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Hyperventilation Enhances Transcapillary Diffusion of Sodium Fluorescein

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Abstract

Voluntary hyperventilation (HV) provokes hemoconcentration due to a loss of fluid from the intravascular space. In 10 healthy male volunteers the hypothesis was tested whether HV increases transcapillary fluid shift into the interstitial compartment. For this purpose, fluorescent light intensity (FLI) alterations after intravenous injection of sodium fluorescein (Na fluorescein) before and during 3 min of HV were determined. Concomitantly, temperature and microvascular skin flux (laser Doppler fluxmetry, LDF) were recorded continuously. Hematocrit and serum proteins, as markers of hemoconcentration, increased significantly from 41.2 ± 2.3 to 42.7 ± 2.0% ($p = 0.0023$) and from 69.5 ± 3.4 to 72.9 ± 3.0 g/l ($p = 0.0005$, respectively). Skin temperature and LDF showed no changes during HV compared to baseline levels. Interstitial FLI indicating transcapillary diffusion of Na fluorescein was significantly higher ($p < 0.001$) during HV compared to the values recorded during the baseline period. The exact mechanism of enhanced transcapillary diffusion of Na fluorescein is not known. The distinct increase in FLI without a significant change in microvascular skin flux suggests an HV-induced increase in capillary pressure or an enhancement in capillary permeability for water and small solutes.

Key Words
Hyperventilation
Hemoconcentration
Transcapillary transport
Sodium fluorescein

Introduction

Hyperventilation (HV), defined as breathing in excess of metabolic requirements is often associated with panic attacks. It has also been observed in somatic diseases, during pregnancy and at delivery [1–3]. Alterations of arterial carbon dioxide, acid-base metabolism and serum electrolytes are involved in the pathogenesis of the typical symptoms of HV. Hyperventilation also provokes a variety of hemodynamic changes, such as increases of heart rate, reduction of cerebral blood and vasodilatation of skeletal muscle blood vessels [1]. In addition, HV induces a reversible loss of protein free solution from the intravascular space and a decrease of circulating plasma volume [4, 5]. In support of this concept, a shift of intravascular fluid to skeletal muscle tissue during HV was demonstrated in animals [6]. In humans, no data exist about the influence of HV on transcapillary diffusion of fluid and small solutes.

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The purpose of this study was to investigate whether HV induces changes of transcapillary diffusion of sodium fluorescein (Na fluorescein) as an estimate for small molecular capillary permeability.

Methods

Subjects and Study Design

Ten healthy male volunteers with a mean age of 27.6 years (range 26–31 years), a mean body weight of 72 kg (range 62–78 kg) and a mean height of 178 cm (range 172–188 cm) were included in the study. They were healthy and had no history of coronary heart disease, bronchial asthma, HV or epilepsy.

The studies were performed with the subjects in the supine position. The right leg was fixed in a vacuum cushion in order to minimize movement artifacts. After an acclimatization period of about 30 min, laser Doppler flux (LDF) measurements at the skin overlying the distal tibia were started. After a recording period of 10 min, 0.2 ml of Na fluorescein per estimated liter of blood volume were injected into an antecubital vein. Transcapillary diffusion of Na fluorescein was measured as described below. Thirty-five minutes after recording baseline transcapillary diffusion, 3 min of HV followed; 30 s after starting HV, the Na fluorescein injection was repeated in order to determine transcapillary diffusion during HV. Studies of repeated Na fluorescein injections have shown to have a good reproducibility [7]. During the whole period LDF and skin temperature (Technoterm 9400, Quartz AG, Basel, Switzerland) were recorded. Room temperature varied between 22 and 24 °C. Venous blood samples (8 ml per sample) from a cubital vein were drawn for determination of hemoglobin, hematocrit, serum protein and plasma viscosity 2 min before as well as 3 min after starting HV.

Hyperventilation

HV (>30 breaths/min) was assumed to be adequate if the end-tidal CO_2 concentration decreased to less than 2.5 kPa within the first 30 s and could be held below 2.5 kPa during the remaining 2.5 min.

