Measuring Uptake and Elimination of Nitrogen in Humans at Different Ambient Pressures

Patrik Sundblad; Oskar Frånberg; Christoph Siebenmann; Mikael Gennser

BACKGROUND: To measure nitrogen (N_2) wash-out and uptake requires elaborate set-ups, especially when doing the measurements at increased or decreased ambient pressure. Here we present a transportable device for quantifying N_2 turnover in humans which can be used at different ambient pressures.

METHODS: A modified close-circuit electronic rebreather was used to assess N_2 turnover. Changes in N_2 volume within the rebreathing circuit, reflecting N_2 uptake or washout, were derived from the continuously monitored total system volume and the calculated volumes of oxygen and water vapor. The calculation of continuous N_2 volume curves was performed off-line using dedicated computer software.

RESULTS: Four subjects participated in the proof-of-concept tests. At steady state, the drift in calculated N_2 volume in the rebreathing circuit over a 1-h duration was minimal. Three of the subjects participated in additional N_2 steady-state measurements where 1019 mL (BTPD) of N_2 was injected into the rebreathing circuit over 20 min and the measured volume increase was 1006 $\pm$ 32 mL. Lastly, N_2 elimination was assessed during decompression to 0.5 atm and while breathing hyperoxic gas. N_2 uptake was measured during compression to 1.8 atm. The elimination and uptake curves were deemed to be realistic.

DISCUSSION: A method for assessing N_2 turnover in humans has been developed and a first evaluation has been performed. It is easy to work with operationally and can be used at different ambient pressures. More research is needed in order to further validate it as a method for assessing N_2 turnover in humans.

KEYWORDS: decompression, nitrogen elimination, pressure, diving.

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Normally, while breathing, the partial pressure of inhaled nitrogen (P_IN_2) is the same as in the tissues, where it exists in a dissolved state. When the P_IN_2 increases, as during diving, pulmonary N_2 uptake occurs and tissue PN_2 increases until a new steady state is reached. The opposite happens when P_IN_2 decreases. If there is a fast and large enough reduction in ambient pressure, the N_2 dissolved in the tissues may form gas bubbles. This is generally accepted as the initiating step in decompression sickness. The rate of N_2 uptake or wash-out secondary to a given change in P_IN_2 is affected by several factors such as temperature, physical exercise, and body position.[2,10] It has also been suggested that bubble formation per se affects inert gas elimination during decompression.[4,6] N_2 elimination has been measured previously, typically during normobaric conditions, using hyperoxic gas or alternatively using argon or helium enriched gas.[2,3,6] N_2 elimination has also been measured in hyperbaric conditions,[1,7] but to our knowledge there exists only a single report on direct measurement of N_2 absorption at pressure.[9] N_2 absorption has instead been investigated indirectly by assessing the elimination, after diving for example. In addition, only a few reports exist regarding actual measurements of direct elimination of nitrogen during hypobaric conditions.[5]

We developed a method, using a similar approach as Dick et al.,[4] where N_2 volume in a closed rebreathing system is

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derived from the total system volume subtracted by calculated volumes of oxygen and water vapor. This method can easily be used at different ambient pressures. In addition to N_2 elimination, absorption can also be investigated. The method is based on a modified commercial rebreather system (Poseidon Se7en, Poseidon Diving Systems AB, Västra Frölunda, Sweden). This paper describes the system and the calculations used for quantifying N_2 turnover in human subjects. The evaluation trials comprised of evaluating the stability of the measurements and showing that a given change in N_2 volume was measured accurately. Measurements of actual N_2 uptake and elimination curves in hyper- and hypobaric conditions, respectively, are also included.

METHODS

Subjects

Four subjects participated in the various sessions. Their age, height, and weight were 44.5 (33–59) yr, 178 (176–183) cm, and 78 (75–92) kg [median (range)]. The study was approved by the Regional Ethics Committee in Stockholm and all subjects gave informed consent to participation.

