Effect of acute hypoxia on vasopressin release and intravascular fluid during dynamic exercise in humans

Akira Takamata, Akira, Hiroshi Nose, Takashi Kinoshita, Munetaka Hirose, Toshiyuki Itoh, and Taketoshi Morimoto. Effect of acute hypoxia on vasopressin release and intravascular fluid during dynamic exercise in humans. Am J Physiol Regulatory Integrative Comp Physiol 279: R161–R168, 2000.—To test the hypothesis that acute hypoxia does not modify the relationship between plasma vasopressin concentration ([AVP]p) and plasma osmolality (P osmol) during exercise and that the increase in [AVP]p during exercise is due mainly to the exercise intensity-dependent increase in P osmol, we examined [AVP]p during a graded exercise in a hypoxic condition (13% O2, N2 balance) in seven healthy male subjects. A graded exercise in a normoxic condition on a separate day served as the control. Hypoxia reduced peak aerobic power (V O2 peak) by 32.4 ± 2.7%. Blood samples obtained during rest and at around 25, 45, 65, 80, and 100% of V O2 peak of each of the respective conditions were used for analyses of intravascular water and electrolyte balance. The pattern of the changes in fluid and electrolyte balance in response to percent V O2 peak was similar between the two conditions. Plasma volume decreased linearly as percent V O2 peak increased while P osmol increased in a curvilinear manner with a steep increase occurring at above ~66% V O2 peak. Above this relative exercise intensity, plasma sodium, potassium, and lactate concentrations also increased, whereas plasma bicarbonate concentration decreased. Thus transvascular fluid movement at above ~66% V O2 peak was due to the net efflux of hypotonic fluid out of the vascular space in both conditions. The relationship between [AVP]p and P osmol during exercise in response to relative exercise intensity was similar between the two conditions. The results indicate that acute mild hypoxia itself has no direct effect on vasopressin release, and it does not modify the relationship between [AVP]p and P osmol during exercise. The results also support the hypothesis that exercise-induced vasopressin release is primarily stimulated by increased P osmol produced by hypotonic fluid movement out of the vascular space in a relative exercise intensity-dependent manner.

arginine vasopressin; plasma osmolality; plasma volume; normobaric hypoxia; exercise intensity

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measured intravascular volume and electrolyte balances and blood pressure responses, as well as [AVP]p.

METHODOLOGIES
After a physical examination conducted by a physician, seven healthy male volunteers gave informed consent before participating in the study. All subjects were sea level residents, and none had recent high-altitude exposure. Their age was 24.1 ± 2.7 (SE) yr, body weight was 66.79 ± 2.21 kg, and plasma volume (PV) was 45.85 ± 1.38 ml/kg body wt. Subjects performed graded exercise under normoxic (room air) and normobaric hypoxic (13% O2) conditions on separate days. Experiments were conducted at the same time of day for each subject, separated by at least a week, and the order of the experiments was randomized. The experimental protocol was approved by the Review Board on Human Experiments, Kyoto Prefectural University of Medicine.

Experimental protocol. On the day of the experiments, subjects reported to the laboratory at 9:00–10:30 A.M. after a light breakfast. Subjects were instructed to refrain from salty food and to drink at least 400 ml of water at home ~1 h before reporting to the laboratory. After voiding, subjects were weighed, and they sat on a chair against the cycle ergometer in an environmental chamber at an ambient temperature of 25°C (relative humidity, 30%). A 21-gauge Teflon-coated catheter for blood sampling was inserted into a superficial vein on the forearm, and electrocardiogram (ECG) electrodes, a blood pressure cuff, and a pulse-oximeter probe were placed during this period. The subjects then wore a Rudolf mask and inspired room air for 30 min. A blood sample was taken and then subjects started to inspire either normoxic (room air) or hypoxic gas mixture (13% O2 and N2 balance) from a reservoir bag containing water to moisten the inspired gas. Thirty minutes after the resting period, by which time oxygen consumption, heart rate, and blood pressures had become stable, a blood sample was drawn, and the subjects began to exercise with the cycle ergometer in a semirecumbent position at 60 rpm. The exercise work load was increased by 29 or 58 W every 3 min (5–7 intensities) until subjects reached exhaustion. Blood samples were taken at the last minute of the resting period and each of the work loads (6–8 samplings). The blood samples obtained at the relative exercise intensities of ~10% (rest), 25%, 45%, 66%, 78%, and 95% of the VO2peak in the respective conditions were used for analyses of intravascular and electrolyte balance. The arm for blood sampling was warmed with a heating pad to maintain high blood flow through the hand (14).

