Voluntary breath-holding in the morning and in the evening

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ABSTRACT

The aim of the present study was to determine whether or not voluntary breath-holding time (BHT) changes with the time of the day. BHT with airways closed at end-expiration was measured in six male subjects in the sitting position during the morning (08.00–12.00 hours, on days 1, 6, 7 and 8) and evening (20.00–24.00 hours, on days 2 and 4). BHT increased with the number of days of testing and, at day 8, the morning values averaged 160% of those on day 1. Also, \( \Delta P_{ACO2} \) [the difference between end-tidal partial pressure of CO\(_2\) \( (P_{CO2}) \) and alveolar \( P_{CO2} \) \( (P_{ACO2}) \) at the breaking point] increased in proportion to BHT. Hence the \( \text{BHT/\( \Delta P_{ACO2} \)} \) ratio remained nearly constant. Voluntary hyperventilation prolonged BHT and increased \( \Delta P_{ACO2} \). Conversely, in hypoxia (13% O\(_2\) for 1–2 h), BHT and \( \Delta P_{ACO2} \) were reduced proportionally. During the evening sessions, most of the \( \text{BHT/\( \Delta P_{ACO2} \)} \) ratios in normoxia, hypoxia or after hyperventilation were higher than the corresponding morning values, with the group difference reaching statistical significance for the measurements in normoxia and hypoxia. In conclusion, voluntary BHT varies in both duration and its relationship with \( \Delta P_{ACO2} \) between the morning and evening hours. The results should also imply that, with an interruption of breathing, changes in alveolar and arterial gases are not the same at different times of the day.

INTRODUCTION

Many physiological variables oscillate throughout the day, following 24-h (or ‘circadian’) patterns. Recent studies have indicated that daily changes also occur in various aspects of the control of breathing (for review, see [1]). In humans, 24-h oscillations in body temperature, ventilatory chemosensitivity and alveolar gases can be detected even in the absence of usual daily changes in activity patterns or in the state of arousal [2–6]. Voluntary breath-holding (BH) is a simple manoeuvre, which has long been used to assess integrated aspects of ventilatory control and its interaction with metabolic rate [7–9]. In fact, in first approximation, BH time (BHT) is determined by the chemical stimuli arising from the metabolic activities and ventilatory responses to these stimuli. In the present study, we investigated whether or not BHT and the associated changes in alveolar gases differ between the morning and evening hours.

During a cessation of breathing or apnoea, including voluntary BH, the progression in metabolic activities causes gradual changes in alveolar gases, i.e. an increase in CO\(_2\) and a fall in O\(_2\). If the upper airways are kept closed, the organism becomes a self-contained unit. An additional factor in determining the values of alveolar and blood gases is the progressive fall in lung volume, which is by itself a source of ventilatory stimuli via the pulmonary vagal reflexes. Mithoefer [10] and Hong et al. [11] have

Key words: alveolar gases, apnoea, circadian pattern, metabolic rate.

Abbreviations: BH, breath-holding; BHT, BH time; FRC, functional residual capacity; \( P_{ACO2} \), alveolar partial pressure of CO\(_2\); \( P_{CO2} \), partial pressure of CO\(_2\); \( P_{ACO2(brp)} \), \( P_{ACO2} \) at the breaking point; \( \dot{V}_{O2} \), oxygen consumption.

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provided a detailed analysis of the events accompanying BH with closed airways. In brief, within the first seconds of BH, the usual pressure gradient of CO₂ between lung capillaries and alveoli (approx. 6 mmHg) is rapidly lowered by the accumulation of CO₂ in the alveoli, therefore reducing CO₂ elimination. This contrasts with the alveolar–capillary O₂ gradient, which, being large (approx. 60 mmHg), does not limit O₂ loading into the blood. Hence the passage of O₂ from the lungs into the capillary exceeds that of CO₂ in the opposite direction, reducing lung volume. The reduction in lung volume concentrates the alveolar gases, raising their partial pressures; this, by further reducing the CO₂ diffusion while protecting the O₂ transfer, aggravates the decrease in lung volume in a self-perpetuating chain of events. After approx. 10 s of BH, CO₂ accumulation in the lungs is only approx. one-third of oxygen consumption (VO₂) and, shortly after, it ceases altogether [11,12].

Once the capillary–alveolar CO₂ pressure gradient is abolished and CO₂ accumulation in the lung ceases, the further reduction in lung volume is closely related to the uptake of O₂ in the pulmonary capillaries. Indeed, it was shown almost half a century ago [7] that, during BH with closed airways, lung volume falls linearly with time, as one should expect with a constant VO₂. Hence, for any given level of VO₂ and for BHT in excess of 15–20 s and, despite the absence of CO₂ accumulation in the lungs, the alveolar partial pressure of CO₂ (Paco₂) at the breaking point [Paco₂(b)Pr] increases progressively with BHT, according to a relationship which depends on VO₂ [13].