Equipment

The basic approach to measure uptake or elimination of gaseous nitrogen (N_2) was to determine changes of the total gas volume in a closed rebreathing system (Poseidon Se7en, Poseidon Diving Systems AB) and then subtract calculated volumes of oxygen and water vapor. Poseidon Se7en is an electronic rebreathing system, developed for diving, that controls the level of oxygen by keeping the partial pressure of oxygen (Po_2) constant at preset levels, with two internal oxygen sensors providing the inputs that guide the dosing of oxygen via a computerized algorithm. CO_2 is removed from the rebreathing gas by a soda lime scrubber (SofnoDive 797, Molecular Products Inc., Boulder, CO). The system includes a pressure sensor (for dive depth) and injects diluent gas (compressed air) when the system volume decreases, e.g., during compression while diving. The injection of diluent gas was switched off during the measurements in the current study since the variation of N_2 volume in the system should solely depend on the nitrogen uptake or wash-out by the man-in-the-loop.

There were three modifications done on the rebreathing system (see **Fig. 1**): 1) replacement of the original two counterlungs with bellows that measured system volume via potentiometers; 2) the incorporation of four instrument pods at different locations in the rebreathing loop for the measurement of Po_2, temperature, and humidity; and 3) the addition of two injection ports. One injection port, located at Bellows 1, was used for injection of N_2 and the other, located in the scrubber container (before the scrubber), allowed provision of O_2 in a bleed-in fashion (Fig. 1).

Before each session, the internal oxygen sensors were automatically calibrated by the rebreathing device, whereas the oxygen sensors in the pods were calibrated against the internal sensors. The measured Po_2 (six sensors), humidity (four sensors), temperature (four sensors), ambient pressure, pressure in the gas bottles, and volume (from the bellows) were collected continuously, with a sampling rate of 10 Hz. The data was analyzed offline using a dedicated program developed in LabView (National Instruments Corporation, Austin, TX).

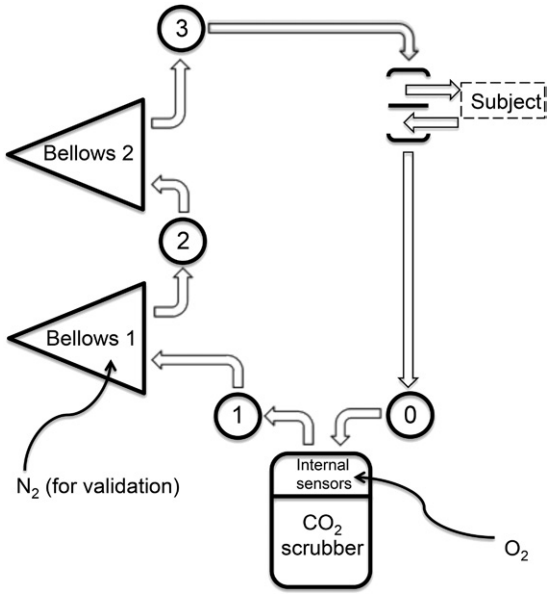

Fig. 1. A schematic description of the rebreathing system. Variations in the system volume (V_{sys}) is measured with potentiometers in the two bellows, and Po_2, temperature, and humidity are measured continuously (10 Hz) in each of the four measurement pods (0–3). Internal sensors in the rebreathing system comprise two Po_2 sensors and a pressure sensor.

System volume. The volume of the rebreathing loop with bellows emptied (minimal system volume) was calculated by measuring the geometry of different parts of the system, also taking into account the packing factor of the soda lime in the scrubber (0.5). The total volume was calculated to 6.9 L, which was confirmed by an assessment using N_2 dilution (7.0 L). The potentiometers of the bellows were calibrated by injection of a preset volume of air; this was done for each bellows separately.

Temperature. The temperature of the rebreathing loop increases over time due to the heat from exhaled air and the exothermic reaction in the soda lime scrubber. This has a significant effect on the system volume at a given pressure. Furthermore, the temperature varied in the different parts of the rebreathing circuit. Therefore, a compartmentalized model was developed in order to calculate a "representative" temperature of the system for the calculation of volume changes. Specifically, the rebreathing loop was divided into three compartments: 1) the exhalation side (exhal) (from mouthpiece to scrubber container); 2) the scrubber container; and 3) the inhalation side (inhal) (from scrubber container to the mouthpiece, including the two bellows). Based on the volume calculations of the system a weighted contribution to a representative temperature (T_{sys}) were defined as:

$$T_{sys} = 0.17*\text{scrubber temp} + 0.69*\text{inhal temp} + 0.14*\text{exhal temp} \quad \text{Eq. 1}$$

The temperature increase in a fresh soda lime scrubber was determined in four subjects breathing for 110 min by placing 10 thermistors in the soda lime and 2 on the outside, in the container. The temperature increase was very similar between trials and a function for the typical average temperature profile for a fresh soda lime canister was determined as:

$$\text{Temperature of scrubber container (°C)} = -0.00125x^2 + 0.296x + 20.767 \quad \text{Eq. 2}$$

where x is minutes of resting breathing in the rebreathing loop.

