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Hemoglobin and Hematocrit Values After Saline Infusion and Tourniquet

Abstract

This study attempted to contribute to standardization of blood testing in sport, and to investigate the effect of artificial dilution with saline. In 10 healthy, physically active males and 3 healthy physically active females hemoglobin (Hb), hematocrit (Ht), and % reticulocytes (%retics) were measured at different time points to look for possible fluctuations during day time, while the subjects had regular coffee breaks and lunch. In 7 of the subjects in a separate experiment 500 ml of saline were infused around 8 am and Hb, Ht, and %retics were measured before and every hour thereafter until 7 hours after infusion. In addition Ht was measured on a hematological analyzer as well as with a centrifuge. In a separate experiment the effect of tourniquet duration on Hb and Ht was studied in 9 of the subjects. The results show that Hb,

Ht, and %retics are stable from 8 am to 4 pm, but that infusion of 500 ml of saline induces an acute decrease in Hb and Ht within one hour (Hb decreased from 15.2 ± 0.9 g/dl to 14.5 ± 1.0 g/dl, and Ht from $45.6 \pm 2.8\%$ to $44.0 \pm 2.5\%$). The decline in Hb and Ht was maintained during the 7-hour observation period. Ht values of the same samples measured with a hematological analyzer and a centrifuge were not different. Application of the tourniquet did significantly affect Hb and Ht values only from two minutes, and thereafter Hb and Ht remained stable during the rest of the 5-minute tourniquet. With blood testing in sport these results have to be taken into consideration.

Key words

Blood testing · hemoglobin concentration · tourniquet · saline infusion

Introduction

A high maximal oxygen uptake is a prerequisite for athletic success in endurance sports. The oxygen transport capacity and consequently maximal oxygen uptake can be increased by increasing the red cell mass and consequently the hemoglobin concentration in the blood [1, 3, 13]. Based on positive doping cases and testimonial evidence it is likely that for this purpose the administration of recombinant human erythropoietine (rh-EPO) has been used by some individuals [11]. Because exogenous erythropoietine (EPO) is difficult to detect, and can only be found in the urine until approximately 5 days after the last injection [6], it has

been attempted to look for effects of rh-EPO in the blood, such as hemoglobin concentration (Hb), hematocrit (Ht), and % reticulocytes [7]. Although cutoff limits have been challenged [9], in some sports above a certain level of Hb (cross country ski; [FIS]; biathlon; [IBU]; speed skating; [ISU]) or Ht (cycling; UCI) an athlete is not allowed to compete [2, 4, 5, 12] and only follow-up investigations that make manipulated high Hb or Ht levels very unlikely, may allow the athlete to compete again [5]. It is obvious that a no start or repeated urine testing do have great impact on the athlete, and therefore the athlete may be tempted to artificially lower the key hematological parameters in an effort to avoid a no start or repeated urine testing. This may be done by

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excessive fluid intake [10] or infusion of saline, in order to remain below the cutoff limit at the time of blood sampling. However, it is unknown to what extent infusion of saline affects hematological variables and how long the possible changes last. Schmidt et al. [10] reported that ingestion of 1000 ml of saline within 15 minutes time lowered Hb and Ht for a duration of 75 minutes after ingestion, however beyond this time no measurements were reported.

Therefore the aim of this study was to investigate hematocrit and hemoglobin concentration before and up to 7 hours after infusion of 500 ml of saline. Since some sport federations measure hematocrit with an automated analyzer whereas others measure it with a conventional centrifuge, measurement of hematocrit in the saline infusion experiment was done not only with a hematological analyzer but also with a centrifuge, which is considered as gold standard. In order to account for possible fluctuations in hematological parameters during the day, in a separate experiment blood was sampled at different time points during the day.

In case of a Hb or Ht value which is slightly above the limit, athletes may try to attribute this to prolonged application of the tourniquet (testimonial evidence). However, no data on the effect of duration of the tourniquet on Hb and Ht are available. Therefore our last aim was to study the effect of tourniquet duration up to five minutes on Hb and Ht values.

Materials and Methods

Subjects

Nine healthy male students (age 21–25), three healthy female students (age 21–24), and one former male elite endurance athlete (54 years), who were all physically active and moderately to well-trained in running or cycling, participated in this study, which consisted of three separate experiments.

