A novel, simplified ex vivo method for measuring water exchange performance of Heat and Moisture Exchangers for tracheostomy application

Cindy van den Boer MD¹, Sara H. Muller PhD², Andrew D. Vincent PhD³, Klaus Züchner PhD⁴, Michiel W.M. van den Brekel MD PhD¹⁵⁶, Frans J.M. Hilgers MD PhD¹⁵⁶

¹ Department of Head and Neck Oncology and Surgery, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands
² Department of Clinical Physics and Instrumentation, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands
³ Department of Biometrics, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands
⁴ Medical Technical Service, University Medical Center, Georg-August University, Göttingen, Germany
⁵ Institute of Phonetic Sciences (ACLC), University of Amsterdam, The Netherlands
⁶ Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Corresponding author: Frans JM Hilgers, f.hilgers@nki.nl; telephone +3120512550; fax +31205122554

Conflict of interest: this research project was funded by an unrestricted research grant of the manufacturer of the tested HMEs, Atos Medical, Sweden. The study sponsor had no involvement in the study design, in the collection, analysis, and interpretation of data, in the writing of the manuscript, or in the decision to submit the manuscript for publication. The authors have no other conflict of interest. The local medical ethical review board approved the study.

The first author presented this study at a poster session at the 8th International Conference on Head and Neck Cancer of the American Head and Neck Society in Toronto on July 21-25, 2012
Abstract

Background: Breathing through a tracheostomy results in insufficient warming and humidification of inspired air. This loss of air-conditioning can be partially compensated for with the application of a Heat and Moisture Exchanger (HME) over the tracheostomy. In vitro (ISO 9360-2:2001) and in vivo measurements of the effects of an HME are complex and technically challenging. Aim of this study is to develop a simple method to measure the ex vivo HME performance comparable with previous in vitro and in vivo results.

Materials and methods: HMEs are weighed at the end of inspiration and at the end of expiration at different breathing volumes. Four HMEs (Atos Medical, Hörby, Sweden) with known in vivo humidity and in vitro water loss values were tested. The associations between weight change, volume and absolute humidity were determined using both linear and non-linear mixed effects models.

Results: The rating between the four HMEs by weighing correlates with previous intra-tracheal measurements ($R^2 = 0.98$), and the ISO-standards ($R^2 = 0.77$).

Conclusion: Assessment of the weight change between end of inhalation and end of exhalation is a valid and simple method of measuring the water exchange performance of an HME.

Keywords: Heat and Moisture Exchanger, humidity, respiration, tracheostomy, total laryngectomy

Introduction

Total laryngectomy is the treatment of choice for advanced (T4) laryngeal cancer and for recurrent disease after prior organ preservation treatment. This surgical procedure results in breathing through a mandatory permanent tracheostoma that profoundly alters respiratory physiology. The bypass of the upper airways leads to insufficient warming and humidification of the inspired air. Consequently, patients develop pulmonary problems such as mucus hyper secretion and frequent involuntary coughing, and become more susceptible to respiratory...
infections. Also breathing through a temporary tracheostomy results in insufficient warming and humidification of inspired air. Compensation for the loss of air-conditioning can be achieved by application of a Heat and Moisture Exchanger (HME) over the tracheostoma. HMEs are passive humidifiers: during exhalation, water is retained onto the core material of the HME and during inhalation this water is released again into inspired air. The core material of HMEs consists of foam, paper, or other substances, usually impregnated with hygroscopic salt to increase the water retaining capacity. Simultaneously to the exchange of water, HMEs store and release heat during the breathing cycle. Use of an HME after laryngectomy has proven to significantly reduce pulmonary complaints and to improve quality of life. Therefore, these medical devices are currently considered as mandatory therapeutic and rehabilitative measures for laryngectomized patients.

Presently, a wide range of HMEs is commercially available, and for medical professionals it is not easy to judge the differences in performance of the various HMEs. Some manufacturers provide water loss values for their HMEs according to ISO 9360-2:2001, but this specification is not universally available. Moreover, water loss values are not easily and intuitively translated into clinical benefits as higher water loss correlates with a less effective HME performance. An additional issue is that the 24-hour ISO measurements require complex and validated machinery, and slight differences in set up specifications (e.g. calibration) of these measurements can lead to considerable outcome variations between laboratories.

Alternatively, clinical decision-making could be based on in vivo measurements of the HME effect on tracheal climate. Since direct intra-tracheal humidity measurements are technically complicated, most studies have opted to sample air from the trachea and to analyze this sample with an external fast humidity sensor. In such measurements extreme care is required to avoid loss of water vapor due to condensation in the sampling tube. No commercial equipment is available for these challenging measurements and experience has shown that custom-built equipment is difficult to develop and maintain. Moreover, only a minority of available HMEs thus far has been analyzed both in vitro and in vivo. Therefore, an easy method for assessing HME performance using commercially available equipment could be very useful for medical professionals. Also for HME development, a universally available tool is required for comparison of newly designed with existing HMEs next to clinical feasibility studies.

