AN ABSTRACT OF THE THESIS OF

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Title: The Effect of Various Breathing Maneuvers on Measurement of Lung Volumes for Hydrostatic Weighing

Redacted for Privacy

Abstract approved: ______________________

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This study was to determine if an increase of $PaCO_2$, after breath-holding prior to full inspiration, could enable a person to reach the same FVC measured on land while submerged. Another purpose of this study was to explore the difference in Db and %BF between HW at RV, TLC and TLCwet with breath-holding. The FVC was measured under the conditions of on land and in water, both with breath-holding and without breath-holding. Secondly, subjects were hydrostatically weighed at RV, TLCwet, and TLCwet with breath-holding to determine the differences in estimation of Db and %BF. The RV was estimated via empirical formula. The TLC was determined by summing FVC and RV. Fifteen male (18 - 25 years old) and 15 female (19 -28 years old) students volunteered for this study. A two-way ANOVA with repeated measures and Scheffe's post hoc test were used for statistical analysis of data. The α level was set at .05 for statistical significance. There

was no indication of increase in FVC with breath-holding. The FVC both with and without breath-holding was significantly reduced in water (5.4 % - 5.5 % in females, 3.4 % - 3.9 % in males). The mean Db calculated from RV was the lowest in both genders and was significantly lower than Db from TLCwet, TLCdry, and TLCwet with breath-holding. The Db at TLCwet was lower than at TLCdry with no statistical significance in males (average 0.002 gm/cc difference) and in females (mean difference was 0.005 gm/cc). The mean %BF difference between TLCwet and TLCdry was 0.75 % in males and 2.01 % in females (p>.05). It is unlikely that a possible increase of $PaCO_2$ attained after breath-holding could facilitate inspiratory motion via stimulus to the chemoreceptors to overcome hydrostatic pressure. The empirical estimate of RV may not be suitable for young adults who have larger lungs. Using TLCdry in HW could be an alternative instead of using RV for males; for females it should be used with greater caution.

THE EFFECT OF VARIOUS BREATHING MANEUVERS ON MEASUREMENT OF LUNG VOLUMES FOR HYDROSTATIC WEIGHING

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Master of Science

Completed April 6, 1990

Commencement June, 1990

APPROVED:

Redacted for Privacy

Major Professor in Human Performance

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Date thesis is presented: April 6, 1990

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DEDICATION

It is with much love and gratitude that I dedicate this thesis to my parents and sisters, especially Jean, my older sister. Their love, encouragement, and constant support has instilled the self-confidence necessary for me to achieve academic success.

ACKNOWLEDGEMENTS

I would like to express appreciation to my committee chairman, Dr. Christian W. Zauner, and my committee members Dr. Jeffrey McCubbin, Dr. Anthony Wilcox, Dr. Robert Houston and Dr. Howard L. Wilson for the guidance, assistance, and valuable criticism. A special thanks to Dr. Zauner for his many hours spent working with me, his encouragement, and his unending patience. Also, a special thanks to Dr. Ribold of the Oregon State University, Veterinary Hospital, who gave me the necessary technical help and advice for my study.

Thanks to Christine Quinn who, during my whole experiment, gave me her constant help as well as sound advice and many suggestions. I am grateful for having access to all equipment needed in this study. Also, I would like to thank Darlene DeShon, who was one of the volunteers for my study and also devoted her time to help with my English writing, which was essential to the completion of my thesis.

Finally, I would like to thank Dr. Michael Maksud, Dr. John Dunn, Dr. Annette M. Rossignol, Dr. Terry Wood, and all those in the College of Health & Human Performance who helped me throughout the year. A special thanks goes out to the subjects who volunteered to be tested. Without their help I would not have been able to complete the objectives of this study.

TABLE OF CONTENTS

Page

CHAPTER

I. INTRODUCTION ---------- 1

Statement of Problems ---------- 3
Research Hypotheses ---------- 4
Statistical Hypotheses ---------- 4
Operational Definitions ---------- 5
Assumptions ---------- 7
Delimitations ---------- 7
Limitations ---------- 8

II. REVIEW OF LITERATURE ---------- 9

Introduction ---------- 9
Changes in Lung Volumes While Submerged ---------- 11
Use of Residual Volume and Total Lung Capacity for HW ---------- 15
Mechanisms of Respiration ---------- 17
Summary ---------- 19

III. METHODS ---------- 21

Subjects ---------- 21
Equipment and Apparatus ---------- 22
Procedures ---------- 23
Study Design ---------- 28
Statistical Treatment of Data ---------- 29

IV. RESULTS AND DISCUSSION ---------- 30

Pilot Study ---------- 30
Lung Volume Measurements ---------- 32

CHAPTER (continued) Page

The Breath-holding Maneuver When Immersed to the Neck ---------- 39
Body Density and Body Fat Percentage ---------- 42

V. CONCLUSIONS ---------- 52

Summary ---------- 52
Conclusions ---------- 52
Recommendations ---------- 53

REFERENCES ---------- 55

APPENDICES ---------- 62

A. Informed Consent Document ---------- 62
B. Hydrostatic Weighing and % Body Fat Data Sheet ---------- 65
C. The Body Weight in Water at Three Lung Volumes ---------- 67

LIST OF FIGURES

FIGURE | Page

1. The Simulated FVC Tracings From the Standard Maneuver and From the Maneuver With Prior Breath-holding ---------------------------- 33

LIST OF TABLES

TABLE Page

1. Means and Standard Deviations For Age and Physical Characteristics of 15 Male and 15 Female Volunteers ---------- 21

2. Intraclass Correlation Coefficient For FVC Measurements Under Four Condition ---------- 31

3. Intraclass Correlation Coefficient For HW at Three Lung Volumes ---------- 31

4. Means and Standard Deviations For FVC measurements ---------- 32

5. Two-factor Repeated Measures ANOVA For FVC Measurements ---------- 35

6. Scheffe's Post Hoc Test For Multiple Comparison Among FVC ---------- 36

7. Means and Standard Deviations For Db Estimation and %BF Estimation ---------- 43

8. Two-factor Repeated Measures ANOVA For Db Estimation and %BF Estimation ---------- 44

9. Scheffe's Post Hoc Test For Multiple Comparison Among Body Densities ---------- 45

10. Scheffe's Post Hoc Test For Multiple Comparison Among % Body Fat ---------- 46

THE EFFECT OF VARIOUS BREATHING MANEUVERS ON MEASUREMENT OF LUNG VOLUMES FOR HYDROSTATIC WEIGHING

CHAPTER I

INTRODUCTION

The outcome of human body composition assessment has been broadly applied for the purposes of estimating nutrition profile, predicting heart disease risk, evaluating the effectiveness of exercise programs and as a means to aid in the selection of physical activity modes. Of the several methods of body composition assessment, densitometry, determined by hydrostatic weighing (HW), is considered to be the most reliable. Behnke, Feen, and Welham (1942) described this method, based upon Archimedes' principle of specific gravity.

With numerous changes and adaptations of Behnke's basic procedures, this technique is now viewed as a standard laboratory procedure for assessing body density and subsequently determining the body fat percentage (Weltman & Katch, 1981). Some other techniques of estimating body composition, such as skinfold methods and electrical impedance, use HW as the validating criteria. Therefore, a minimal measurement error is important for use of the HW approach.

Traditionally, HW is taken while the subject is in the respiratory condition of residual volume (RV) because RV is generally thought to be the lung volume least affected by hydrostatic pressure (Welch & Crisp, 1958). However, the RV is usually determined on land and then assumed to be the same during HW. Since lung volumes could substantially influence the magnitude of buoyancy, and therefore, the body weight in water, the measurement of RV could be a major source of error in the determination of body density (Db) by HW (Oppliger, Looney, & Tipton, 1987). Therefore, unusual care must be exerted when assessing RV for later use in estimating HW. As Lohmen (1981) pointed out, the difference between body densities where RV is determined on land and underwater is not well established. It appears important whether or not one can indeed reach land-assessed RV when in water. If the subject can not reach land-assessed RV while submerged, the error of measurement of Db by using RV established on land could be substantial. If more air remains in the lung when the subject is submerged than when on land, the Db will be underestimated, and vice versa. It is believed that expiring under water to the point of land-established RV may be an unfamiliar and sometimes impossible technique for many individuals to master (Weltman & Katch, 1981).

For the purpose of minimizing this source of error, techniques for HW at total lung capacity (TLC) have been developed in different populations (Behnke, 1961; Napper, Vogler, Joseph, & Donnelly, 1988; Weltman & Katch, 1981), even without head submersion (Donnelly, Brown, Israel, Smith-Sintek, O'Brien, & Caslavka, 1988). Once again, since the TLC is usually obtained based upon the forced vital capacity (FVC) measured on land, the Db could be overestimated due to the hydrostatic pressure effect on the relatively larger lung volume, that is, the hydrostatic pressure causes smaller FVC in water, so less bouyancy. The subjects therefore would weigh more under water, subtracting the larger volume (a land-assessed FVC) in the computation of Db then causes an overestimation. This overestimation could also be significant (Timson & Coffman, 1984). In the proposal of this thesis project, it was hypothesized that if a subject was able to obtain the true status of total lung capacity, that is, the same as achieved on land, while underwater, the HW and resulting estimation of Db should have the greatest accuracy.

Statement of the Problems

The purposes of this study were to determine if: (a) humans, while submerged, could reach the same TLC as measured on land by employing two breathing maneuvers; and (b) the Db calculated in the condition of RV differed significantly from the Db calculated in the status of TLC.

The two breathing maneuvers were: (a) forced expiration following a maximal inspiration from FRC preceded by tidal breathing, and (b) forced expiration following a maximal inspiration from RV preceded by maximal breath-holding.

Since the changes of FVC reflect the changes of TLC (assuming RV stays constant), FVC was measured and compared in this study. The FVC measurement was designed to have four testing conditions: (a) FVC measured on land without breath-holding (FVCdry), the standard FVC measurement; (b) FVC measured on land with prior breath-holding (FVCdry, breath-holding), (c) FVC measured in water without breath-holding (FVCwet), and (d) FVC measured in water with prior breath-holding (FVCwet, breath-holding). Consequently, computed TLC was TLCdry, TLCwet, TLCdry with breath-holding, and TLCwet with breath-holding.

Research Hypotheses

A review of the literature led to the hypotheses that: (a) While a subject is submerged, maximal breath-holding prior to forced inspiration may yield a FVC which is very close to the FVC measured on land; and (b) if the difference in Db between using RV and as opposed to TLCdry is substantial, the Db using TLCwet, and TLCwet with breath-holding should be between the other two.

Statistical Hypotheses

The statistical hypotheses were expressed as:

1. $H_{0(1)}$: $\mu1=\mu2=\mu3=\mu4$,

   $H_{a(1)}$: at least one of the following means differs from the others

where $\mu1$= mean of FVC on land in males and females

$\mu2$= mean of FVC in water in males and females

$\mu 3$= mean of FVC on land with prior breath-holding in males and females

$\mu 4$= mean of FVC in water with prior breath-holding in males and females

2. $H_{0\,(2)}$: $\mu 5=\mu 6=\mu 7=\mu 8$,

$H_{a\,(2)}$: at least one of the following means differs from the others

where $\mu 5$= mean of Db with RV in males and females

$\mu 6$= mean of Db with TLCwet in males and females

$\mu 7$= mean of Db with TLCwet with breath-holding in males and females

$\mu 8$= mean of Db with TLCdry in males and females

Operational Definitions

ATPS: the volume of gas at the specific conditions of measurement, which are therefore at ambient temperature (273 ^{o}K + ambient temperature ^{o}C), ambient pressure, and saturated with water vapor (McArdle, Katch, & Katch, 1986).

BTPS: a volume of a gas expressed at body temperature (usually 273 ^{o}K + 37 ^{o}C or 310 ^{o}K), ambient pressure (whatever the barometer reads), and saturated with water vapor with a partial pressure of 47 mmHg at 37 ^{o}C (McArdle, Katch, & Katch, 1986).

Vital Capacity (VC): the largest volume of air that can be expired after a maximal inspiration (Mohler, 1982).

Forced Vital Capacity (FVC): This is one of the ways to express VC, and is generally recommended (Boushey, Jr., & Dawson, 1982). The FVC is the volume of air expelled from the lungs during a maximal forced expiration starting after a maximal forced inspiration (Levitzky, 1986). It is measured and displaced on a spirometer and the volume should be converted to BTPS condition.

Residual Volume (RV): The RV is the volume of gas left in the lungs after a maximal forced expiration (Levitzky, 1986).

The unit for RV and FVC is liters. In this study, the RV was estimated from the FVC by the following equations:

Males: RV = 0.24 x VC (BTPS), (Wilmore, 1969)

Females: RV = 0.28 x VC (BTPS), (Wilmore, 1969)

Total Lung Capacity (TLC): TLC equals RV plus FVC (Levitzky, 1986).

Tare Weight: the weight of all the equipment suspended in the water filled hydrostatic water tank (Sinning, 1975).

Body Density (Db): defined as the weight per unit of volume. Density is usually expressed in grams per cubic centimeter (gm/cc) when accounted with determination of body composition (Sinning, 1975). The body weight or mass (Ma) is determined by weighing in air. The difference between Ma and submerged weight (Mw) is equal to the body volume when the appropriate water temperature correction (Dw) is applied. The formula for calculating the Db is:

$$Db = Ma \times Dw / (Ma - Mw - RV \times Dw),$$ (Goldman & Buskirk, 1961)

Assumptions

Some basic assumptions were made for application in this study:

(a) RV estimated on land represented the true RV,

(b) water pressure had no influence on RV, therefore, RV in water was the same as that on land; and

(c) all subjects performed the necessary pulmonary maneuvers for estimating lung volume to the best of their ability.

Delimitations

The study was delimited as follows:

(a) The population sampled in this study were male and female students in attendence at the Oregon State University, aged from 18 to 28 years;

(b) based on the informed consent subjects with restrictive and/ or obstructive lung diseases or chest abnormalities were not utilized,

(c) no attempt was made to accept or eliminate volunteers from serving as subjects due to their swimming ability,

(d) all subjects were nonsmokers, and

(e) to avoid the fatigue factor resulting from repeated assessment of lung volumes, FVC measurement and HW were conducted on separate days.

Limitations

The study was limited in that:

(a) RV was estimated rather than directly measured, this could introduce and/or augment measurement error;

(b) subjects were college students and may not well represent those non-college students in the same age category,

(c) volunteer subjects were used and therefore, the randomly sampling principle was not met; and

(d) after breath-holding, there was no $PaCO_2$ analysis; therefore, the extent to which breath-holding increased $PaCO_2$ is unknown.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

The HW approach for Db assessment is established based upon Archimedes' specific gravity principle, which says that a body immersed in a fluid is acted on by a buoyancy force, made evident by a loss of weight equal to the weight of the displaced fluid (Behnke, 1961). Therefore:

body specific gravity = weight in air/loss of weight in water
(Behnke, 1961)

Density is defined as the concentration of matter, measured as the mass per unit volume:

body density (Db) = mass/volume, (Brozek, Henschel, & Keys, 1961)

The difference (Ma - Mw) between the weight of the body in air (Ma) and the weight when completely submerged in water (Mw) is the weight of the displaced water. To obtain the volume corresponding to the mass of water displaced by the body, a correction is made for the density of the water (Dw) at the time of HW. So:

$$D = (\text{weight in air} / \text{loss of weight in water}) \times \text{density of water}$$

(Behnke, 1961)

The volume of air in the lungs (RV) should be subtracted from the gross body volume:

$$D = \frac{Ma}{\frac{(Ma - Mw)}{Dw} - RV}$$

(Miller, & Blyth, 1953)

Air in the gastrointestinal tract is also a concern. However, it is small in volume and difficult to measure and can be ignored (Goldman & Buskirk, 1961).

Determinations of body volume and body density, together with information on constants obtained from cadaver and other studies, permit the estimation of total body fat and thus, "fat free" body weight (Goldman & Buskirk, 1961). For this concept of fat-free body, Siri (1961) developed the following equation to convert body density into body fat percentage:

$$\%Fat = (4.95 / \text{body density} - 4.50) \times 100$$

In the present study, Siri's formula was used to calculate % body fat (%BF) for all the subjects.

The precision of body fat determination using the densitometry approach has been questioned, due to the fact that residual air in the lungs and gastrointestinal tract may affect measurements (Weltman & Katch, 1981). The scope of this study is such that only the potential influence of lung volumes on hydrostatic weighing will be discussed.

Traditionally, RV has been employed as the estimation of air in the lungs when calculating Db in the HW method, while TLC is suggested as being a viable substitute (Behnke, 1961; Weltman & Katch, 1981). Relative to the accuracy of Db estimation, it is rational to argue that the lung volumes used in the calculation (RV or TLC) should reflect the conditions that exist during HW. In most circumstances both RV and TLC are land-assessed. Since RV and TLC may change while subjects are submerged, the Db could be inaccurate if lung volumes measured on land are used for calculation rather than lung volumes measured in water. The questions to be answered are: (a) Can RV or TLC measured on land be achieved while submerged; (b) do lung volumes change while submerged, and if so, how?

Changes in Lung Volumes While Submerged

Researchers believe that the variations in lung volumes during submersion might be caused by two major factors: the hydrostatic force counteracting the action of the inspiratory muscles, and the shift of blood into the thorax (Agostoni, Gurtner, Torri, & Rahn, 1966; Bondi, Young, Bennett, & Bradley, 1976; Hong, Cerretelli, Cruz, & Rahn, 1969). The blood shift into the thorax could certainly take over space in the thoracic cavity and therefore reduce lung volumes (VC and/or RV). Moreover, a shift of blood into the thorax may cause a pulmonary vascular engorgement (Girandola, Wiswell, Mohler, & Barnes, 1977), which may induce a trapping of air in the lung, thus altering volumes.

Decreased RV

Early studies have produced varied findings. It has been reported that RV is decreased from 4% to 16% when submerged (Agostoni, Gurtner, & Torri, 1966; Brozek,Henschel, & Keys, 1949; Jarrette, 1965). Bondi, et al (1976) reported an average decrease of 9.35 % in RV in their study with ten subjects while underwater. The explanation offered was that the transdiaphragmatic pressure increased, leading to an increased pressure gradient between extra- and intrathoracic compartments. The changed pressure gradient caused more venous blood return to the thorax. As a result of increased blood volume, thoracic outward recoil force was decreased. Moreover, blood shifted into the thorax is competing with air for space in the thoracic cavity (Bondi et al, 1976).

Hydrostatic pressure exerts approximately a 20 cmH_2O increment on the chest wall when a subject is immersed at neck level (Craig & Ware, 1967). This pressure increment is believed to be capable of reducing RV mechanically by assisting the subject to expire more fully (Bondi et al, 1976). Because of hydrostatic pressure, blood pooling in peripheral veins is eliminated. This initiates venous blood redistribution. Another major factor influencing the decrease of lung volume is the hydrostatic pressure on the abdomen. Hydrostatic pressure, which causes loss of gravitational effect on the abdomen, also causes the diaphragm and the abdominal contents to move upward (Agostoni et al, 1966). This upward movement will restrict diaphragmatic exursion thereby reducing measured lung volumes (Agostoni et al, 1966; Bondi et al, 1976).

Increased RV

Dahlback and Lundgren (1972) pointed out that it is possible to have increased air-trapping when the lung volume is relatively low during submersion. Due to the trapped air in the airways, RV could be increased. The mechanics of air-trapping is due to pulmonary vascular engorgement which induces a swelling of blood vessels, therefore closing small air ways. At full expiration during immersion, airways both large and small are being compressed, the smaller ones being compressed to the point of possible collapse (Bondi et al., 1976). Generally, the RV may increase 5 % - 6.7% while underwater (Carey, Scheafer, & Alvis, 1956; Girandola et al, 1977). As mentioned earlier, hydrostatic pressure leads to blood redistribution, that is, increased thoracic blood return. This considerable blood volume increase (about 500 ml) can cause pulmonary vascular engorgement which will yield a "stiffness" of the lung tissue, reducing its compliance and elasticity (Girandola et al, 1977).

Unchanged RV

Prefant, Lupi, and Anthonisen (1976), and Robertson, Engle, and Bradley (1978) proposed that RV will not be changed significantly when submerged because of the counterbalance between hydrostatic pressure and vascular congestion. McGarty (1982), in her research, used a nitrogen dilution technique measuring RV in both dry and submersed (wet) conditions. The results showed no significant reduction in RV land measured and RV measured in water.

Reduced VC

Hydrostatic pressure and blood shift into the thorax, the same factors influencing RV, have a significant effect on FVC. In an early study, Hong, Ting, and Rahn (1960) stated that there was no significant reduction in VC between dry and submersion conditions in the standing position. This result supported that of Carey, Schaefer, and Alvis (1956). However, later research suggests that VC is reduced when tested while the subject is submerged in water. Approximately 60 % of the reduction in VC is due to an increased intrathoracic blood volume and the rest is due to hydrostatic forces (Hong et al., 1969). The same workers reported that VC was reduced an average of 360 ml with subjects seated in water (Hong et al., 1969). A review of findings indicated that VC is reduced in a range of 3 % - 9 % while immersed (Agostoni et al., 1966; Hong et al., 1969; Carey et al., 1956; Craig & Ware, 1967; Morgan, 1983; Robertson, Engle, & Bradley, 1978).

Use of Residual Volume and Total Lung Capacity for HW

The measurement of RV in the submerged condition is a difficult procedure. The commonly used technique to estimate RV is gas dilution, using methods such as helium dilution, oxygen washout, and nitrogen washout. In most cases, the dilution technique is reliable and accurate. Unfortunately, it is not sensitive when airway obstruction exists (Levitzky, 1986; Wilmore, 1969). Robertson, Engle, and Bradley (1978), by using a whole body plethysmograph, found the measurement of submerged RV by dilution technique to be underestimated by about 200 ml, but when measuring submerged VC the difference was less. Because of air trapped in the lungs when submerged (which is more likely to happen at lower lung volumes), equilibrilium of helium within the trapped airway will not be achieved. It remains uncertain whether or not RV measurement underwater with a gas dilution technique can be truly representative of an ideally minimized error.

From previous review, it could be reasonable to argue that RV may not change significantly when submerged. That is, hydrostatic pressure has less influence on the lungs when the volume is low due to the counterbalance between hydrostatic pressure and vascular congestion. Researchers suggest that RV measured on land can be used for calculating Db (Welch & Crisp, 1958). McGarty (1982) reported no significant change in the RV between on land and in water conditions in both males and females; in contrast, the FVC was significantly reduced submerged as opposed to on land, with a greater reduction seen in males ($p<.01$).

Some other factors may also affect the underwater RV. McArdle, Katch, and Katch (1986) noted that psychological fear of submersion may lead to a higher RV and that before obtaining an ideal underwater RV, the subject may require a learning period.

Researchers have searched for an ideal way to estimate Db more accurately by using different lung volumes. An alternative way is to use TLC to calculate Db by asking the subject to perform HW with full inspiration. The major drawback in using TLC is that the subject may not be able to achieve the TLC which was measured on land, due to hydrostatic pressure on the chest wall and blood shift into the thoracic cavity. An overestimation of Db would be resulted.

McGarty (1982) measured FVC, from which TLC was estimated, in an underwater condition (TLC-wet) and looked at the differences in resulting mean Db as compared with those when using RV-wet, TLC-wet, and TLC-dry. The results showed that the Db with HW at RV-wet was lower than at TLC-wet, but the Db with HW at TLC-wet was lower than at TLC-dry. Since TLC-wet is measured at the same condition as HW, the Db calculated based upon TLC-wet may be more accurate than based upon TLC-dry. Timson and Coffman (1984) did a similar experiment and reported comparable results. However Weltman and Katch (1981) compared mean Db at RV-dry and at TLC-dry, and stated that while there was a statistical significance between the two, the difference in %BF using RV and TLC was negligible (0.5 % for men and 0.9 % for women).

In summary, when comparing Dbs at RV, TLC-wet and TLC-dry, the result is generally that Db at TLC-wet is between Db at RV and Db at TLC-dry. However, from the practical point of view, the TLC-wet method is not normally used since it requires more time and equipment, and it is more complicated. The focus of this study was upon whether or not the TLC measured on land by the prior breath-holding technique (TLC with breath-holding), could be reached while submerged to chin level, as well as the potential difference in Db among using RV, TLCdry, TLCwet, and TLCwet with breath-holding.

Mechanics of Respiration

An important breathing control mechanism is the Hering-Breuer reflex, which is the inflation-deflation stretch receptor reflex. The receptors are distributed within the smooth muscle of large and small airways (Levitzky, 1986). When lungs are inflated to a large volume (more than 1,000 ml), the receptors send greater stimuli in order to inhibit lung overinflated. When lungs are deflated to a lesser or lower volume, the receptors have less inhibition control on inspiration, that is, inspiration is more forceful. In the normal situation, the Hering-Breuer reflex has a minimal effect on breathing because it is overridden by higher respiratory centers (Levitzky, 1986).

Agostoni et al (1966) reported that during submersion and negative-pressure breathing, the diaphragm was relaxed at the end of spontaneous expirations. It was also reported that there was no electrical activity originating from the diaphragm at the end of spontaneous expirations on tracings obtained with needle electrodes (Murphy, Koepke, Smith, & Dickinson, 1959; Taylor, 1960). The suggestion is that the mechanism leading to strong contraction of the diaphragm when subjects expire maximally is not elicited by a reflex originated by relative collapse of airways or by the stretching of the diaphragm, but it is in some way related to the simultaneous contraction of the abdominal muscles (Agostoni et al., 1966). Generally, the Hering-Breuer stretch reflex is weak in the conscious subject. (Agostni, 1962; Widdicombe, 1961; Widdicombe, 1964).

An increased $PaCO_2$ could reduce the discharge of stretch receptors, that is, increase the tendency for inspiration (Berger, Mitchell, & Severinghause, 1977; Mitchell, 1976). Responding to the changes of $PaCO_2$, peripheral chemoreceptors play a more important role than central receptors (Lambersten, 1980; Levitzky, 1986). The effect of $PaCO_2$ on respiration is that ventilation increases due to increased tidal volume and frequency when $PaCO_2$ increases. It is obvious that a $PaCO_2$ increase can cause increased depth of respiration and tidal volume. On the other hand, an increase of $PaCO_2$ reduces the discharge of stretch receptors, thereby, increasing the tendency for deeper inspiration. Holding one's breath for an extended period of time may build up $PaCO_2$. This increase of $PaCO_2$ may be able to stimulate the respiratory center (by peripheral chemoreceptors) resulting in a deeper inspiration.

One purpose of this study was to see whether, after a breath-holding following a maximal expiration, an increased $PaCO_2$ caused a deeper inspiration when submerged to chin level. If so, the increase of $PaCO_2$ could be said to be a sufficient influence to overcome the negative effect of water pressure on the chest wall and blood redistribution in the thorax. Such an effect might allow land-assessed FVC to be achieved.

Summary

Hydrostatic weighing is the standard measurement used to determine %BF in most laboratories. The RV is traditionally used in performing HW and calculating Db. However, when a person is submerged lung volumes are subject to change. Research indicates that RV may increase, decrease, or remain unchanged, while VC tends to be reduced.

A more accurate way to assess body density may be to measure the specific lung volume at the time of HW. This way, the measurement error from RV is reduced. Using land-assessed RV to calculate Db form HW could lead to an underestimation of Db, whereas the method of using land established TLC to estimate Db in HW could lead to an overestimation. Both deviations from the true Db are due mainly to hydrostatic pressure on the chest wall and the blood shift or redistribution.

A high $PaCO_2$ can increase the depth of breathing by stimulating peripheral chemoreceptors thus enhancing inspiration force by decreasing the discharge rate of Hering-Breuer stretch receptors. It was proposed in this study that there was a possibility that enhanced inspiration force caused by $PaCO_2$ increase due to breath-holding could partially compensate for the effect of hydrostatic pressure on the chest wall so that submerged subjects would be able to reach the point of TLC measured on land. This proposal was the major purpose of the study.

CHAPTER III

METHODS

This study employed 30 subjects, 15 male and 15 female, all of whom were volunteers. The basic procedures for the measurements of FVC and HW, as well as the equipment utilized in the study are also discussed in this Chapter. The results of FVC from different testing conditions (on land and in water, with and without breath-holding), and the Db from HW at different lung volume (RV, TLC and TLCwet with breath-holding) were statistically analyzed with ANOVA.

Subjects

Table 1 describes the physical characteristics of the subjects. Subjects were recruited for this study with the use of posters throughout the university campus. Subjects were all students enrolled at Oregon State University. The age of the males ranged from 18 to 25 years, with an average age of 20.70 years. The age of the females ranged from 19 to 28 years, with an average age of 22.27 years. All but four students were originally from the state of Oregon. Twenty six of the subjects had never been either hydrostatically weighed before, nor had their lung volumes assessed by spirometer. All the subjects were non-smokers. The subjects were randomly assigned to the order of FVC measurement (on land and in water, with breath-holding and without

Table 1.
Means and Standard Deviations (S.D.) For Age and Physical Characteristics of 15 Male and 15 Female Volunteers.

Gender	n	Age (years)/S.D.	Height (cm)/S.D.	Weight (kg)/S.D.
F	15	22.27 / 2.76	170 / 8.7	61.74 / 5.73
M	15	20.70 / 2.36	177 / 9.1	74.14 / 8.99

breath-holding). Also, they were randomly assigned to the order of lung volume performance for HW (RV, TLC, TLCwet with breath-holding).

Based upon questions on an informed consent document, all the subjects were free of any restrictive and/or obstructive lung disease or chest abnormality. Before the study began, the participants were informed of the procedures and benefits of body fat assessment, as well as the potential hazards of hydrostatic weighing. Each subject then signed an informed consent statement (see Appendix A).

Equipment and Apparatus

The lung volume measurement was performed on the Universal Computer Interface (UCI) system (Vacumed Inc., Ventura, California). This computer-integrated system includes: an Airco Ohio 827 dry spirometer (Vacumetrics, Model 1615), an analog-to-digital converter (ADC), a cathode-ray tube (CRT) screen, and a printer output for the calculated VC which was converted to the BTPS status. For the purpose of this study, the microprocessor-assisted spirometry system was acceptable. It saved time and minimized the possibility of calculation errors in converting ATPS to BTPS . Before lung volume was measured, calibration was done with a standard three liter calibration syringe (Vacumed Inc. Ventura, California). Three liters of room air was injected twice with the syringe, once in 2 s and once in 6 s (Morris Kanner, Crapo, & Gardner, 1984) to assure that the instrument accurately represented the volumes.

The apparatus used for HW was as follows:

Homs Full Capacity Beam Scale: for weighing body weight on land, Model: 500 AD, Type: ounce and decimal, pounds (Douglas Homs Corp., Belmont, California).

Chatillon Toledo Scale: for weighing the body in water, Model: 314 D, Type: MI 2210, 15 Kilos x 25 grams (American Scale Corp., Eugene, Oregon).

The water tank was made by Oregon State University Human Performance Laboratory personnel, with a water capacity of 1.5 m^3. A stainless steel tube seat was suspended on the Chatillon Toledo scale. The height of the seat was adjustable in order to have the water at chin level when the subject was in the seat.

Procedures

The procedures to be described include FVC measurement and HW. Subjects had two separate visits to complete FVC measurement and HW.

The data collection sheet for FVC measurement and HW is provided in Appendix B. To minimize the potential measurement error, between trials of FVC measurement or HW, subjects had a 3 to 5 minute break. The formula from Goldman and Buskirk (1961) was used for calculation of Db, and the equation from Siri (1961) for estimation of %BF.

$$Db = Ma \times Dw / (Ma - Mw - RV \times Dw), \qquad \text{(Goldman \& Behnke, 1961)}$$

$$\%BF = (495 / Db) - 450, \qquad \text{(Siri, 1961)}$$

Originally, in Goldman and Behnke's equation, the amount of air to be subtracted is RV. In this study, RV was replaced by TLC for computing Db at TLC condition. Therefore, the equation from Goldman and Behnke could be rewrite as:

$$Db = Ma \times Dw / (Ma - Mw - \text{appropriate lung volume} \times Dw)$$

where the appropriate lung volume could be RV, TLCwet, TLCdry, or TLCwet with breath-holding.

The water temperature within the HW tank was maintained at 36 ^{o}C to 37 ^{o}C during testing. The water density (Dw), then, was corrected for the temperature at which the HW was conducted. After HW, the tare weight (seat and weight belt, if it was applied) were subtracted from the total weight read on the scale.

Measurement of FVC

Each subject was measured with four testing conditions (on land and in water, with and without breath-holding). A nose clip was worn by each subject while the measurements were taken. The subjects breathed through a mouth piece which was connected to the computer integrated Ohio 827 spirometer (Vacumetrics, Model 1615). The system was checked for leaks before the measurements began. All the FVC measurements were performed when the subject was in the seated position. The order of measurements of FVC under four testing conditions was randomly assigned. According to the statement from the Snowbird Workshop of the American Thoracic Society (Gardner, Baker, & Broennle, Jr., 1979), each measurement had a minimum of three trials of acceptable FVC maneuvers, and the highest value was used for data analysis. The displacement of FVC was observed and recorded.

FVCdry. This was performed with a standard FVC maneuver or standard forced expiratory effort. As Miller (1987) described, the subject took a full inspiration from a normal breathing pattern and exhaled as rapidly, and completely as possible.

FVCwet. Subjects were submerged to chin level sitting on the suspended seat. They performed FVC in the same way as they did on dry land with the standard FVC maneuver.

FVCdry with prior breath-holding. Subjects were asked to expire maximally after tidal breathing. Then, they held their breath at the end of full expiration as long as possible. When they could no longer hold the breath, they inspired forcefully, and immediately continued to the

FVC maneuver which was a full inspiration followed by a rapid and complete exhalation. The displacement of the latter expiration was the measurement of FVC with prior breath-holding.

FVCwet with prior breath-holding. The subject performed FVC in the same way as that of FVCdry with prior breath-holding except for being submerged to chin level.

Hydrostatic weighing

On the second visit, hydrostatic weighing was performed by each subject at three lung volumes (RV, TLC, and TLCwet with prior breath-holding). The order of lung volume assessment performed was randomly assigned. With each type of lung volume, the subject was hydrostatically weighed at least three times until three nearly identical readings were obtained in succession (Katch, Michael, & Horvath, 1967; Weltman & Katch, 1981). A mean value was derived from these measurements for estimation of Db and %BF. Therefore, a minimal total of nine trials of HW were conducted with each volunteer. A Chatillon autopsy scale (American Scale Corp., Eugene, Oregon) was used for assessing the underwater body weight. Before entering the water, the subjects were weighed on a Homs beam scale (Douglas Homs Corp., Belmont, California), while wearing only their swimming suits.

The subjects carefully entered the water tank after a thorough shower. Both hair and swimming suit were soaked. He or she assumed a comfortable sitting position and became suspended in the tank. All air bubbles were removed from the body and from inside the swimming garment by tapping or pulling the swimming garment. Subjects performed the breathing maneuvers to the best of their ability.

HW at RV. After several normal breaths, the subject was asked to forcefully expel all air from the lungs. After reaching maximal expiration, she/he held the breath and immersed completely by leaning forward with the head and torso. After the scale reading was taken, the tester gave a tap on the side of the tank, signaling the subject to emerge from the water. Few subjects failed to hold their breath at RV long enough at the beginning of the trials before the scale reading finished. Those trials then were abandoned. More trials were added until the subjects were capable of holding their breath to complete the HW trial.

HW at TLC. After a few normal breaths the subject inhaled maximally, and then held their breath at the end of full inspiration. The subject then submerged completely until the tester read the scale. When the buoyancy was too much to have a stable reading on the scale, a weight belt was fastened to the waist of the subject.

HW at TLCwet with breath-holding. The difference between this method and HW with TLC was that before performing TLC, subjects held their breath as long as possible after exhaling maximally. When the subjects needed to breathe in, they inhaled forcefully. At the point of maximal inhalation, they held their breath again and submerged into the water for HW.

Study Design

The study was composed of two parts: (a) measurement of FVC under four conditions (on land and in water, with prior breath-holding and without prior breath-holding); and (b) HW at different lung volumes (RV, TLC, and TLCwet with breath-holding). Each subject had two separate visits, one for VC measurements, and one for HW.

For FVC measurements, each single measurement under a given condition (e.g. dry with prior breath-holding) had three trials and the highest value was used for data analysis. Therefore, twelve trials were conducted for each subject. The order of testing conditions to measure FVC was randomly assigned to avoid bias due to fatigue or learning. Thus, a repeated measures design for each gender group was accomplished. The FVC measurements for a given subject were completed on one day.

The HW was conducted on another day. The RV was obtained by using empirical formula from FVC measured on land without breath-holding. The TLC then calculated from RV and FVC measured at different conditions (dry without breath-holding, wet without breath-holding, and wet with breath holding). The order of lung volumes (RV, TLC, and TLCwet with prior breath-holding) was randomized for each person. Thus the same repeated measures design was carried out for each gender group. Each HW at a given lung volume (e.g. RV) took at least three trials until three nearly identical readings were obtained in succession and a mean score from these identical readings was used for data analysis. Based upon HW, the Db for each subject was computed. There were four Db values associated with four lung volumes (RV, TLCwet, TLCdry, and TLCwet with breath-holding). The body weight in water from TLVwet was used for Db calculation at TLCdry and TLCwet.

The TLCdry with breath-holding was not used to compare with other TLCs or RV for estimation of Db and %BF. For the purpose of this study, it was assigned as a quality control to see if FVCdry with breath-holding would differ from standard FVCdry significantly.

Statistical Treatment of Data

The dependent variables for FVC measurements were the scores of FVC and the independent variables included different testing conditions such as on land, in water, and with or without breath-holding. For HW, the dependent variables were the means of Db and the independent variables included the different lung volumes such as RV, TLCdry, and TLCwet with breath-holding. