurements (r = 0.351, p < 0.05). β-endorphin and hysteria had a negative correlation (r= 0.370, p < 0.05) in the winter.
DISCUSSION
Our study dealt with hormonal adaptation in winter swimmers (WSs) during one winter, with simultaneous comparison of personal traits and the hormonal results. The study was an extension of a former investigation on a smaller group of females and males, who acclimatised themselves to cold water indoors for three months. In that study noradrenaline (NA) and cortisol were elevated in females after one month, but NA had decreased at three months while the level of cortisol remained high. In males no significant changes were observed (3).

In another study on WSs we observed that catecholamine response to a dip in 4°C water was attenuated in December after three months of regular winter swimming, indicating adaptation to cold, and NA and A values were highest in the autumn, as was the case in the present study (2).

In the present material the NA and A concentrations were highest in the first assay in September and after that the levels decreased in both groups. This result complies with cold adaptation in the WSs, but on the other hand, since the NA and A levels were also lowered in the non-swimmers, the lowering could also depend on adaptation to the research protocol as such. The higher levels of stress hormones in the first sample may be due to nervousness caused by the new situation. Thus it is impossible to tell whether the attenuated sympathetic reaction indicated adaptation to cold during the winter swimming season or mental adaptation to the long lasting investigation, or both.

The degree of sympathetic nervous reactivity to cold stress as indicated only by plasma NA content seemed to remain unchanged during repeated cold water immersion for 6 weeks. However, peripheral vasoconstriction was increased, indicating better ability to save body heat (9).

In an experiment with WSs and controls who spent 1 h in 13°C water it was observed that the WSs were better able to reduce the heat loss and shivering and withstand mild hypothermia than the non-adapted controls. These observations are indicative of changes in central thermoregulation (9,10).

Systolic blood pressure decreased in both our groups, but significantly so only in the WSs group, which had more test persons the control group only having 9. The difference between the first (autumn) and third (spring) measurements was on average 6 mmHg, which is in accordance with the drop in catechol values and can be regarded as a sign of adaptation to repeated cold exposure or the
research protocol. Diastolic pressure did not change in our material, but in a long distance cold water (10-14°C) swimming experiment diastolic pressure was lowered on the fourth day (11). Some winter swimmers have reported that swimming without any medication, has lowered their elevated blood pressure.

Acute cold water exposure increases the blood pressure, but it does not remain elevated (12).

Dopamine is one of the transmitters in the brain that regulates mood (13), but it was measurable only in three blood samples. The level of its metabolite, homovanilic acid (HVA), remained unchanged over the winter in both groups and there were no differences between the groups. The average content of HVA in the combined material showed correlation with the anxiety factor in the autumn, and it also correlated with blood pressure.

The plasma serotonin (5-HT) values decreased during the winter. In the spring there was correlation between 5-HIAA, HVA and obsessionality. The correlations thus indicated that the more intensive the metabolism of 5-HT and DA, the more anxious and obsessive the person. It also appeared in another WSs group that plasma HVA content correlated with anxiety (Huttunen, unpublished results).

One interesting observation was reported on the 5-HT content in plasma of melancholia patients. Those who were more melancholic had lower 5-HT values and a tendency to low 5-HIAA values, but not lowered 5-HT content in platelets (16). Regarding the interpretation of the 5-HT assays in plasma one must keep in mind that platelets store 5-HT and part of them can remain in plasma after centrifugation increasing the variation of the results.

β-endorphine plasma contents in both groups remained quite stable through the winter. In the winter measurement the endorphin content and the hysteria score had a negative correlation, which might be interpreted so that if the blood levels of this "mood hormone" are low, inclination to hysterical reactions is increased.

Thus in the present study some changes of the measured parameters appeared during winter and only a few correlations between biochemical and psychological factors could be found. The general observation emerged that the persons in both groups were psychologically healthy (1), and the biochemical parameters in blood turned also out to be quite similar in WSs and controls.

The authors are unaware of earlier reports where WSs have been studied over winter and where both psychological traits and neural
transmitters and hormones in plasma have been measured, which have an effect on nervous functions. On the other hand, one may claim that plasma stress hormone and transmitter concentrations and the traits of mood cannot have correlations. Therefore the present results should be interpreted with caution. The decrease of NA and 5-HT content in plasma during the winter swimming season can be regarded as signs of adaptation to regular cold exposure, which might have a connection with psychic functions or central adaptation to cold.

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