Since an HME stores and releases water with each in- and exhalation, the HME gains and loses weight during breathing. The amount of water, and therefore the weight change depends on
the breathing volume. We postulate that this change of weight could be an alternative measure for HME performance. For weight measurements a suitable balance could be used and for volume assessment a commercial spirometer should be sufficient. The purpose of this study was to establish whether the weight change of an HME during between end of inhalation and end of exhalation could be a measure for HME performance comparable to in vitro ISO measurements and previous in vivo measurements.

Methods

HME Devices
Two commercially available HME devices and two prototype devices (Atos Medical Hörby, Sweden) were tested in this study, identical to the HME devices used in the recent in vivo study of Scheenstra et al.\textsuperscript{6} HME specifications of water loss values according to the ISO standards and the in vivo absolute humidity values according to Scheenstra et al. are shown in table 1. The two commercially available HMEs are the R-HME (short for Provox Normal) and the L-HME (short for Provox Hi flow). The prototype versions are the Rplus-HME and its lower resistance version the Lplus-HME. These prototypes have a different closure mechanism and more foam material in order to improve performance (see figure 1)

Humidity and breathing volume measurement
Measurements were performed with the test configuration shown in figure 2. A healthy volunteer (female, 29 years of age, first author CvdB) breathed through a spirometer (Flowhead MLT300 ADInstruments GmbH Oxfordshire, UK). The other side of the spirometer was connected to a T-shaped tube. Inside this T-tube, humidity was measured with a fast heated capacitive hygrometer that measures the absolute humidity (AH sensor) with a response time of 0.1 - 0.2 s.\textsuperscript{16} The fast humidity AH sensor is calibrated as described previously using a reference humidity sensor (Testo BV, Almere, the Netherlands). Inaccuracy of the absolute humidity after this calibration is less than 5%.\textsuperscript{5, 13} At the far end of the T-tube an HME was connected. The ‘dead-space’ of the T-tube is 30 ml and the dead space volume of the spirometer flow-head is 70 ml according to the manufacturer’s manual.
The spirometer was calibrated before each use and the resulting data were corrected for baseline and volume drift according to the recommendations of the manufacturer. Volume drift is mainly caused by the increased volume of the warmer and moister exhaled air, therefore drift correction was only applied to the exhaled volumes. Integrating the flow over the duration of the breath yields the observed breathing volume. Spirometer data were recorded and analyzed with Powerlab software (ADInstruments GmbH Oxfordshire, UK).

Humidity values were registered and saved with Acquis 2.8 software and were exported to Microsoft Excel. Environmental humidity and temperature were monitored with a commercial, calibrated humidity sensor (Testo BV, Almere, The Netherlands). Body temperature of the volunteer was monitored with an electronic aural thermometer (Genius2, Kendall, Tyco Healthcare Mansfield, U.S.A).

**Weight measurement**

Weight was measured with a Micro Balance (Sartorius MC210P, Göttingen, Germany). Stability of the balance and of the measurement procedure was monitored by repeated measurements of a calibration weight. Accuracy and repeatability were within 0.1 mg.

During weight measurements, HMEs were stored in an airtight cylindrical box (diameter: 35 mm, height: 19 mm, weight 4 grams) immediately after in- or exhalation to prevent evaporation into environmental air. The time required to remove the HME from the T-tube and to store in the box was 3±1 seconds. Control measurements of HMEs without enclosure in a box showed that weight loss due to evaporation occurred primarily in the first seconds and was less than 5% of the weight change of an HME in the first 3 seconds. Error in the weight measurements due to the variation in storage time was less than 2% of the weight change. During reconditioning of the HME (see below) in between weight measurements, the boxes were left open to prevent built up of condensation inside the box.

Preferably measurements should be performed under clean room conditions to prevent contamination with e.g. dust particles. Because this was not possible in the laboratory where the balance was positioned, we used optimal hygienic measures instead. Aluminium foil was used to keep the surface around the balance as clean as possible, and the empty boxes were
weighed before and after each experiment to ensure that no contamination had occurred. If contamination had occurred the measurement sequence was excluded from further analysis.

**Preparation of the HMEs**

The foam in the HMEs is impregnated with a hygroscopic salt (CaCl$_2$). Absorption of the amount of water by the CaCl$_2$ depends primarily on environmental relative humidity (RH). Therefore, HMEs were kept overnight at a constant RH of 40-45 % and room temperature of 20-22°C. To prevent excessive drifting during the measurements, HMEs were conditioned towards equilibrium water saturation at the start of the measurement sequence by continuous breathing in rest by the volunteer (frequency 14.5 +/-0.5 breaths per minute). For each HME the length of the conditioning period required for equilibrium water saturation (stable weight) was determined by verifying the weight increase over time (10-20 minutes).