The temperature in the exhalation and inhalation parts was measured continuously in the pods (Fig. 1) during the experimental runs. The temperature in the inhalation side was determined as the average of the temperatures in pod 1 and pod 3. Temperature in the exhalation side was determined as the average of 35°C (temperature of exhaled air) and pod 0.

The system volume at body temperature at atmospheric pressure (BTPD) was calculated as:

$$V_{BTP} = \left(V_{sys} * P_{sys} * \left(273.15 + 37\right)\right) \Big/ \left[\left(T_{sys} + 273.15\right) * 1.013\,\text{bar}\right] \quad \text{Eq. 3}$$

where V_{sys} is the minimal system volume and the volume measured by the potentiometers, P_{sys}, is the measured pressure (bar), and T_{sys} is the representative temperature of the system, as described above. A filter was applied to the calculated V_{BTP} curve, which removed variations induced by ventilation, but not variations of lower frequency.

Partial pressure of oxygen. Similar to temperature, the partial pressure of oxygen varies in different parts of the rebreathing loop. A two-compartment model was used to compute an oxygen level that would be representative for the whole system, which then was used when calculating the volume of oxygen. The first compartment was the inhalation side, including the bellows, and the second was the exhalation side, including the scrubber (Fig. 1). As with temperature the weighted contribution based on parts of the system volume for a representative Po_2 were defined as:

$$Po_{2sys} = 0.3*\text{scrubber\&exhale } Po_2 + 0.7*\text{inhal } Po_2 \quad \text{Eq. 4}$$

The scrubber&exhale Po_2 was given by measurements in pod 0, and the inhal Po_2 was given by a weighted average of measurement in pod 1, 2, and 3, with a relative weight of 0.1, 0.45, and 0.45, respectively.

Water vapor pressure. This was calculated as:

$$P_{vap} = \left(\left(e\text{^}\left(18.956 - 4030.18 \Big/ \left(T_{sys} + 235\right)\right)\right) \Big/ 10\right) * \left(\text{Humidity}\,(\%) * 0.01\right) \quad \text{Eq. 5}$$

where P_{vap} is the water vapor pressure and humidity measured at pod 3. The humidity was usually close to 100%.

N_2 volume in the rebreathing system. This was calculated as:

$$V_{N2} = \left(1 - \left(P_{vap} + \left(PO_{2sys} * 100\right)\right) \Big/ \left(P_{sys} * 100\right)\right) * V_{BTP} \quad \text{Eq. 6}$$

The calculations above were done in sequence in a sample-by-sample manner, which then yields a continuous signal of N_2 volume in the system (**Fig. 2**). In conditions with wash-in or wash-out of N_2, a simple monoexponential function ($y = a + b * (1 - \exp(-x / c))$) was fitted to the N_2 curve for the assessment of the rate and magnitude of the uptake or elimination.

Procedure

The system was assessed in three different ways: 1) evaluation of the stability of the computed N_2 volume by collecting data during N_2 steady state; 2) measurement of an increase in N_2 volume by injection of a given N_2 volume during steady state; and 3) demonstration of measurements of actual N_2 uptake or wash-out induced by compression, decompression, or an elevated oxygen concentration (at normal ambient pressure). All measurements were performed in the sitting position, with an ambient temperature of 22−23°C. At the start of a session the subject exhaled to functional residual capacity and then inhaled 3 L of air from a bag before exhaling into the rebreathing circuit (that were at minimum volume, i.e., closed bellows). Oxygen could be provided in two different ways, either by using the automatic oxygen dosing of the rebreathing apparatus or by bleeding in oxygen via a manually controlled flow meter.