For measuring end-tidal pCO_2 ($PetCO_2$), an infrared capnograph (Ohmeda 5000, BOC Health Care, Boston, Mass., USA) was calibrated with 5% carbon dioxide before each investigation. Gas was sampled continuously from a side hole of a mouthpiece using a plastic catheter with a diameter of 3 mm and a length of 60 cm. The capnogram was recorded by a paper chart recorder running at 5 mm/s (Gould Recorder 2200 S, BOC Health Care). All measurements were performed by the same person.

Before, during and up to 6 min after HV $PetCO_2$, LDF and skin temperature were recorded.

Fluorescence Video Microscopy

Transcapillary diffusion of Na fluorescein was measured over the anterior tibial compartment using fluorescence video microscopy as described previously in detail [8, 9]. The equipment used for intravital video microscopy includes an adjustable fluorescence microscope (SM-Lux, Leica AG, Glattbrugg, Switzerland) with epillumination mounted on a heavy support (Foba AG, Wettswil, Switzerland), a low light level 2/3 inch CCD camera (DXC-930, Sony, Tokyo, Japan), a mercury vapor lamp (HBO 100 W, Osram Eichstett, Germany), a video monitor (Picture Monitor model PM-171T, Ikegami, Tsushinki, Japan), a tape recorder (S-VHS, Panasonic, Tokyo, AG-7500 Japan), a video densitometer (Colorado Video Inc., Boulder, Colo., USA), and a strip chart recorder (Gould 2600S, Gould Brush, Cleveland, Ohio, USA).

The microscope was adjusted to the skin surface of the distal tibial area. After an intravenous bolus injection of 0.2 ml 20% Na fluorescein per liter of estimated blood volume, the appearance of the fluorescent dye was visualized in the skin capillaries at the region of interest. Capillary filling and transcapillary diffusion were recorded and stored on video tape for off-line evaluation. A planar objective 2.5 × /0.08 (Leica AG) was used, resulting in an optical magnification of 4 times and a final magnification of 160 times on the video monitor (screen diagonal 17 inches). Fluorescent light intensities (FLI) were measured using the 'large-window' technique [7] in a skin area of 1.4 × 1.4 mm (1.96 mm^2) and quantitated at 5, 10, 20, 30, 60, 120, 180 and 300 s after the first appearance of the dye. The values were expressed in arbitrary units (AU). In addition, the appearance time, defined as the interval from the bolus injection to the first appearance of the dye at the site of observation, was measured. Moreover the first increase of FLI after the appearance of Na fluorescein in the cutaneous capillaries was evaluated by calculating the tangent of the slope of the densitometer curve.

Before the second injection of Na fluorescein during HV, a new baseline of FLI was determined. The rise in FLI after repeated Na fluorescein injection was compared to this second baseline.

Laser Doppler Fluxmetry

Skin blood flux was measured using a helium-neon laser Doppler device (PF3, Perimed, Stockholm, Sweden). An angle probe (PF314, Perimed, Sweden) was positioned on the skin of the ventral aspect of the calf (in the middle between ankle and knee joint), approximately 2 cm from the site where transcapillary diffusion of Na fluorescein was determined.

LDF was recorded continuously on a multichannel strip chart recorder (Gould Brush) with a paper speed of 1 cm/s. In addition, the flux data were digitized at a sample rate of 5 Hz and stored on a personal computer. Zero calibration of the laser Doppler fluxmeter was performed by fixing the probe to a white static surface. Gain setting was carried out using the standard motility solution provided by the manufacturer. Since the laser Doppler instrument does not provide absolute, but relative values of blood flow, flux values are expressed in AU. In the present study, 1 mV corresponds to 1 AU. The bandwidth of the laser Doppler fluxmeter was set to 4 kHz, the time constant was 0.2 s. A blood pressure cuff was positioned distally on the thigh for transient arterial occlusion to obtain the biological zero of the LDF signal [10]. The value obtained was subtracted from all flux values determined.

Hemoglobin and hematocrit were measured with an automatic analyzer (H-1-Technicon, Bayer Diagnostic, Munich, Germany), serum protein with an autoanalyzer (Hitachi 474, Böhringer, Mannheim, Germany) according to the Biuret method. The variability of measurement was below 3% for both parameters.