**Measurement protocol**

After conditioning of an HME, a measurement sequence of 25 weight observations was performed, alternatingly at the end of inhalation and at the end of exhalation. In order to obtain observations at a range of volumes the volunteer breathed with different breathing patterns: 13 observations of breathing in rest (tidal volume), 6 of deep breathing, and 6 of shallow breathing. Before each successive weight observation, a short period of tidal breathing (at least five breathing cycles) was used to recondition the HME, immediately followed by an in- or exhalation of the prescribed breathing patterns. One breath (observation) is defined as one inhalation or one exhalation. The breathing cycle is the sum of one inhalation and one exhalation. Absolute humidity was registered at the end of inhalation just before removal of the HME (the end-inspiratory absolute humidity: AHinsp). Total duration of one measurement sequence was between 40 to 50 minutes.

For all four HMEs this measurement sequence was performed by the same volunteer. To assess intra-individual variation, five additional HMEs of the same type (R-HME) were measured 11 times (weight, volume and absolute humidity) during rest breathing. To assess inter-individual variation, one measurement sequence of 25 observations with this HME type (R-HME) was performed by 5 additional healthy volunteers (mean age 36 years, three males and two females). To establish the baseline HME capacity of the test set configuration (spirometer and T-
shaped tube), fourteen additional humidity observations were performed without HME by the volunteer. The study was approved by the Protocol Review Board of the Institute, and informed consent was obtained from all volunteers.

Data normalization

The measured end-inspiratory absolute humidity ($AH_{inspM}$) depends on the environmental absolute humidity ($A_{He}$). $AH_{inspM}$ cannot be lower than $A_{He}$ and if $A_{He}$ is equal to the alveolar absolute humidity ($A_{Ha}$), the $AH_{inspM}$ will be equal to $A_{Ha}$.

In order to compare measurements on different days with varying $A_{He}$ (measured in this study), the measured $AH_{inspM}$ was converted into a normalized $AH_{insp}$ at $A_{HeR}$ (the reference environmental humidity chosen to be 5 mg/L), using the following formula (comparable to figure 2 in Wilkes 2004):

$$AH_{insp \ at \ A_{HeR}} = A_{HeR} + \ (AH_{inspM} - A_{He}) * (A_{Ha} - A_{HeR}) / (A_{Ha} - A_{He}) \quad \text{(formula 1)}$$

The formula is a simplification, which does only normalize for the actual measured $A_{He}$ variation and does not take any other dependencies of $AH_{insp}$ such as dead space into account.

For $A_{Ha}$ the saturation humidity at body temperature of the volunteer was used (using the formula given in Zuur et al. or http://www.humidity-calculator.com/index.php).

Similarly, weight change ($\Delta W$) will also depend on environmental humidity. The measured $\Delta W$ ($\Delta WM$) was normalized to a normalized weight change $\Delta W$ at $A_{HeR}$ using the formula:

$$\Delta W \ at \ A_{HeR} = \Delta WM * (A_{Ha} - A_{HeR}) / (A_{Ha} - A_{He}) \quad \text{(formula 2)}$$

Using the body temperature to calculate $A_{Ha}$ is only justified when $A_{Ha}$ is much larger than both $A_{HeR}$ and $A_{He}$. Normalization for body temperature of the volunteers could have been done in a similar way, but was not performed as body temperature of the volunteer was constant within 0.3 °C (the corresponding error in the weight change is less than 2%).

Statistical Methods
Associations

The association between breath volume and both HME weight and absolute humidity were modeled using mixed effects regression. Given the different measurement protocols, only the change in HME weight was available, so two different models were constructed. Scatter plots suggested a linear relation between weight change and log-transformed breath volume. Consequently the weight change mixed-effects model included log-transformed volume, HME-type and their interaction as predictor variables. Random intercepts were included for each measurement period (see appendix 2, equation 1). For absolute humidity the scatter plots suggested an exponential decay with non-zero asymptote. Consequently, the association between end-inspiratory absolute humidity and inspiratory breathing volume was determined using an exponential-decay nonlinear least-squares regression. The end-inspiratory absolute humidity values were the outcome with initial absolute humidity levels set at the end-expiratory humidity values from the previous breath. Exponential decay asymptote, initial intercept and the decay rate were allowed to vary with HME-type (see appendix 2, equation 2).

Repeatability

The intra-individual variation of the R-HME during tidal-breathing was measured for one volunteer (CvdB) at 6 distinct assessment periods. Systematic differences across assessment periods were tested for using Kruskal-Wallis tests for three parameters: end-inspiratory absolute humidity, end-expiratory absolute humidity, and the ratio of weight change and breathing volume.

Weight and corresponding volume measurements were taken from 6 volunteers to assess the inter-individual variation of the R-HME. In the weight-change vs. (log-transformed) breath volume linear mixed effects model, random intercepts and slopes were included per individual. The inter- and intra-individual variations were assessed in weight-change model at breathing volumes equal to 0.5L and 1.0L.