Before commencement of the study the subjects were medically screened by a questionnaire and had an interview with a physician. The subjects were fully informed about the purpose and the nature of the study, and were aware of possible inconveniences of the experiments. After approval of the local ethics committee and before commencement of the study the subjects signed a written informed consent form.

Procedures and experiments

The three experiments were separated by at least one week.

Experiment 1: normal Hb and Ht fluctuations during the day

All thirteen subjects participated in this experiment to study possible fluctuations in Hb and Ht during the day. After having eaten breakfast at home the subjects reported to the laboratory at 8 am. Before taking the 3-ml blood samples the subjects sat for 5 minutes, after which a tourniquet was applied and the needle was inserted. Blood was drawn from an antecubital vein using a vacuum system (vacutainer 0.8 × 38 mm, Becton Dickinson, Plymouth, UK) and a K3-EDTA vacuette tube (Greiner-bio-one, Austria). After the blood started to flow the tourniquet was released. Only 5–10 seconds elapsed between application of the tourniquet and the blood starting to fill the tube. Subsequently

the subjects reported to the lab for blood sampling every hour until 8 hours after the first sample. Between blood sampling the subjects were allowed to drink and eat ad libitum and had regular coffee and lunch breaks, and to do their regular administrative activities. The blood samples were taken alternately from an antecubital vein in the left and right arm, and analyzed within 60 minutes after blood withdrawal. The same procedure was used for every sample, and sampling analysis was done within one hour.

Experiment 2: saline infusion

Seven males who also took part in the first experiment, participated in experiment 2. After consuming a regular breakfast at home the subjects reported to the lab around 8 am, lay down and a catheter (Quick Cath-II, Baxter, USA) was inserted into an antecubital vein, and connected to an infusion system. As soon as the catheter with the infusion system was in place, a 3-ml blood sample was obtained from an antecubital vein of the contra-lateral arm using a vacuum system and a K3-EDTA vacuette tube (Greiner-bio-one, Austria), as in experiment 1.

After taking the first blood sample the infusion system was opened and 500 ml of physiological saline was infused in 10–13 minutes (range). After the infusion was completed the catheter was removed and the subjects resumed their daily activities and returned to the lab every hour for taking a 3-ml blood sample up to 7 hours post-infusion. The left and right arm were alternately used for blood sampling. Before every blood sampling the subjects sat for 5 minutes, after which a tourniquet was applied and the needle was inserted. After the blood started to flow the tourniquet was released. The time elapsed between the application of the tourniquet and starting the blood to fill the tube was 5–10 seconds. As soon as the blood started to flow, the tourniquet was released. Between blood sampling the subjects were allowed to drink and eat ad libitum at regular times, and to do their regular administrative activities. The blood samples were analyzed within 60 minutes after the blood draw. After analysis on the hematological analyzer the rubber cap of the tube was removed and two capillaries (Haematokrit Kappillaren, 75 mm, 75 µl, OD 1.5–1.6 mm, Hirschmann, Germany) coated with Na-heparin were filled with blood for measurement of hematocrit with a hematocrit centrifuge (microhematocrit centrifuge type 346, MSE scientific instruments, UK). After filling one side of the tube it was sealed with wax (wax plate for hematocrit tubes, Hirschmann, Germany), and put in the centrifuge. The samples were centrifuged for 5 minutes at approx. 13310 g. The lengths of the total blood column and plasma column were measured with a millimeter ruler, and mean Ht was calculated from the two tubes. The within-sample coefficient of variation was 1.3%.

Experiment 3: effect of tourniquet

Seven male and two female subjects who also took part in experiment 1, participated in experiment 3, in which the effect of duration of the tourniquet was studied. After 5 minutes sitting in a chair the tourniquet was applied, and released when the blood started to flow. After taking the first 3-ml sample, the tourniquet was tightened again and maintained for 5 minutes, and a vacuum tube was filled with 3 ml of blood every minute until 5 minutes. After inserting the needle for taking the first sample, it was left in place for the remainder of the experiment. After tak-

Table 1 Mean hemoglobin concentration (Hb) and hematocrit (Ht) before infusion of 500 ml of saline and every hour thereafter. Ht2 is hematocrit as measured with the conventional centrifuge method