Measurements. Heart rate was counted from ECG recordings (Nihon Kohden), and blood pressure was measured by ECG-triggered sphygmomanometry (Colin STBP). O2 and CO2 fractions of expired gas were collected from a mixing chamber and continuously measured with a gas analyzer (magnetopneumatic O2 analyzer and infrared CO2 analyzer; Horiba IA-200) and expired flow with a respiratory flowmeter (Minato). Arterial blood O2 saturation (SaO2) was estimated by pulse oximetry with a probe placed on the index finger (Nihon Kohden).

Aliquots of blood samples for the measurement of plasma bicarbonate concentration ([HCO3]p) were immediately transferred into heparin sodium-coated glass capillaries (Corning) and sealed tightly. Aliquots for measurements of other electrolytes and osmolality were immediately centrifuged, and separated plasma was stored at −20°C until measurement was performed. Blood for [AVP]p assay was transferred into an ice-chilled EDTA tube and centrifuged at 4°C, and separated plasma was stored at −80°C until each assay was performed. The remaining blood was immediately prepared for the measurement of hematocrit and Hb concentration.

Hematocrit was determined by the microcapillary centrifugation method, [Hb] by the cyanometohemoglobin method (Sigma Hb kit), and plasma protein concentration by refractometry (Atago). PaCO2 was measured by freezing point depression (Vogel OM801), plasma Na+ and K+ concentrations ([Na+]p and [K+]p) by flame photometry (Corning 480, Medfield, MA), and plasma Cl− concentration ([Cl−]p) with a chloride titrator (Corning 925). Plasma lactate concentration ([Lact−]p) was determined with fixed-enzyme electrode (YSI model 27, Yellow Springs, OH) and [HCO3]p by a blood gas analyzer (Corning 178).

[AVP]p was determined by RIA (AVP RIA kit; Mitsubishi, Yuka, Japan). Intra- and interassay coefficients of variation for 3.3 pg/ml AVP were 4.1 and 8.5%, respectively. The minimal detection limit of the AVP assay was 0.21 pg/ml in this experiment (0.063 pg/tube). All samples from a given subject were determined within the same assay kit.

PV was determined by the Evans Blue dye dilution technique on a separate day after an overnight fast. Measurements were performed in the environmental chamber at an ambient temperature of 25°C and at a relative humidity of 30% in a seated position after 1 h seated control period. Calculations and statistics. Percent change in PV (ΔPV) was calculated from hematocrit and [Hb] (8). Changes in PV (ml/kg) were calculated from the ΔPV and the initial PV determined by Evans Blue dye dilution. Plasma water content was calculated by subtracting the plasma solid fraction from the previous PV. Plasma solid fraction was calculated from the plasma protein concentration using the predetermined regression equation showing the relationship between plasma solid fraction (determined from dry weight) and plasma protein concentration (16). The regression equation was

\[ \text{Plasma solid (g/dl)} = 0.93 \times \text{PPC} + 1.81 \quad (r = 0.96) \]

where PPC is plasma protein concentration (in g/dl). Plasma electrolyte concentrations are presented as the concentration in the plasma water (meq/kgH2O).

ANOVA values for repeated measures (2 within factors) were used to determine the effect of the conditions (hypoxia vs. normoxia) and relative exercise intensities of each condition on the measured variables. Multiple comparisons of specific data points of interest were performed by Fisher’s least significant test when appropriate. P values <0.05 were considered significant. Values are presented as means ± SE of the seven subjects unless indicated otherwise.