Validation

The estimated end-inspiratory AH at inspiratory breathing volume 0.5L of this study for the four HME types were compared with previously published in vivo results. The correlation coefficient
was determined from a weighted linear regression using inverse variances. Similarly the estimated weight changes at breathing volume 1L were compared with ISO water loss at 1L.

Results

Figure 3 shows in detail how the measurement sequence was performed. The figure shows (parts of) the raw data trace of the AH sensor (upper trace) and the spirometer flow sensor (lower trace) for the Lplus-HME. Section A shows the first breaths of the conditioning period of the HME. The end-inspiratory absolute humidity values show a slow increase at the start due to the increasing moistening of the inspired air by the HME (see * in figure 3). Section B, C and D show examples of observations at different breathing volumes (tidal, deep, and shallow breathing, respectively), an in- or exhalation are marked with "in"/"ex" respectively. In the raw data trace in figure 3 the difference in AHinsp for the different breathing levels can be seen: AHinsp at the end of deep inhalation #18 is lower than AHinsp of the tidal breaths which in turn are lower than AHinsp of shallow inhalation #22 (see the arrows in the figure).

Insert Figure 3 about here

Figure 4a and 4b show respectively volume and weight for the measurement sequence (25 observations) of the Lplus-HME, as an example. The observations numbers on the X-axis correspond with the observation numbers of figure 3 above the raw AH trace (#1, #2, #3 etc.). From the observed weights, 21 weight changes of the HME are calculated between the maximum and minimum weights (respectively end of exhalation and end of inhalation) and are shown by the line segments in figure 4b. The average volume was calculated for corresponding consecutive weight observations. Weight changes between in and exhalations belonging to different breathing types were discarded, as well as the first weight observation of each measurement sequence, which were systematically higher due to the different conditioning history (see first data point in figure 4b).

Insert Figure 4 about here
Weight change as a function of average breathing volume is shown in figure 5 for all four HME types. All measurements were normalized to an AHeR of 5 mg/L (range measured AHe from 7.22 to 9.62 mg/L). For each HME the estimated model fit is shown.

The model fit of the AHinsp as a function of inspiratory breathing volume is shown in figure 6 for 21 HME measurements per type and for 14 without HME measurements. These measurements were also normalized to an AHeR of 5 mg/L.

In figure 5 the weight changes at an average breathing volume of 0.5 liter and 1.0 liter, and in figure 6 AHinsp values at 0.5 liter are marked per HME (vertical dashed line). These values are summarized in table 2, together with the differences relative to the R-HME. Values at 0.5L are required for comparison with the in vivo measurements in patients, for whom previously an average breathing volume of 0.5L was reported.\textsuperscript{20} For comparison with the ISO standards weight changes at a volume of 1.0L are required.

Repetitions of the R-HME at tidal volume breathing showed no systematic differences across measurement periods for AH values (Inspiration p = 0.18; Expiration p=0.12), or the weight change volume ratio (p = 0.58). The inter-individual variation for the 6 volunteers at a breathing volume of 0.5 liter and 1.0 liter was estimated as 0.45 mg/L and 0.78 mg/L, respectively, and the intra-individual variation was 0.18 mg/L for both breathing volumes. Consequently the differences observed between R-Plus HME and other HME types for a single observer (see Table 2) exceed the estimated 95% confidence interval upper limit of 1.34 at 0.5L and 2.22 at 1.0L, when inter-individual variation is included.

Figure 7a shows a comparison of the in vivo AHinsp measurements in patients observed by Scheenstra et al.\textsuperscript{6} and the values obtained in this study at a volume of 0.5L (table 2) at AHeR of 5 mg/L. Comparison of the HME weight change in this study and the ISO standards is shown in figure 7b. The weight changes at a volume of 1.0L from table 2 have been normalized to the standard ISO humidity conditions of 0 mg/L using formula 2.
Discussion

This study shows that HME performance can be determined by measuring the weight difference between end-inspiration and end-expiration using a regular balance and a standard spirometer. Results correlate well with earlier in vivo measurements using complex custom-built equipment to measure intra-tracheal humidity, and with in vitro values provided by the manufacturer based on 24-hour ISO 9360-2:2001 assessments.

As can be seen in figure 7a, the AHinsp as measured in one volunteer correlates well with the in vivo intra-tracheal measurements of AHinsp in a group of laryngectomized patients ($R^2=0.98$). It should be noted that the in vivo curve is shifted upward by about 14 mg/L. This shift represents the HME effect of the upper part of the trachea, since the measurements in the laryngectomized patients were performed one cm inside the trachea. In contrast, the additional HME effect from the short distal tube in the test configuration in the present study is only minor, as can be seen in figure 6, where the AHinsp ‘without HME’ almost immediately and completely reaches the environmental humidity value.