Viscosimetry

The Contraves Low Shear viscosimeter (Contraves, Oerlikon, Switzerland) used allows measurements of plasma viscosity at a rotational laminar shearing in Couette flow. A MGW Lauda RC 3 thermostabilizator held the temperature constant within ± 0.01 °C. All measurements were performed at a temperature of 20 °C. Viscosity

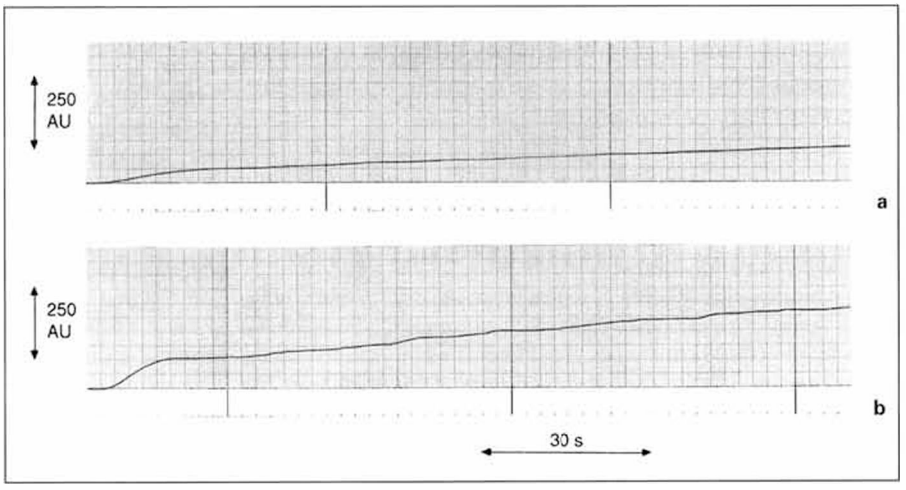

Fig. 1. Typical example of original densitometry curves demonstrating the steep increase in FLI after Na fluorescein injection before (**a**) and during (**b**) hyperventilation.

Table 1. Mean values of LDF hemoglobin, hematocrit, serum proteins and plasma viscosity at different time intervals of the hyperventilation maneuvre

	Before HV	During HV	Significance
LDF, AU	6.9 ± 4.6	6.1 ± 3.8	n.s.
Hemoglobin, g/dl	14.2 ± 0.8	14.7 ± 0.8	n.s.
Hematocrit, %	41.2 ± 2.3	42.7 ± 2.0	p = 0.0023
Serum protein, g/l	69.5 ± 3.4	72.9 ± 3.0	p = 0.0005
Plasma viscosity, 94.5 s^{-1}	1.8 ± 0.1	1.9 ± 0.1	n.s.

values were obtained at 3 different shear rates (94.5, 11.0 and 4.4 s). To obtain a steady state of flow, the measuring times were 60 s [11, 12].

Statistical Analysis

Data are presented as means ± SD. The analysis of variance (ANOVA) for repeated measurements was used to compare FLI over time and the two-tailed paired t test to compare FLI between control and test period.

The study was approved by the Ethical Committee of the Department of Medicine, University Hospital Zürich, and all subjects gave informed consent.

Results

All 10 volunteers participating in the study fulfilled the criteria of adequate HV with a decrease of $PetCO_2$ to values below 2.5 kPa during the first 30 s of HV and remained below this value during the rest of the HV period.

Systemic Effects of Hyperventilation

Hematocrit and total serum protein increased significantly ($p < 0.01$, $p < 0.001$, respectively) during HV. The values for plasma viscosity and hemoglobin tended to increase during the maneuver ($p = 0.067$ and $p = 0.059$, respectively). The mean and SD are listed in table 1. Mean skin temperature at the measuring site was 32.7 ± 0.6 °C at the beginning and 32.6 ± 0.7 °C at the end of the investigation (n.s.).

Laser Doppler Flux

LDF values showed no significant change during HV compared to the baseline values. The values recorded 3 min after HV decreased significantly compared to baseline values ($p < 0.05$). The mean LDF values and the standard deviations are shown in figure 1.