RESULTS
The VO2peak in the hypoxic condition was 32.4 ± 2.0 and 48.0 ± 2.5 ml·min−1·kg body wt−1 in the normoxic condition. Hypoxia reduced the VO2peak by 32.4 ± 2.7% in our experimental conditions. Heart rate at any given relative exercise intensity was similar in the two conditions except ~100% VO2peak at which relative exercise intensity heart rate under hypoxia was slightly but significantly lower than that under normoxia (Table 1). The response of mean arterial pressure (MAP) to relative exercise intensity was similar between the two conditions but MAP at ~66% of VO2peak was significantly lower under hypoxia (Table 1). SaO2 was significantly lower during hypoxia throughout the experiment. SaO2 under normoxia was
relatively unchanged, whereas SaO₂ under hypoxia gradually decreased with the increase in relative exercise intensity (Table 1).

Body weight loss was 463 ± 51 g during hypoxia and 386 ± 41 g during normoxia. The body weight loss during hypoxic exercise tended to be smaller, but no significant differences were demonstrated between these conditions (P = 0.098).

Figure 1 shows ΔPV, P_osmol, [Na⁺]_p, and [Lact⁻]_p as functions of the relative (left) and absolute exercise intensity (%VO₂peak; left) and absolute exercise intensity (VO₂; right). Values are means ± SE of 7 subjects at each exercise intensity. *Significant difference between hypoxic and normoxic conditions. †Significant difference from normoxia at rest. "Significant difference from hypoxia at rest. V˙O₂, oxygen consumption; V˙O₂peak, peak oxygen consumption; HR, heart rate; MAP, mean arterial pressure; SaO₂, arterial blood oxygen saturation.

Values are means ± SE of 7 subjects. V˙O₂, oxygen consumption; V˙O₂peak, peak oxygen consumption; HR, heart rate; MAP, mean arterial pressure; SaO₂, arterial blood oxygen saturation. *Significant difference between normoxic and hypoxic conditions. †Significant difference from rest within the condition.
intensities (right). The response of PV to the increased relative exercise intensity was similar between the two conditions except for ~66% \( \text{VO}_{2\text{peak}} \), and therefore, the decrease in PV at a given oxygen consumption was larger during exercise under hypoxia (Fig. 1, right). The pattern of changes in \( P_{\text{osmol}} \) in response to relative exercise intensity was essentially similar between the two conditions, with a steep rise in \( P_{\text{osmol}} \) occurring above ~66% \( \text{VO}_{2\text{peak}} \) (Fig. 1, left). The increase in \( P_{\text{osmol}} \) above ~77% \( \text{VO}_{2\text{peak}} \), however, was lower under hypoxia than normoxia. The response patterns of \([\text{Na}^+]_p\), \([\text{K}^+]_p\), and \([\text{Cl}^-]_p\) to the relative exercise intensity were also similar between the two conditions, but the increases under hypoxia were lower than under normoxia at the higher exercise intensities (Fig. 1 and Table 2). The increases in \( P_{\text{osmol}} \) and electrolyte concentrations except \([\text{Cl}^-]_p\) under hypoxia at above \( \text{VO}_{2\text{peak}} \) of ~25 ml · min⁻¹ · kg body wt⁻¹ were larger than normoxia (Fig. 1, right, and Table 2).

\([\text{Lact}]_p\) increased markedly whereas \([\text{HCO}_3^-]_p\) decreased above ~66% \( \text{VO}_{2\text{peak}} \) as the relative exercise intensity increased (Fig. 1 and Table 2). The responses of \([\text{Lact}]_p\) and \([\text{HCO}_3^-]_p\) to absolute exercise intensity were larger at above \( \text{VO}_{2\text{peak}} \) of ~25 ml · min⁻¹ · kg body wt⁻¹ during hypoxic exercise (Fig. 1, right, Table 2). The increase in \([\text{Lact}]_p\) and decrease in \([\text{HCO}_3^-]_p\) were identical at lower exercise intensities, but the decrease in \([\text{HCO}_3^-]_p\) tended to become smaller than the increase in \([\text{Lact}]_p\) above ~66% \( \text{VO}_{2\text{peak}} \), and the difference in the changes of these anions at ~100% \( \text{VO}_{2\text{peak}} \) was significant in both conditions (Fig. 2). This response was similar in the two conditions (Fig. 2).