Both weight changes and AHinsp values result in the same ranking levels for the four tested HMEs (table 2), with the Rplus-HME being significantly better than the R-HME. This result matches those reported by Scheenstra et al (see figure 8).

Comparing figure 5 and 6 (weight change and AHinsp, as function of breathing volume, respectively), it is interesting that the curves in both figures show a very different shape. The AHinsp (figure 6) decays exponentially similar to previously described AHinsp in vivo measurements.\textsuperscript{6,13,15} Weight change (figure 5) increases linearly for small volumes and then levels off. This difference can be understood qualitatively as follows. The difference in AHinsp with HME and the AHinsp without HME represents the amount of water that is evaporated from the HME. For small volumes the difference in AHinsp with HME and without HME is relatively large (figure 6) so that the HME weight changes quickly (see figure 5). For larger volumes the
constant, but small AHinsp difference causes a slower weight change with increasing volume. This qualitative description has been quantified in Appendix 1.

The correlation between weight change at 1.0L and water loss using the ISO standard technique (figure 7b, $R^2=0.77$) is smaller than the correlation between weight change at 0.5L and in vivo observations (figure 7a, $R^2 =0.98$). In particular the weight change at 1.0L of the L-HME is unexpectedly high compared to the ISO value. The measurements at high volumes may be less reliable due to the use of a standard flowhead suitable for tidal volume breathing in rest, but not intended for high air velocities. Also, it cannot be excluded that the velocity of the water exchange on the foam depends on airflow velocity, which could also be an explanation for the difference between the volunteers during forced breathing.

The repeatability of the measurements on different samples of the R-HME exhibited no statistical differences between these samples. Furthermore, the measurements with the volunteers showed that the inter-individual variation is smaller than the significant differences between the R-plus HME and the other HMEs. This is in agreement with Scheenstra et al\textsuperscript{15}, who have shown that the inter-individual variability in patients is small. Combining these results, we can conclude that the impact of an HME on absolute humidity and weight changes can be measured reliably with one volunteer. Only the weighing measurement error limits the power of this ex vivo method, as e.g. can be noted from the cluster of R-HME measurements at tidal breathing in figure 5. In table 2 all differences of the AHinsp values compared to the R-HME are significant, but for weight only the Rplus-HME is significantly different to the R-HME.

The advantage of this ex vivo weighing method is that it is simple to perform with a balance (accuracy 0.1 mg or better) and a spirometer to register volume (note that the humidity sensor in the T-tube only was needed to compare the test results with earlier in vivo measurements). Requirements for reliable measurements are preparing the HME to equilibrium humidity saturation, careful and clean handling of the HME and rapid measuring and storing the HME in a closed box to avoid evaporation. The environmental humidity should be low, since at high environmental humidity the HME will hardly collect water (see formulas 1 and 2). Body temperature should be monitored to exclude fever of the volunteer. Also environmental
absolute humidity measurements (or relative humidity and temperature) are required to normalize the results by applying formulas 1 and 2.

This ex vivo weighing of water exchange of HMEs is a reliable method to measure HME performance without the need for complex technology, and without the additional in vivo problems such as oversaturation of the fast humidity sensor by condensation or sputum. It enables the fast measurement of the performance of various commercially available HMEs for tracheostomy application and is directly useful for medical professionals to obtain information about the performance of different types and e.g. newly designed HMEs, in addition to clinical data obtained from feasibility studies. It also might be a useful supplementary tool for developers of HMEs. Moreover, it is conceivable that this method could be adapted for measurements of other types of HMEs, e.g. those used in the intensive care.

Finally, water retention in the HME (more is better) measured under human breathing circumstances is more intuitive, and might be better understandable for medical professionals than the present water-loss standard (higher value is less effective HME) measured with the ISO equipment.

In summary, this ex vivo weighing method is the "missing link" between the existing in vitro data using an artificial lung and the clinical in vivo data from patients.

**Conclusion**

This study presents a new and straightforward ex vivo method to measure HME performance by weighing the HME at the end of inspiration and the end of expiration, independent of complex technology. The results strongly correlate with previous in vivo measurements and are similar to in vitro data, using the ISO 9360-2:2001 standard equipment.
   Evidence-based review of treatment options for patients with glottic cancer.

   Physical and psychosocial consequences of total laryngectomy. Clin Otolaryngol

   effect of a resistive heat moisture exchanger (Trachinaze) on pulmonary function
   and blood gas tensions in patients who have undergone a laryngectomy: a
   randomized control trial of 50 patients studied over a 6-month period. Head

4. Hilgers FJ, Aaronson NK, Ackerstaff AH, Schouwenburg PF, van Zandwijk N. The
   influence of a heat and moisture exchanger (HME) on the respiratory symptoms

5. Scheenstra RJ, Muller SH, Vincent A, Ackerstaff AH, Jacobi I, Hilgers FJ. Short-
   term endotracheal climate changes and clinical effects of a heat and moisture
   exchanger with an integrated electrostatic virus and bacterial filter developed for

   heat and moisture exchanger for laryngectomized patients: endotracheal
   temperature and humidity. Respir Care 2011;56(5):604-611.