In the present study we investigated whether or not BHT changed between morning and evening and, if it did, whether Paco₂(b)Pr changed in proportion to it. First, we constructed the average BHT/ΔPaco₂ [where ΔPaco₂ represents the difference between end-tidal partial pressure of CO₂ (Paco₂) and Paco₂(b)Pr] curve during the morning. The relationship has been obtained taking advantage of the fact that BHT changes greatly over successive days, due to practice and psychological factors [9]. Subsequently, we plotted the BHT/ΔPaco₂ data obtained in the evening. Three possibilities were anticipated. First, the morning and evening data may coincide, which would suggest that circadian changes in physiological variables did not appreciably affect BHT and the associated changes in alveolar gases. Alternatively, the evening data may differ from morning, but fall on the same BHT/ΔPaco₂ curve. This result would be compatible with morning and evening differences in chemosensitivity playing a dominant role in BHT. Finally, the evening data points may fall off the BHT/ΔPaco₂ curve. This would indicate that differences in chemosensitivity alone could not explain the morning and evening differences in BHT. Additional measurements have been collected during hypoxia, to enhance ventilatory chemosensitivity, and after voluntary hyperventilation, which, by prolonging the BHT, increases the ventilatory stimuli originating from the volume shrinkage.

### METHODS

#### General protocol

The study was performed on six male subjects, ranging from 17–51 years of age (Table 1). They gave a written consent to partake in the study after being fully informed about the protocol, although the aim of the study was not disclosed. Two of the subjects were moderate smokers, and all lived permanently at sea level. With one exception, they were active members of a scuba diving club; hence they were familiar with voluntary breathing manoeuvres, including BH, and holding a mouthpiece. The subjects abstained from caffeine-containing drinks from the day before and throughout the whole experimental week.

All measurements were performed in a 12-seat hyperbaric chamber. In fact, although the barometric pressure remained close to 1 atmosphere (approx. 101 kPa) in all circumstances, the confined environment of the chamber was considered appropriate to maintain very similar conditions of light and external stimuli among the various sessions. In addition, it made it practical to vary the oxygen content for the sessions in hypoxia.

Measurements were conducted on days 1, 2, 4, 6, 7 and 8 either in the morning (between 08.00 and 12.00 hours, on days 1, 6, 7 and 8) or evening (between 20.00 and 24.00 hours, on days 2 and 4). Hence in no cases were two sessions performed within the same day. Approx. 30 min before each session, the subjects were invited to have a light snack, whereas the main meal was eaten upon termination of the measurements.

Ambient temperature and humidity were monitored and recorded with a data logger (HOBO H8; StowAway) and averaged 20.0 ± 2 °C and 53 ± 10 % respectively, in the morning, and 22.7 ± 0.5 °C and 60 ± 5 % in the evening. Barometric pressure was 101.1 ± 0.5 kPa in the morning and 100.1 ± 0.3 kPa in the evening. In addition to the tests in normoxia at rest and following hyperventilation and in hypoxia, measurements in hypercapnia were also performed. However, because of an error in the CO₂ concentration and the control of ambient temperature in one of the two sessions, the morning–evening

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**Table 1** Characteristics of the six subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
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<th>4</th>
<th>5</th>
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<td>Age (years)</td>
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<td>23</td>
<td>26</td>
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<tr>
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<td>70</td>
<td>84</td>
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<td>87</td>
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<tr>
<td>Height (cm)</td>
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<td>167</td>
<td>175</td>
<td>177</td>
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comparison became unwarranted, and those data have been excluded.

**BH test**

Subjects were seated and maintained a straight posture whilst breathing through a mouthpiece, with the nostrils closed by a nose-clip. The mouthpiece was attached to a short flexible rubber tube (inner diameter, 12 mm and length, 150 mm). The tube airflow resistance was 0.4 cm of water · litre⁻¹ · s⁻¹, which is approx. 17 % of the total resistance of the respiratory system at functional residual capacity (FRC) [14]. The total volume of the added dead space was approx. 18 ml. An O₂ and a CO₂ gas analyser (OM-11 and LB-2 respectively; Beckman) were sampling continuously at a rate of 500 ml/min through a side port at the peripheral end of the flexible tube, and their output was recorded on a polygraph. The subject was invited to breathe steadily for approx. 1 min and then relaxed at the FRC, before active BH began by bending the tube, effectively sealing the airways. At the breaking point, the subject was instructed, first, to release the tube back into its straight position; then, to breathe out forcibly to allow for alveolar gas sampling. BHT was monitored with a stopwatch from the moment of airway closure until the final expiration, and this time corresponded to the duration of the zero CO₂ signal on the polygraph recording. For each session, the subjects performed between three to five tests at not less than 5-min intervals. The full completion of the tests required approx. 2 h.