Transcapillary Diffusion of Na Fluorescein

A typical example of FLI registration during the control period and HV is given in figure 1. FLI as a marker of transcapillary diffusion of Na fluorescein increased significantly during HV. Already 5 s after the first appearance of the dye, a significant difference was found ($p < 0.05$). At that time, during HV, FLI was 44.5 ± 19.0 AU compared to 24.5 ± 10.1 AU during the control period. The values at different times are shown in figure 2. The rate of rise (tangent α) was significantly greater ($p < 0.05$) during the HV period (tangent $\alpha_{hv} = 0.73 \pm 0.45$) than during the control measurement (tangent $\alpha_{cr} = 0.41 \pm 0.27$). The mean appearance time of Na fluorescein was 56.9 ± 25.5 s during the resting period, compared to 22.8 ± 5.4 s during HV. The difference between the two mean values was significant ($p < 0.001$).

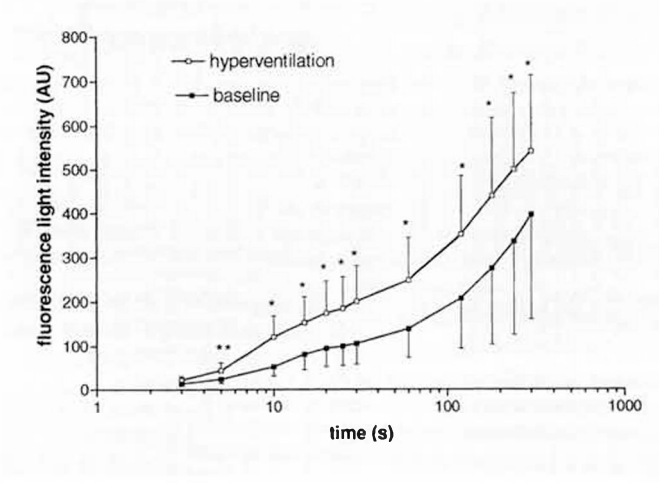

Fig. 2. Mean ± SD values of FLI (AU) during normal breathing (baseline) and hyperventilation (** $p < 0.05$, * $p < 0.001$ compared to baseline values).

Discussion

The main finding of our study is that short-term voluntary HV lasting 3 min enhances transcapillary diffusion of Na fluorescein. Na fluorescein diffuses through the capillary wall even under physiologic conditions [8]. The intravital dye accumulates in increased amounts in the interstitial space where it may be detected by videomicroscopy and assessed quantitatively by videodensitometry [8, 13]. In some diseases, such as long-term diabetes, transcapillary diffusion of Na fluorescein is significantly increased [13]. Moreover, the technique has been used to evaluate small molecular permeability enhanced by insulin-like growth factor I [14].

The finding of increased transcapillary diffusion is accompanied by hemoconcentration, which reflects intravascular fluid loss [4, 5]. Moreover, the increased intravascular concentration of proteins is caused by enhanced transport of water through the capillary wall. The tendency towards higher values of plasma viscosity is in good agreement with these findings.

There are several possible mechanisms for an increase in transcapillary diffusion. A well-known cause is enhanced blood flow. Transport of water and small solutes across the capillary wall is directly proportional to microvascular flow [15]. In our study, microvascular flow was measured by laser Doppler fluxmetry. Although LDF does not directly reflect skin blood flow, a close agreement between LDF and skin blood flow measured by plethysmography was documented [16]. In our study, there was no significant change in the LDF values measured before and during HV. Therefore, increased flow is a very unlikely explanation for the observed increase in the transcapillary diffusion of Na fluorescein. Only after the end of HV does a significant drop of flux indicate a post-HV vasoconstrictor response.

Though LDF did not change during HV, changes in blood flow through the thermoregulatory and nutritive compartment cannot be excluded. However, the capillary surface area available for diffusion is assumed to be unaltered during HV, because capillary density did not change.

Capillary hypertension could be an alternative explanation for intravascular fluid loss and interstitial edema formation. It has been observed in patients with type I diabetes in whom edema formation occurs [17]. Augmentation of capillary pressure might develop during HV even without any significant changes in flow. Deep breathing induces reflex vasoconstriction which could involve pre- and postcapillary segments [18, 19]. Increased vascular resistance may elevate capillary pressure and consequently favor fluid transport through the capillary membrane.