Figure legends

Figure 1. Heat and Moisture Exchangers: R-HME (right) and Rplus-HME (left). The closure mechanism of the Rplus-HME is different to the R-HME: where foam acts as the spring mechanism (left) instead of the plastic spring (right).

Figure 2. Test configuration. From left to right: spirometer connected to a T-tube containing an Absolute Humidity (AH) sensor with application of an HME. Ruler in centimeters.

Figure 3. Raw data trace of absolute humidity (mg/L) and Spirometer Flow (L/10s) of the Lplus-HME. 1 ex: exhalation, in: inhalation.

A: Start of the HME conditioning showing the increasing humidity (asterisk).
B: Tidal volume in rest observations, example of observation #1-4.
C: Deep breathing, example of observation #18 and #19.
D: Shallow breathing, example of observation #22 and #23.

For a detailed explanation see the text.

Figure 4. Observations for the Lplus-HME are given as an example of one complete test series. (a) Volume; (b) Weight. Blue squares: inhalations; red dots: exhalations. The line segments connecting the weight observations represent the weight changes used in the analysis (see text). Observation numbers (#) correspond to the numbers in figure 3.

Figure 5. Weight change as function of average breathing volume, normalized at AHeR = 5 mg/L.

For each HME type, 21 weight changes are shown, except for R-HME, wherefore also the

1 Note that the maximum values do not show an immediate conditioning effect, because the humidity sensor is located upstream from the HME. Only during inhalation the humidity sensor is downstream from the HME and able to register the instantaneous impact of the HME on the absolute humidity. Therefore only the end-inspiratory humidity data were analyzed.
repeatability measurement points (40) are depicted. A vertical dashed line indicates the weight changes at 0.5L and 1.0L average breathing volume per HME (see table 2). The parameters of the fit are given in Table 1 Appendix 2.

**Figure 6.** AHinsp as a function of inspiratory breathing volume. Normalized at AHeR = 5 mg/L. 21 AHinsp values corresponding to the weight changes shown in figure 5 and for 14 without HME measurements are used to calculate the fits of the model, due to the chosen scale on the horizontal axis, not all measured points are visible. A vertical dashed line indicates the AHinsp per HME at 0.5L inspiratory breathing volume (Table 2). The parameters of the fit are given in Table 2 Appendix 2.

**Figure 7a.** The AHinsp from this study and the AHinsp from the in vivo study of Scheenstra et al. (shown in table 1) show a correlation of \( R^2 = 0.98 \).
SD is presented as vertical and horizontal bars.\(^{10}\)

**Figure 7b.** The weight change from this study and water loss according to the ISO specifications (shown in table 1) show a correlation of \( R^2 = 0.77 \).
SD of the weight change is shown in as vertical bars, the SD of the ISO values are not known. The ISO values are transformed to negative values for optimal visualization of the comparison data together in this figure.

**Figure 8.** Measurement of the HME-effect: weight change (table 2) versus in vivo AHinsp change (AHinsp(HME) minus AHinsp(without HME) table 1).

\(^{2}\) The offset of the estimated model fits on the average breathing volume axis shows the impact of the dead space. During inhalation weight decrease immediately starts, but during exhalation HME weight begins to increase after the air in the dead space has passed the HME. Therefore, the offset on the average breathing volume axis is equal to half of the combined dead space of T-tube, spirometer and volunteer.
Table 1. HME specifications: in vitro ISO values (Atos Medical Hörby, Sweden) and in vivo end-inspiratory absolute humidity (AHinsp) values and HME effect defined as intra-tracheal increase of AHinsp compared to breathing without HME\(^1\).

<table>
<thead>
<tr>
<th>Variable:</th>
<th>Water loss (\text{mg H}_2\text{O/L}) Tidal volume of 1L AHeR of 0 mg/L H(_2)O (ISO 9360-2:2001)</th>
<th>AHinsp (\text{mg/L H}_2\text{O}) Tidal volume of 0.5L AHeR of 5 mg/L H(_2)O (Scheenstra et al.)</th>
<th>AHinsp(HME) – AHinsp (without HME) (\text{mg/L H}_2\text{O}) Tidal volume of 0.5L AHeR of 5 mg/L H(_2)O (Scheenstra et al.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without HME</td>
<td>-</td>
<td>17.7</td>
<td>-</td>
</tr>
<tr>
<td>L-HME</td>
<td>25.4</td>
<td>20.4</td>
<td>2.7</td>
</tr>
<tr>
<td>R-HME</td>
<td>23.7</td>
<td>21.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Lplus-HME</td>
<td>24.0</td>
<td>22.2</td>
<td>4.5</td>
</tr>
<tr>
<td>Rplus-HME</td>
<td>21.5</td>
<td>24.8</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Table 2. Overview of HME performance in AHinsp and weight change (normalized for AHeR = 5mg/L). In brackets, the Standard Error (SE) or P-value for comparison with the R-HME (grey high lighted).