For the measurements in hypoxia, the hyperbaric chamber was flushed with N₂ until the ambient O₂ concentration averaged approx. 13 %, a process that took approx. 35–45 min to complete. For the measurements after hyperventilation (in normoxia), the subjects were invited to increase the depth of breathing and to continue hyperventilation until the end-tidal PCO₂ fell below 20 mmHg. The BH manoeuvre was then carried out.

**Data analysis**

The polygraph data were analysed with the help of a graphics tablet connected to a minicomputer. For each test, BHT, end-tidal PCO₂ and PACO₂(brp) were measured and, subsequently, ΔPACO₂ and the BHT/ΔPACO₂ ratio were calculated. The first two tests of the experimental session on day 1 were excluded from the analysis. Results are presented as means ± S.E.M. Mean results of the evening and morning sessions were compared statistically by two-tailed paired Student’s t test, with the level of significance being at *P* < 0.05.

**RESULTS**

**Resting conditions**

The intra-subject variability was rather large, between 10 and 20 %, for BHT and PACO₂(brp) (alveolar partial pressure of O₂ at the breaking point), and small, approx. 2–3 %, for PACO₂(brp), and in all cases it remained stable throughout the whole experimental week.

On day 1, the six subjects had very different BHTs, spanning from 21 to 99 s (60 ± 13 s, mean ± S.E.M.). In the last three morning sessions (days 6, 7 and 8), all subjects had a longer BHT than in the first session, an increase of 40–60 % (Figure 1, top panel). For all sessions, the end-tidal PCO₂ remained steady at approx. 35 mmHg. However, PACO₂(brp) increased from 46 ± 2 to 52 ± 2 mmHg (Figure 1, middle panel); hence ΔPACO₂ increased from day 1 to day 8. This increase occurred approximately in proportion to BHT; in fact, the ratio between BHT and ΔPACO₂ remained constant at approx. 5 s/mmHg (Figure 1, bottom panel, open squares).

During the evening sessions, BHT was either higher than (day 2) or close to (day 4) the morning curve. In the former case, PACO₂(brp) was close to, and in the latter case below, the corresponding values expected for those days from the morning relationship (Figure 1, filled symbols). Because the values of end-tidal PCO₂ in the evening and morning sessions were the same, the BHT/ΔPACO₂ ratio was significantly higher in the evening hours (*P* < 0.02; Figure 1, bottom panel, filled symbols). When examined

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![Figure 1](image-url)  
**Figure 1** BHT (top panel), end-tidal PCO₂ and PACO₂(brp) (middle panel) and BHT/ΔPACO₂ (lower panel) in the morning and evening on various days

BHT is represented as the percentage of the value at day 1, when it averaged 60 s. In the morning sessions (AM, open symbols), BHT/ΔPACO₂ ratio remained close to the value of the first day (5.1 s/mmHg; dotted line). Evening sessions (PM; filled symbols). Symbols represent the means of the six subjects, with bars indicating S.E.M.
individually for each subject (Figure 2, left-hand panel), the evening and morning difference in BHT/ΔPaco₂ ratios showed a large variability in the evening hours ranging from 101–191 % of the morning value (on average, 132 ± 14 %; \( P < 0.02 \)).

**Hyperventilation**

The subjects were requested to hyperventilate until end-tidal \( P_{CO₂} \) was less than 20 mmHg. Because of this, BHT increased drastically on average to 191 % (day 1) and 221 % (day 8) of that measured on day 1 without previous hyperventilation. The increase in BHT was accompanied by an increase in \( P_{ACO₂}(brp) \), which averaged 42 ± 2 and 50 ± 2 mmHg on days 1 and 8 respectively. Hence the BHT/ΔPaco₂ ratios in the morning hours averaged 3.6 ± 0.4 s/mmHg and did not differ significantly between day 1 and day 8.

During the evening hours, the average value of the BHT/ΔPaco₂ ratio (4.2 ± 0.9 s/mmHg) was only slightly, and not significantly, higher than the average value in the morning sessions. On an individual basis, the BHT/ΔPaco₂ ratio in the evening hours was lower than the morning value in one subject and higher in the remaining five subjects (Figure 2, right panel, filled squares).