Figure 3 shows the relationship between intravascular H\textsubscript{2}O content and osmotic content calculated from \( P_{\text{osmol}} \) and plasma H\textsubscript{2}O content. The slope of this relationship indicates the osmolality of moved fluid across the vascular wall. At exercise intensities below ~45%, the osmolality of moved fluid was on the 300 mosmol/kg\textsubscript{2}O line (nearly isosmotic), but above this relative exercise intensity, the moved fluid became hyposmotic, i.e., a relatively large amount of free water shifted out of the vascular space compared with the solute. This response was similar in both conditions (Fig. 3).

The \([\text{AVP}]_p\) response to relative exercise intensity was similar between the two conditions, although \([\text{AVP}]_p\) at ~100% \( \text{VO}_{2\text{peak}} \) under hypoxia was lower than it was under normoxia (Fig. 4A). \([\text{AVP}]_p\) at above \( \text{VO}_{2\text{peak}} \) of ~25 ml · min⁻¹ · kg body wt⁻¹ was higher under hypoxic exercise compared with normoxic exercise (Fig. 4B). The relationship between \([\text{AVP}]_p\) and

<table>
<thead>
<tr>
<th>Relative Exercise Intensity, %( \text{VO}_{2\text{peak}} )</th>
<th>Rest</th>
<th>~25%</th>
<th>~45%</th>
<th>~65%</th>
<th>~80%</th>
<th>~100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Na}^+]_p) normoxia</td>
<td>153.2 ± 0.7</td>
<td>153.9 ± 0.5</td>
<td>154.9 ± 0.7</td>
<td>156.2 ± 0.7†</td>
<td>158.9 ± 0.8†</td>
<td>160.6 ± 0.9†</td>
</tr>
<tr>
<td>([\text{Na}^+]_p) hypoxia</td>
<td>153.5 ± 0.8</td>
<td>153.5 ± 0.7</td>
<td>154.1 ± 0.6</td>
<td>154.4 ± 1.4</td>
<td>156.4 ± 1.1†</td>
<td>159.6 ± 1.5†</td>
</tr>
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</table>

Values are means ± SE of 7 subjects in meq/kgH\textsubscript{2}O. \([\text{Na}^+]_p\), plasma sodium concentration; \([\text{K}^+]_p\), plasma potassium concentration; \([\text{Cl}^-]_p\), plasma chloride concentration; \([\text{Lact}]_p\), plasma lactate concentration; \([\text{HCO}_3^-]_p\), plasma bicarbonate concentration; \( \text{VO}_{2\text{peak}} \), peak oxygen consumption. *Significant difference between normoxic and hypoxic conditions. †Significant difference from rest within the condition.
DISCUSSION

To clarify the regulation of AVP release under hypoxia, we examined the effect of acute mild hypoxia on [AVP]p during a graded exercise. Hypoxia rather inhibited AVP secretion at higher relative exercise intensity. The [AVP]p at ~100% VO2peak was lower under hypoxia than normoxia (Fig. 4A). However, the relationship between [AVP]p and Posmol was similar between the two conditions, and thus the smaller increase in [AVP]p during hypoxic exercise was due to the lower increase in Posmol at this exercise intensity. The present study demonstrated that acute hypoxia did not stimulate AVP secretion at rest (Fig. 4), and it did not alter the relationship between [AVP]p and Posmol during exercise (Fig. 4C). Thus acute hypoxia per se does not enhance AVP secretion, and there exists no altering effect of hypoxia on the relationship between [AVP]p and Posmol during exercise.

Blume et al. (2) reported that [AVP]p and urinary AVP excretion did not increase, although Posmol increased in subjects at 5,400 or 6,300 m, suggesting that osmosensitivity was impaired at high altitudes. However, the characteristics of the time course of this change in osmotic sensitivity are unknown.