<table>
<thead>
<tr>
<th>Variable:</th>
<th>AHinsp at 0.5L (mg/L)</th>
<th>Weight change at 0.5L (mg)</th>
<th>Weight change at 1.0L (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without HME</td>
<td>5.3 (0.1)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>L-HME</td>
<td>7.3 (0.11)</td>
<td>1.8 (0.15)</td>
<td>3.6 (0.19)</td>
</tr>
<tr>
<td>difference L-HME and R-HME</td>
<td>-1.2 (p&lt;0.0001)</td>
<td>-0.2 (p=0.36)</td>
<td>0.1 (p=0.21)</td>
</tr>
<tr>
<td>R-HME (reference)</td>
<td>8.5 (0.11)</td>
<td>2.0 (0.10)</td>
<td>3.6 (0.11)</td>
</tr>
<tr>
<td>Lplus HME</td>
<td>9.4 (0.19)</td>
<td>2.3 (0.15)</td>
<td>3.7 (0.17)</td>
</tr>
<tr>
<td>difference Lplus-HME and R-HME</td>
<td>0.9 (p=0.0003)</td>
<td>0.3 (p=0.09)</td>
<td>0.1 (p=0.19)</td>
</tr>
<tr>
<td>Rplus HME</td>
<td>12.9 (0.28)</td>
<td>4.3 (0.16)</td>
<td>7.5 (0.20)</td>
</tr>
<tr>
<td>difference Rplus-HME and R-HME</td>
<td>4.4 (p&lt;0.0001)</td>
<td>2.3 (p&lt;0.0001)</td>
<td>3.9 (p&lt;0.0001)</td>
</tr>
</tbody>
</table>

\(^1\) The L-HME was measured in a different in vivo dataset\(^2\) as the Rplus-HME and Lplus-HME\(^6\), therefore the AHinsp value of the L-HME is converted using the corresponding difference between the L-HME and R-HME (reference value) for both datasets. In vivo data are normalized to environmental Absolute Humidity (AHeR) of 5 mg/L H\(_2\)O using formula 1.
Figure 1

Figure 2
Figure 3

![Graph showing flow vs. absolute humidity over time](image-url)
Figure 4a and b

**Volume observation sequence Lplus-HME**

![Volume graph]

**Observation numbers (#)**

4a. Volume

**Weight observation sequence Lplus-HME**

![Weight graph]

**Observation numbers (#)**

4b. Weight
Figure 7a

Figure 7b

Figure 8.
Appendix 1

A novel, simplified ex vivo method for measuring water exchange performance of Heat and Moisture Exchangers for tracheostomy application. Respiratory Care. Cindy van den Boer, Sara H. Muller, Andrew D. Vincent, Klaus Züchner, Michiel W.M. van den Brekel, Frans J.M. Hilgers. Corresponding author: Frans JM Hilgers, Department of Head and Neck Oncology and Surgery, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands; f.hilgers@nki.nl

Comparison of the measured weight decrease with the theoretical weight decrease calculated from End-inspiratory Absolute Humidity.

From the measured end-inspiratory absolute humidity (AHinsp) a theoretical weight decrease can be calculated by integration in two different ways.

Method 1. Integration over volume (dimensional analysis: [mg/L] * [L] = [mg])
\[ dWth (V) = \int_0^V (AHinsp \text{ with HME (v)} - AHinsp \text{ without HME (v)}) * dv \]
\[ dWtn (V) = \text{theoretical weight increase at volume V.} \]

This method is illustrated in figure 1: the grey surface area between the curves represents the theoretical weight decrease at 0.5L: dWth (0.5L).

![Figure 1. AHinsp Lplus-HME and without HME.](image)

This approach is a simple straightforward integration, but does not take into account the actual flow velocity. Therefore we also performed a more fundamental integration.

Method 2. By integration of the product of AH insp and flow over time (dimensional analysis: [mg/L] * [L/s] * [s] = [mg]):
\[ dWth = \int AHinsp(t) * \text{Flow(t)} * dt \]

This method takes into account that at higher flow velocities more water vapor will be collected per unit of time. Integration was performed over the full duration of the inspiration, but the first 0.2
seconds of each inhalation were discarded to minimize the impact of the time constant of the humidity sensor. Observations of AHinsp and Flow were done in different computer systems, and the curves were manually overlaid using the slight discontinuities in the AH measurements which show the start and end of the inspiration (see figure 2, which illustrates the calculation of the integral similar to figure 1).

**Figure 2.** Calculation of the integral of AHinsp and breathing volume. The Red curve is AHinsp (full scale 35 mg/L); the blue curve the flow (full scale 0.7 L/s) and the black curve the product of AHinsp and flow (mg/s). The grey area under the black curve is the theoretical weight change (mg) (without correction for the situation without HME). The figure shows a single (tidal) breath using the Lplus-HME.