	Before	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs
Hb (g/dl)	15.2±0.9	14.5**±1.0	14.4**±0.8	14.6**±1.0	14.3**±1.1	14.38**±1.1	14.6*±1.4	14.6**±1.2
Ht (%)	45.6±2.8	44.0**±2.5	43.5**±1.8	44.0**±2.5	43.4**±3.2	42.9**±3.3	43.8*±4.0	43.4**±3.6
Ht2 (%)	45.8±2.7	43.8**±2.2	44.0**±2.1	44.3**±2.5	43.4**±3.5	43.3**±3.7	43.7**±3.6	43.8**±2.9

* $p < 0.05$ compared to pre-infusion value. ** $p < 0.01$ compared to pre-infusion value

Table 2 Mean (\pm SD) hemoglobin concentration (Hb) and hematocrit (Ht) (before and after 1, 2, 3, 4, and 5 minutes of tourniquet)

	Before	1 min	2 min	3 min	4 min	5 min
Ht (%)	44.6±3.3	45.2±2.8	45.8±2.9 [#]	46.2±3.3*	46.3±3.7*	46.3±3.2*
Hb (g/dl)	14.8±1.2	15.0±1.1	15.1±1.2 [#]	15.3±1.3*	15.4±1.3*	15.3±1.2*

* $p < 0.01$ compared to pre-tourniquet value, [#] $p < 0.05$ compared to pre-tourniquet value

ing the last sample the tourniquet was released and the needle was removed. Analysis was done within 60 minutes after sampling.

Analysis and variables

After taking the blood samples, they were analyzed within 60 minutes after sampling. For the hematological analysis an ADVIA 120 (Bayer Corporation, Tarrytown, NY, USA) was used whereas hematocrit in every sample was also measured on a centrifuge (microhematocrit centrifuge type 346, MSE scientific instruments, UK).

Data handling and data analysis

The data was imported in Microsoft Excel, and analyzed with SPSS 8.0 software package (SPSS, Inc Chicago, IL, USA). After testing for skewness and kurtosis, the samples at various time points were analyzed with a one-way ANOVA for repeated measures. To locate a difference a Scheffé post-hoc test was done. A difference was considered statistically significant at $p < 0.05$.

Results

Taking blood samples during the day showed that the mean hemoglobin concentration at 8 am was 14.6 ± 2.8 g/dl and Ht was $44.2 \pm 1.1\%$. The samples taken at every hour afterwards until 8 hours after the first sample did not show any significant changes in Hb or Ht. Also the percent reticulocytes remained stable during the day ($1.1 \pm 0.4\%$ and $1.2 \pm 0.3\%$ in the sample at 8 am and 4 pm, respectively).

Saline infusion did affect Hb and Ht, whereas no effect on %retics was found. As shown in Table 1 at one hour after saline infusion there was a significant drop in both hemoglobin concentration and in hematocrit. This decrease in Hb and Ht was consistent in all subjects and the drop in Hb varied between subjects from 0.4 to 1.4 g/d. The decrease in Ht varied between individuals from 0.9 to 3.7%. There was no relationship between the initial levels of

Hb and Ht and the extent of the drop in these values after saline infusion. Seven hours after infusion both variables were still significantly lower than the starting values. The results show (Table 1) that the measured Ht with the Advia-120 and the centrifuge were not different.

Application of the tourniquet did affect Hb and Ht values (Table 2). The mean Hb in a blood sample obtained following the regular procedure was 14.8 ± 1.2 g/dl, whereas the Hb concentration increased to 15.0 ± 1.1 g/dl and 15.3 ± 1.2 g/dl after 1 and 5 minutes, respectively. Hematocrit also changed during tourniquet application and varied from 44.6 ± 3.3 before tourniquet to 45.2 ± 2.8 and 46.3 ± 3.2 after 1-, and 5-minute tourniquet, respectively ($p < 0.01$).