Hypocapnia typical of HV could also directly influence fluid and small molecular transport through the capillary wall by increasing its permeability. There are no reports in the literature on this subject. It could be argued that the observed increase in glomerular permeability at high altitude is due not only to hypoxemia, but also to HV [20].

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References

1 Magarian G: Hyperventilation syndromes. Infrequently recognized common expressions of anxiety and stress. Medicine 1982;61:219–236.

2 Gardner W: Hyperventilation disorders. J R Soc Med 1990;83:755–757.

3 Bass C, Gardner W: Respiratory and psychiatric abnormalities in chronic symptomatic hyperventilation. BMJ 1985;290:1387–1390.

4 Stäubli M, Rohner F, Kammer P, Ziegler W, Straub P: Plasma volume and proteins in voluntary hyperventilation. J Appl Physiol 1986; 60:1549–1553.

5 Straub P, Bühlmann A: Reduction of blood volume by voluntary hyperventilation. J Appl Physiol 1970;29:816–817.

6 Eichelberger L, Hastings A: The exchange of salt and water between muscle and blood. II. The effect of respiratory alkalosis and acidosis induced by overbreathing and rebreathing. J Biol Chem 1937;118:197–204.

7 Baer-Suryadinata C, Bollinger A: Transcapillary diffusion of Na-fluorescein measured by a 'large window technique' in skin areas of the forefoot. Int J Microcirc Clin Exp 1985;4:217–228.

8 Bollinger A, Jäger K, Roten A, Timeus C, Mahler F: Diffusion, pericapillary distribution and clearance of Na-fluorescein in the human nailfold. Pflügers Arch Ges Physiol 1979;382: 137–143.

9 Franzeck U, Isenring G, Frey J, Jäger K, Mahler F, Bollinger A: Eine Apparatur zur dynamischen intravitalen Videomikroskopie. Vasa 1983;12:233–238.

10 Caspary L, Creutzig A, Alexander K: Biological zero in laser Doppler fluxmetry. Int J Microcirc Clin Exp 1988;7:367–371.

11 Graf C, Barras J: Rheological properties of human blood plasma. A comparison of measurements with three different viscosimeters. Experientia 1975;35:224–225.

12 Barras J, Jung T, Haberthür C, Koerner K: Vergleichende Untersuchungen der rheologischen Eigenschaften von Erythrozytenkonzentraten während einer Lagerungszeit von sechs Wochen in verschiedenen Konservierungslösungen. Vasa 1989;18:122–127.

13 Bollinger A, Frey J, Jäger K, Furrer J, Seglias J, Siegenthaler W: Patterns of diffusion through skin capillaries in patients with long-term diabetes. N Engl J Med 1982;307:1305–1310.

14 Franzeck U, Dörffler-Melly J, Hussain M, Wen S, Froesch E, Bollinger A: Effects of subcutaneous insulin-like growth factor-I infusion on skin microcirculation. Int J Microcirc 1995;15: 10–13.

15 Intaglietta M, Johnson P: Principles of capillary exchange; in Johnson P (ed): Peripheral Circulation. New York, Wiley, 1978, pp 141–166.

16 Johnson J, Taylor W, Shepherd A, Park M: Laser-Doppler measurement of skin blood flow: Comparison with plethysmography. J Appl Physiol 1984;56:798–803.

17 Sandeman D, Shore A, Tooke J: Relation of skin capillary pressure in patients with insulin-dependent diabetes mellitus to complications and metabolic control. N Engl J Med 1992;327: 760–764.

18 Brown E: Physiological effects of hyperventilation. Physiol Rev 1953;33:445–471.

19 Bolton B, Carmichael E, Sturup G: Vasoconstriction following deep inspiration. J Physiol (Lond) 1936;86:83–94.

20 Hansen J, Olsen N, Rasmussen B, Kanstrup I, Dechaux M, Dubray C, Riachalet J: Albuminuria and overal capillary permeability of albumin in acute altitude hypoxia. J Appl Physiol 1994;75:1922–1927.