Also in this case the background situation without HME should be subtracted. However, we did not have observations without HME with the same flow rate as the observations with HME. Therefore we used method 1 to calculate the theoretical weight change without HME at the inspiratory breathing volume \( V = \int \text{Flow}(t) \, dt \)

The resulting theoretical weight decrease calculated with method 1 is shown in figure 3 as a function of measured weight for an inspired volume of 0.5L. Similar to what can also be seen in figure 7 in the paper, the measured weight of the L-HME is larger than expected. However, \( dWt \) correlates well with measured weight confirming that \( dW \) and AHinsp both measure water absorption of the HME.
Figure 3. Theoretical weight change and measured weight change for integration method 1 (at a volume of 0.5L) $R^2=0.92$

In figure 4 the result of integration method 2 is shown. In this case we obtain a separate data point for each separate inspiration observation. Theoretical weight decrease correlates well with the measured weight decrease both for each HME separately and for all HMEs combined confirming the data integrity of our humidity and weight and flow observations. The variation is likely due to the errors, which will have been introduced by the manual co-registration of humidity and flow registration.

Figure 4. Integrals of $AH_{\text{insp}}$ to delta weights, axes in milligrams.
Appendix 2

A novel, simplified ex vivo method for measuring water exchange performance of Heat and Moisture Exchangers for tracheostomy application. Respiratory Care. Cindy van den Boer, Sara H. Muller, Andrew D. Vincent, Klaus Züchner, Michiel W.M. van den Brekel, Frans J.M. Hilgers. Corresponding author: Frans JM Hilgers, Department of Head and Neck Oncology and Surgery, The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands; f.hilgers@nki.nl.

Parameters of the fit of figures 5 and 6.

Table 1. Parameters of the fit of figure 5, weight versus average breathing volume using all inspiration data and expiration data during tidal breathing (as the estimate of initial value).

<table>
<thead>
<tr>
<th></th>
<th>$\beta_{H_1}$</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-HME</td>
<td>2.56</td>
<td>0.31</td>
</tr>
<tr>
<td>L-HME</td>
<td>2.24</td>
<td>0.19</td>
</tr>
<tr>
<td>Lplus-HME</td>
<td>1.98</td>
<td>0.28</td>
</tr>
<tr>
<td>Rplus-HME</td>
<td>4.64</td>
<td>0.36</td>
</tr>
</tbody>
</table>

SE = standard errors of the estimated slopes

Equation 1: \(\text{Weight change} = \beta_{H_0} + \beta_{M} + \beta_{H_1} \times \log(\text{Volume}) + \varepsilon_i\)

where
1. $\beta_{H_0}$ and $\beta_{H_1}$ are the fixed-effect intercepts and slopes for the HME types.
2. $\beta_{M} \sim N(0,\sigma_{M}^2)$ are the random intercepts per measurement period, and
3. $\varepsilon_i \sim N(0,\sigma_{R}^2)$ are the residuals.

Table 2. Exponential decay model of $AH_{insp}$ versus inspiratory breathing volume (figure 6), during tidal breathing (as the estimate of initial value)

Equation 2: \(AH = \beta_{H_0} + (\beta_{AS} - \beta_{H_0}) \times \exp[-\exp(\beta_{DR} \times \text{Insp Vol})] + \varepsilon_i\)

where
1. $\beta_{H_0}$, $\beta_{AS}$ and $\beta_{DR}$ are the intercept, asymptote and the log of the decay rate for each HME.
2. $\varepsilon_i \sim N(0,\sigma_{R}^2)$ are the residuals.

<table>
<thead>
<tr>
<th></th>
<th>$\beta_{AS}$ Est</th>
<th>SE</th>
<th>$\beta_{H_0}$ Est</th>
<th>SE</th>
<th>$\beta_{DR}$ Est</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-HME</td>
<td>7.83</td>
<td>0.11</td>
<td>32.81</td>
<td>0.08</td>
<td>2.00</td>
<td>0.06</td>
</tr>
<tr>
<td>L-HME</td>
<td>6.95</td>
<td>0.14</td>
<td>32.51</td>
<td>0.16</td>
<td>2.18</td>
<td>0.07</td>
</tr>
<tr>
<td>Lplus-HME</td>
<td>7.88</td>
<td>0.28</td>
<td>34.5</td>
<td>0.23</td>
<td>1.75</td>
<td>0.06</td>
</tr>
<tr>
<td>Rplus-HME</td>
<td>10.46</td>
<td>0.45</td>
<td>35.45</td>
<td>0.32</td>
<td>1.54</td>
<td>0.08</td>
</tr>
<tr>
<td>Without</td>
<td>5.12</td>
<td>0.05</td>
<td>31.67</td>
<td>0.07</td>
<td>2.27</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Est = estimated value; SE = standard error of estimate.