**Hypoxia**

In 13 % \( O_2 \) (end-tidal partial pressure of \( O_2 \), approx. 55 mmHg), hypoxic hyperventilation lowered the end-tidal \( P_{CO₂} \) to approximately the same value in the morning (32 ± 2 mmHg) as in the evening (31 ± 1 mmHg). BHT was shorter than in normoxia, and similar between morning and evening hours. In the morning and evening hours, \( P_{ACO₂}(brp) \) averaged 43 ± 2 and 39 ± 2 mmHg respectively, a statistically significant difference (\( P < 0.005 \)), which was found in all subjects. Because of this difference, the BHT/ΔPaco₂ ratio was also significantly higher in the evening compared with the morning hours (\( P < 0.05 \)). On an individual basis, the BHT/ΔPaco₂ ratio in the evening hours was higher than in the morning hours in five out of the six subjects (Figure 2, right-hand panel, triangles).

A comparison of the mean values of the BHT/ΔPaco₂ ratios for the three conditions (normoxia, hypoxia and hyperventilation) is shown in Figure 3 in which the results of all the experimental days are combined. The mean BHT/ΔPaco₂ values were similar in normoxia and

Figure 2  BHT/ΔPaco₂ in the evening against the corresponding values in the morning for each of the six subjects during normoxia (left panel) and hypoxia or hyperventilation (right panel) Solid lines indicate the evening/morning ratio in percentage, with 100 % being represented by the line of identity. Symbols are the mean values of the various sessions for each subject, and bars indicate S.E.M.

Figure 3  BHT/ΔPaco₂ ratio in normoxia and hypoxia and after hyperventilation during the morning and evening Values are means ± S.E.M. * \( P < 0.05 \) between morning and evening sessions.
hypoxia, whereas they were smaller after hyperventila-
tion. For each condition, the tendency for higher values
in the evening hours reached statistical significance in
normoxia \((P < 0.02)\) and hypoxia \((P < 0.05)\).

**DISCUSSION**

The main results of the present study can be summarized
as follows. First, during normoxia in the morning, the
BHT/\(\Delta P_{\text{aco}}\) ratio remained constant throughout
the various sessions, despite the approx. 60 % change in
BHT between day 1 and day 8. Secondly, the BHT/
\(\Delta P_{\text{aco}}\) ratio was increased, on average by approx.
35 %, in the evening compared with the morning
sessions. Thirdly, during hypoxia in the morning, the
BHT/\(\Delta P_{\text{aco}}\) ratio was close to the normoxic value, whereas it
was increased in the evening session. Finally,
following hyperventilation, we did not find a statistically
significant difference in the BHT/\(\Delta P_{\text{aco}}\) ratio between
the morning and evening sessions, although five out
of six subjects presented higher evening values. Taken
together, therefore, and irrespective of the conditions,
the BHT/\(\Delta P_{\text{aco}}\) ratio was higher in the evening than
the morning.

**Sessions in the morning**

These results were predictable [10,11] and, therefore, only
a few aspects will be highlighted. The gradual lengthening
of BHT with repeated tests is a phenomenon consistently
reported, although not fully explained [9,10,15]; possibly,
it reflects the progressive familiarization with the man-
oeuvre and overcoming of the discomfort at the breaking
point. As expected for BHTs longer than 20 s, \(P_{\text{aco}}\) (brp)
increased in proportion with BHT. In fact, once the CO2
transfer into the lungs ceases, the increase in \(P_{\text{aco}}\) is
determined by the lung volume shrinkage, which is a
function of \(\dot{V}_{\text{O}}\) [7,10,11].

During hypoxia, BHT was shortened, but the
BHT/\(\Delta P_{\text{aco}}\) ratio was essentially unaltered. In fact,
hypoxia, just like any ventilatory stimulus which shortens
BHT, should not modify the BHT/\(\Delta P_{\text{aco}}\) ratio as long as
\(V_{\text{O}}\) remains constant. In humans, hypoxia does not
alter metabolic rate [16], which is different than in smaller
species. Because the level of hypoxic exposure was rather
modest, as indicated by the fall in end-tidal \(P_{\text{CO}}\) of
only 2–3 mmHg, BHT lasted approx. 1 min, which is
a duration well within the range of the BHT/\(\Delta P_{\text{aco}}\)
proportionality.

Hyperventilation reduced the BHT/\(\Delta P_{\text{aco}}\) ratio. Be-
cause of the low end-tidal \(P_{\text{CO}}\) after the hyperventilation,
BHT can be quite long and lung volume shrinkage is
marked. In these cases, the vagal reflex of pulmonary
origin becomes the paramount stimulus for breathing,
breaking the apnoea at a \(P_{\text{aco}}\) (brp) lower than without
previous hyperventilation [10].