Our results suggest that the increased AVP release during exposure to hypoxia of longer duration could be a secondary effect of hypoxia, including hypotension and nausea (13). Our results also suggest that hypoxia enhances AVP release more than normoxia at a higher absolute exercise intensity (power output) (Fig. 4B) because of the greater increase in Posmol at a given absolute exercise intensity under hypoxia (Fig. 1, right). For example, moderate- to high-intensity exercise at a higher altitude could result in an enhanced secretion of AVP compared with exercise of the same absolute intensity at sea level. Meehan (15) reported that hypoxia did not stimulate AVP secretion during 6 h of mild exercise, but the exercise intensity they used was too low to stimulate transvascular fluid movement or to elevate Posmol (Fig. 1). Thus physical activity has an impact on the regulation of AVP secretion in an exercise intensity-dependent manner.

Stimuli that cause AVP release during exercise are not sufficiently understood (23). In the present study, we examined the regulation of AVP release during exercise under hypoxia. Hypoxia lowered the VO2peak, but the relationship between [AVP]p and Posmol was not altered by the modification of absolute exercise intensity induced by hypoxia (Fig. 4). Thus our results support the hypothesis that the primary stimulus of AVP release during exercise is increased Posmol (6, 17, 25) or relative exercise intensity but not absolute exercise intensity (Fig. 4). The increase in [AVP]p per unit rise in Posmol during exercise tended to be higher than that induced by hypertonic saline infusion (24), suggesting that mechanisms other than osmoregulation are involved in AVP secretion during exercise (23). One possible mechanism is the effect of muscle chemoreceptor stimulation (18, 23). Because free water movement out of the vascular space was facilitated above ~66% VO2peak with the increase in [Lact]p, this effect could be significant during exercise, especially at higher relative exercise intensities (20). Another possible mechanism is increased body temperature. Takamata et al. (24) reported that AVP secretion is enhanced by increased body core temperature when Posmol was higher than 295 mosmol/kgH2O. Anyway, our results support the hypothesis that the primary stimulant of AVP secretion during exercise is increased plasma osmolality.

Another important finding in this study was that the changing pattern of the intravascular water and electrolyte balance with the increase in relative exercise intensity was essentially similar between the two conditions. Bouissou et al. (3) reported a similar finding, that the change in Posmol and electrolyte concentrations in response to relative exercise intensity was similar...
between exercise under normoxia and under hypoxia. Convertino et al. (7) reported that exercise training attenuated the PV, $P_{\text{osmol}}$, and [AVP]$_p$ responses to absolute exercise intensity, but these responses to relative exercise intensity were not modified by physical training. However, exercise training increases PV and muscle mass, which is a complicating factor to interpret the data. Along with those of the study conducted by Bouissou et al. (3), the results of the present study suggest that fluid movement across the vascular space is related more with the relative exercise intensity than the absolute exercise intensity or muscular work per se. Nose et al. (17) suggested that the increase in $P_{\text{osmol}}$ mainly due to increase in [Na$^+$]$_p$, was partly attributed to by the increased [Lact$^-$]$_p$. They postulated that the increased [Lact$^-$]$_p$ contributes to the restriction of movement of the major cation, Na$^+$, out of the vascular space. In the present study, $P_{\text{osmol}}$ as well as [Lact$^-$]$_p$ started to increase further at exercise intensities above ~66% $V_{02}\text{peak}$. In addition, the reduction in HCO$_3^-$ at above ~66% $V_{02}\text{peak}$ tended to become smaller compared with the increase in [Lact$^-$]$_p$, and a significant difference occurred at ~100% $V_{02}\text{peak}$ in each condition (Fig. 2). By modifying the lactate threshold using hypoxia without any change in PV and muscle mass, we found that the increase in [Na$^+$]$_p$ at a higher relative exercise intensity was accompanied by an increase in [Lact$^-$]$_p$. Therefore, our results confirm the hypothesis that the increase in [Na$^+$]$_p$, and consequently $P_{\text{osmol}}$, during exercise is partly due to the increase in [Lact$^-$]$_p$ that restricts Na$^+$ movement out of the vascular space.

Estimated from body weight loss, the water loss was 463 ± 51 g during normoxic exercise and 386 ± 41 g during hypoxic exercise. Respiratory and sweat water loss would cause plasma hyperosmolality. Assuming that 1) respiratory and sweat water loss is 500 ml, 2) there are 42 liters of total body water (60% of 70 kg), 3) the mean osmolality of lost fluid is 100 mosmol/kg$H_2O$, and 4) water can move across cell membranes quite freely, and therefore osmolality of intra- and extracellular space is similar (16), the increase in $P_{\text{osmol}}$ will be <3 mosmol/kg$H_2O$. Thus the cause of the increase in $P_{\text{osmol}}$ during exercise can hardly be explained by the evaporative water loss. The main cause of the increased $P_{\text{osmol}}$ during moderate to heavy exercise of a short duration seems to be the hypotonic fluid movement from intra- to extravascular space. The hypotonic
water movement from the intravascular to the extracellular space, induced by metabolite accumulation in the muscle tissues, is the most plausible factor resulting in the elevation of $P_{\text{osmol}}$ (21, 22).

The increase in $P_{\text{osmol}}$ at a higher relative exercise intensity during hypoxia was slightly but significantly lower than that during normoxia. Although similar findings were reported by Bouissou et al. (3), they did not comment on this issue. Because the water moved out of the vascular space was not as hypotonic as during normoxia, osmotic driven fluid movement is less under hypoxic condition (Fig. 1). The possible reason for this could be that the measured $V_{\text{O}_2} \text{peak}$ during hypoxia may be underestimated; thus the increase in $P_{\text{osmol}}$ was lower at a given relative exercise intensity. In fact, the maximal heart rate during hypoxia was lower than that during normoxia (Table 1). Early studies have shown that maximal heart rate at high altitudes was lower than at sea level (12), suggesting that the measured $V_{\text{O}_2} \text{peak}$ in the present study was not underestimated. Thus the factor or factors determining $V_{\text{O}_2} \text{peak}$ under hypoxia might be different from that under normoxia. This hypothesis is supported by the results that $[\text{Lact}]_p$ and $[\text{K}]_p$ at any given higher exercise intensity (greater than ~66% $V_{\text{O}_2} \text{peak}$) were also lower during hypoxia (21) (Table 2 and Fig. 1).

Before the experiment, the intake of sodium and fluid was not strictly controlled in the present study; however, the subjects were instructed to refrain from salty food and to drink at least 400 ml of water at home ~1 h before reporting to the laboratory. In addition, the subjects were instructed to maintain their usual diet the night before the studies in both of the two conditions. This procedure ensured that the baseline $P_{\text{osmol}}$ (Fig. 1), hematocrit (43.3 ± 1.2% in normoxia and 43.1 ± 0.7% in hypoxia), and [HB] (14.4 ± 0.6 g/dl in normoxia and 14.7 ± 0.3 g/dl in hypoxia) were similar between the two conditions. Thus the impact of sodium and fluid intake before the experiment was not likely to complicate the results with regard to the transvascular fluid and electrolytes balance and AVP secretion in the present study.

Blood pressure response to relative exercise intensity was similar in both conditions except for that at 66% $V_{\text{O}_2} \text{peak}$ (Table 1), suggesting that chemoreceptor reflex in addition to mechanoceptor reflex are, at least in part, involved in the exercise pressor response because mechanical output at any given relative exercise intensity during hypoxia was significantly lower than in normoxic exercise. Although systemic hypoxia tonically stimulates carotid body chemoreceptor, the initial level and changing pattern of MAP to the increased relative exercise intensity was similar; thus muscle chemoreflex would be involved in the regulation of MAP during exercise (20).

In summary, the present study demonstrated that AVP release during exercise was primarily stimulated by increased $P_{\text{osmol}}$ and hypoxia did not modify the relationship between $[\text{AVP}]_p$ and $P_{\text{osmol}}$ during exercise. The increased $P_{\text{osmol}}$ during exercise was essentially dependent on relative exercise intensity and may be related to the increase in $[\text{Lact}]_p$. At a higher absolute work load, hypoxia enhances $[\text{AVP}]_p$ secretion more than normoxia because of larger increase in $P_{\text{osmol}}$.

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REFERENCES

19. Rose CE Jr, Dixon BS, and Anderson RJ. Effects of hypoxemia and hypercapnic acidosis on renal water excretion and...