Four subjects performed rebreathing sessions of 60 min during steady state. This was done twice, giving eight recordings in total. The potential drift in the computed N_2 volume was assessed in two ways: by defining the slope of a linear regression line to the calculated N_2 curve, and by taking the average volume over the first 50% of the recording and comparing with the last 50% (Fig. 2).

In a separate session N_2 was added to the system by bleeding in gas using a manually controlled flow meter, with a rate of 70 mL · min^{-1} for 2 min followed by 1 min of no flow; this was repeated seven times for a total time and volume of 20 min and 980 mL, respectively (**Fig. 3**). Three subjects participated in these sessions. Finally, one subject participated in three demonstration runs with actual N_2 uptake and washout. Each session was around 45 min. Wash-out was demonstrated by reducing the pressure in a hypobaric chamber to 0.5 atm with Po_2 in the circuit maintained at 21 kPa and by breathing 63% oxygen at normal atmospheric pressure. Uptake was demonstrated by increasing the ambient pressure to 1.8 atm in a hyperbaric chamber, with Po_2 maintained at 42 kPa in the breathing circuit (**Fig. 4**).

Statistical Analysis

Data is presented as mean ± SD.

RESULTS

The four subjects completed two steady state rebreathing runs each. The average slope of the computed N_2 volume was

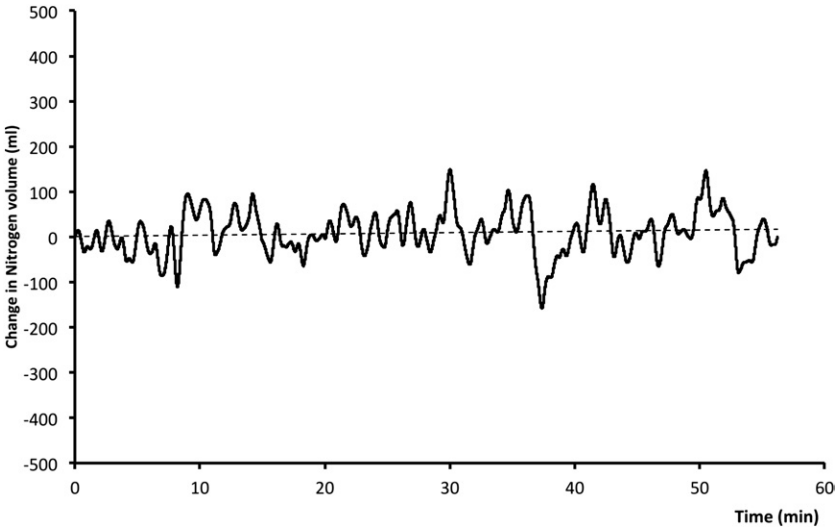

Fig. 2. One continuous N_2 (BTPD) volume measurement during steady state, e.g., no N_2 uptake or elimination. Dashed line denotes a linear regression line, which was used to assess drift.

$6.25 \times 10^{-3} \pm 2.97 \times 10^{-2}$ mL · s^{-1}, which corresponds to a drift over 1 h of 22 ± 107 mL. When comparing the mean volume of the first 50% with the last 50% during these steady state recordings, the difference was 4 ± 64 mL. Thus, despite continuous variations of ± 100 mL as seen in Fig. 2, there was no apparent overall drift.

Three subjects performed rebreathing sessions during which 980 mL of N_2 (equivalent to 1019 mL at BTPD) were injected over 20 min. There was a distinct increase in N_2 volume over the injection periods that were similar for all three subjects (Fig. 3). The calculated increase in N_2 volume (BTPD) was 1006 ± 32 mL.

The measurements of actual N_2 turnover were demonstrated, as indicated in Fig. 4, with N_2 wash-out during decompression and hyperoxia, respectively, and N_2 uptake during compression. The N_2 wash-out during hyperoxia and decompression had fairly similar time courses and magnitudes. During compression a somewhat larger N_2 volume was absorbed compared to the N_2 wash-out with decompression and hyperoxia. In addition, there were larger fluctuations in BTPD at pressure