**Sessions in the evening**

Analysis of the individual responses (Figure 2) and
of the group mean results (Figure 3) indicated that
the BHT/\(\Delta P_{\text{aco}}\) ratio tended to be higher in the
evening, a difference statistically significant in normoxia
and hypoxia. Differences in the state of alertness or
motivation, possibly with greater sleepiness at night,
cannot be an explanation. In fact, they would affect
BHT and \(P_{\text{aco}}\) (brp), but not the BHT/\(\Delta P_{\text{aco}}\) ratio.
The same can be said about the fall in chemosensitivity,
known to occur during the night hours [4,5], or about the
posibility of a morning and evening difference in neuro-
muscular inputs, which play an important role in the
termination of voluntary BH [17]. Indeed, as indicated
above, even in hypoxia, which represents a change in
chemoreceptor input larger than can occur at different
times of the day, the BHT/\(\Delta P_{\text{aco}}\) ratio was as in
normoxia.

Because the value of \(P_{\text{aco}}\) (brp) for a given metabolic
rate depends on lung volume, the possibility of a
systematic difference in lung volume between morning
and evening needs to be considered. The end-expiratory
lung volume has been observed to decrease by approx.
200 ml or approx. 8 % of FRC in the evening [18].
However, if that occurred in the evening sessions of our
experiment, it would have increased the alveolar \(P_{\text{aco}}\)
and lowered the BHT/\(\Delta P_{\text{aco}}\) ratio; this is in contrast
with the results found. Systematic differences in posture,
for example, from a seated-relaxed to a seated-straight
position, causing a morning and evening difference in
FRC seem unlikely. Furthermore, it can be calculated
that, for this possibility to explain the approx. 30 %
evening and morning differences in the BHT/\(\Delta P_{\text{aco}}\)
ratio, FRC would need to vary by more than 600 ml.
This is an unrealistic situation if one considers that FRC
changes by less than 300 ml from the sitting to the
standing position [19].

Of course, differences in end-tidal \(P_{\text{aco}}\) between
morning and evening would have altered the correspon-
ding BHT/\(\Delta P_{\text{aco}}\) ratio. However, despite the circadian
pattern of metabolic rate and pulmonary ventilation, end-
tidal \(P_{\text{aco}}\) remains almost constant throughout the day
[1]. In fact, for each experimental condition, we could
not see any significant difference in end-tidal \(P_{\text{aco}}\)
between morning and evening sessions. It is known that
differences in metabolic rate alter the relationship be-
 tween BHT and \(\Delta P_{\text{aco}}\). For example, the increase in
\(\dot{V}_{\text{O}}\) with muscle exercise increases the \(P_{\text{aco}}\) (brp) for
any given BHT, lowering the BHT/\(\Delta P_{\text{aco}}\) ratio [13].
Our subjects were resting and awake and, in adult
humans, the circadian oscillations in metabolic rate are
almost totally contributed by changes in the state of
arousal. In fact, in awake subjects during bed rest,
oscillations in \(\dot{V}_{\text{O}}\) are small and difficult to detect [1].
This, however, does not exclude the possibility that
uncontrolled factors, other than activity and state of
arousal, may have introduced small differences in $\dot{V}O_2$. Furthermore, because the rise in $P_{ACO_2}$ during closed-airway BHT depends on the $\dot{V}O_2$-dependent fall in lung volume [10,11], changes $P_{ACO_2}$ can disproportionately exceed those in $\dot{V}O_2$.

Conclusions

Because voluntary BH is a simple test integrating metabolic, mechanical and regulatory functions, it has attracted the attention of many investigators (for review, see [10]). The present study demonstrates that the integration of the various functions involved in the BHT/$P_{ACO_2}$ relationship is not the same throughout the day, and the duration of the voluntary apnoea during the night can exceed that during the morning. The reasons for this difference need to be explored, although it is possible that they may reflect small differences in metabolic rate, whereas it is unlikely that they are contributed by differences in ventilatory chemosensitivity. Irrespective of the mechanisms causing it, the fact that the BHT/$\Delta P_{ACO_2}$ ratio differs between night and morning hours also implies that naturally occurring episodes of central apnoea or episodes of obstructive sleep apnoea can have different consequences on blood gases at different times of the day. Whether this may have any relationship to the fact that symptoms of cardiorespiratory diseases and fatal failures have circadian patterns [20–24] is a matter of speculation.

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