Discussion

The present study shows that infusion of 500 ml of physiological saline induced an acute decrease in hemoglobin (Hb) concentration and in hematocrit (Ht), whereas the percentage of reticulocytes was not affected by saline infusion. The mean decrease in Hb concentration was 0.7 g/dl with values ranging from a low of 0.4 g/dl to a high of 1.4 g/dl. For Ht the mean decrease was 1.6% with values ranging from a low of 0.9% to a high of 3.7%. These decreases in Hb and Ht may seem relatively small, however for blood testing in sport even a drop in Hb of 0.4 g/dl as observed in one subject may be enough to make the difference between start or no start. It is likely that the effect of 500-ml saline infusion may be larger in women because of the smaller plasma volume in women compared to men. Because endurance training is associated with higher plasma volumes, the effect of saline infusion may be less pronounced in trained subjects. The subjects of this part of the study were physically active and moderately- to well-trained and four of the subjects were competitive cyclists at regional to national level, and one subject was a retired but still active and a well-trained former elite athlete. Therefore, the effects of saline infusion are expected to be similar in elite athletes.

The data show that the Hb and Ht levels remained fairly stable from one hour after infusion throughout the whole 8-hour observation period. One might argue that the decrease in Hb and Ht is not only attributable to the saline infusion, but also to daily fluctuations since Wisser and Breuer [14] and Manfredini et al. [8] have shown that Hb and Ht show a diurnal rhythm, with a decline during the day. However, the results of the experiment in which blood was sampled repeatedly during the day, failed to show any significant fluctuations in Hb and Ht. The diurnal changes as found by Wisser and Breuer [14] and Manfredini et al. [8] may be attributable to changes in fluid intake and different hydration status during the day. Especially during sleep when subjects may have an increased diuresis and no fluid intake, hemoconcentration is likely to occur. The subjects who participated in the present study were well aware of the importance of adequate fluid intake, and had their normal breakfast with *ad libitum* fluid intake at home, before reporting to the laboratory. This also stresses the importance of instructions to athletes about sufficient fluid intake, not only for optimal performance but also for blood testing. Because our subjects had a normal breakfast with *ad libitum* fluid intake before reporting to the lab the drop in Hb and Ht after infusion is most likely attributable to the infusion of saline. An interesting and comparable study was done by Schmidt et al. [10] who had 4 subjects drink 1 liter of saline in 15 minutes. The decrease in Hb and Ht they found was similar as in the present study, but the subjects were not studied beyond 75 minutes after drinking and had to consume double the amount of salty fluid, compared to the 500 ml of intravenous administration of saline. It is likely that infusion of 500 ml of saline in a 15-minute time span is more feasible than drinking one whole liter of salty fluid in the same time span, which may result in gastric discomfort and nausea.

Infusion of saline did not affect percentage reticulocytes. This absence of any change in reticulocytes is to be expected since dilution of blood with saline does decrease the number of cells per volume, however the number of reticulocytes relative to the number of erythrocytes will not change. So, infusion with saline may cause an acute decrease in Hb and Ht compared to previous individual hematological data, without a change in percentage reticulocytes. This stresses the importance of repeated hematological testing, in order to detect acute changes or fluctuations in hematological parameters. It has to be emphasized that the observation period in the present study was only 8 hours, and it could be argued that changes in the evening cannot be excluded. However, hematological data from athletes who were tested both in the morning and in the evening, did not show any significant differences (Kuipers, unpublished observation).

Another aim of the present study was to compare measurement of Ht with a hematological analyzer and with the conventional centrifuge method, which is considered as gold standard. The results of the present study show that there is no difference between the two methods, implying that accurate Ht measurements can be done with different methodologies.

The results of the present study show that application of a tourniquet does augment hemoglobin concentration and hematocrit, and the increase reached the level of statistical significance after 3 minutes of tourniquet application. The increase in Ht and Hb

concentration can be explained by increased venous pressure and a fluid shift towards the extra vascular space. The data show that after approximately 2 minutes the Hb and Ht values levelled off. Usually the tourniquet is applied for 10–15 seconds and blood sampling is generally completed within one minute. Even if the tourniquet would be maintained for a whole minute during the sampling procedure, it would not significantly affect Hb and Ht values.

In conclusion, the results of the present study show that Hb and Ht are stable during the day, but can be manipulated by infusion of 500 ml of saline, which lasted for at least 7 hours. This emphasizes the importance of repeated and unannounced blood testing in athletes in order to deter and detect blood manipulation. Application of a tourniquet did not affect Hb and Ht until application of the tourniquet for 3 minutes.

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