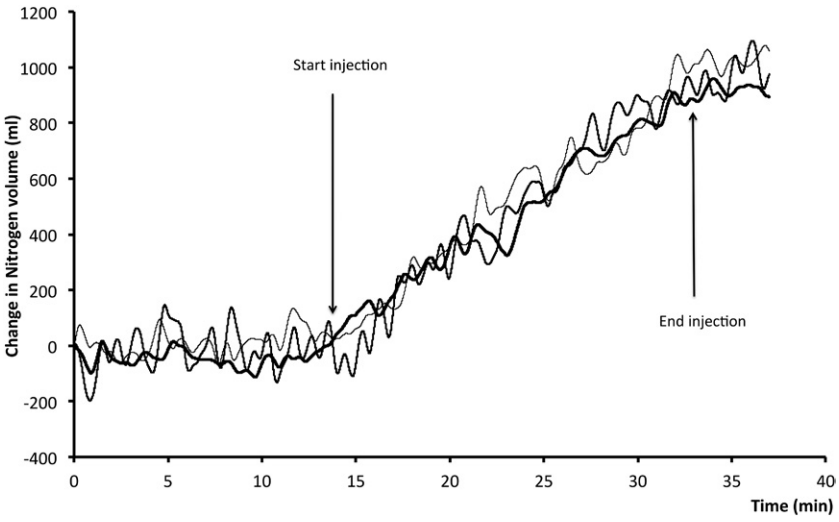

Fig. 3. N_2 (BTPD) volume curves from three subjects. Over 20 min, 980 mL N_2 was injected. The arrows indicate start and end of the injection period.

DISCUSSION

Nitrogen elimination has been evaluated before both in normo- and hyperbaric conditions,[2,6,10] but a search of the literature has only revealed one technical report where direct measurements of N_2 uptake were performed during a sojourn at elevated pressure.[9] Only a few direct measurements of N_2 elimination in hypobaric conditions are known to the authors.[5]

The present method resembles the approach of Dick et al.,[4] where a spirometer was used as a counter-lung and the elimination of N_2 was calculated from the increase in total system volume. As pointed out by these authors there are two caveats to take into account using this approach: 1) drift over time, which is not coupled to N_2 turnover, and 2) the fact that the lungs are a part of the system volume. Regarding drift, we could demonstrate that the system volume remained stable over 1 h with minimal drift. The fluctuations in calculated N_2 volume of the magnitude of ± 100 mL (Fig. 2) are mainly due to variations in the volume of air retained in the lungs during breathing, e.g., due to physiological variations. These variations appear random and can be largely eliminated by averaging the N_2 volume over 1,[4] 5,[6] or 10^{10} minutes. However, since averaging also eliminates information, our approach was to maintain the continuous calculation of N_2 volume and use a computer-aided curve fitting directly on the raw curve when defining the rate and magnitude of N_2 turnover (Fig. 4).

In order to evaluate the method, 980 mL of N_2 ambient temperature at atmospheric pressure dry gas corresponding to 1019 mL N_2 at BTPD was injected over 20 min into the system during steady state breathing. This was done with three different subjects and, as can be seen in Fig. 3, the changes in calculated N_2 volume were very similar with an average increase of 1006 mL at the end of the injection period. Thus, by keeping Po_2 constant and compensating for the added water vapor, 99 ± 3% of the injected N_2 volume was captured.

Assessment of actual uptake and elimination of N_2 was demonstrated in one subject using conditions that aimed at providing similar differences in PN_2 between inhaled gas and the tissue PN_2 during compression and decompression. N_2 wash-out with decompression and hyperoxia were similar and the uptake of N_2 after compression showed a reciprocal response. The total uptake was somewhat larger than the washout. The reason for this is probably two-fold: a shorter period before the measurements started at increased pressure, and the fact that,

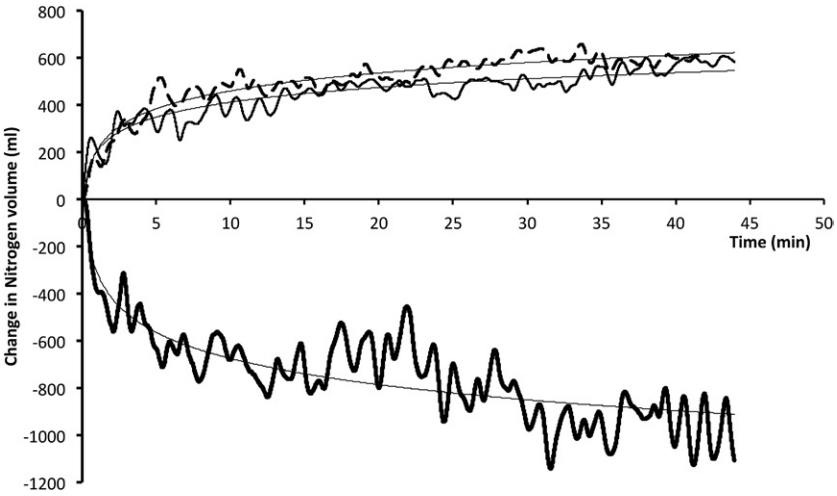

Fig. 4. N_2 (BTPD) elimination during breathing of 63% oxygen at normal pressure (1028 mbar) and during sojourn at 0.5 atm with a Po_2 of 21 kPa, respectively. The upper curve denotes the elimination during hyperoxic breathing, which was slightly larger than the elimination during the hypobaric condition. Also denoted is N_2 (BTPD) absorption during 1.8 atm with inspired Po_2 of 42 kPa. These measurements were done with the same subject.

although the Po_2 was set to 50 kPa during the hyperbaric exposure, the average inhaled Po_2 was only 42 kPa, causing a slightly larger nitrogen pressure gradient in the hyperbaric condition. This indicates that the algorithm for the automatic oxygen dosage system can be improved to better fit the conditions of these measurements, or a manual dosage system can be used.

The fluctuations in N_2 volume (BTPD) at 1.8 atm (Fig. 4) had almost twice the amplitude compared to the variations noted in the steady-state condition (Fig. 2). The reason is that, as discussed above, these variations are largely physiological and will increase in magnitude when occurring at increased pressure and subsequently being transformed to BTPD. However, this amplification effect will not affect the time course or magnitude of the calculated N_2 uptake.

The evaluations in the current study were done during resting conditions, but there is no obvious reason to assume that it should not also work in situations with increased oxygen uptake and ventilation, although new measurements of the carbon dioxide scrubber temperature must be carried out at the elevated metabolic rate. In principle, it would also be possible to calculate N_2 turnover during actual decompression and compression. In these settings gas has to be added or withdrawn from the system given the limitations of system volumes that can be handled by the rebreathing system. However, if the added or withdrawn volumes are measured accurately, also taking into account the prevailing pressure, the N_2 turnover could still be captured using the current calculations.

Limitations

The calculations assume a stable end-tidal CO_2 and, indeed, CO_2 was not measured. However, hypo- or hypercapnia would only affect a part of the system volume (lungs and the exhalation side) and a change in Pco_2 of one %-unit would imply an error of roughly 30 mL when calculating the N_2 volume. However, by adding measurements of CO_2, this uncertainty could be reduced.

The levels of humidity used in the calculations were measured at pod 3 in the inhalation side of the rebreathing loop (Fig. 1). The humidity was high and stable overall, and it is unlikely that it would vary in other parts of the loop in a fashion that would have any significant effects on the calculations.

The duration of the measurements in the current evaluation were between 45–60 min. With respect to the stability of the system, it seems unlikely that longer sessions would provide drifts that would be significantly different from those demonstrated here. The calculations of actual N_2 turnover (Fig. 4) reflect relatively fast tissues[5] and, if slower tissues are of interest, the sessions have to be longer. To properly measure wash-out or absorption of tissues with a certain half-time ($t_{1/2}$), the measurement period should be 6 * $t_{1/2}$. However, it could be argued that, with proper curve fitting (Fig. 4), the time for measuring the rate and magnitude of N_2 turnover could be shorter.

The presented method only measures N_2 turnover via the lungs, hence N_2 exchange over the skin[8] is not taken into account. In the current activities both manual and automatic oxygen injection were used. No notable difference was seen between these two modes when assessing the changes in N_2 volume.

In conclusion, a new system for assessing N_2 turnover has been presented. It can be operated at hyper- and hypobaric pressures and the rate and magnitude of both N_2 uptake and wash-out can be calculated. Its ease of use makes it attractive when investigating a multitude of questions related to the risks of decompression, either in conjunction with diving or aerospace activities. However, one weakness in the present proof-of-concept study is the low number of tested subjects; thus, additional research is needed to support the method for assessing N_2 turnover in humans.

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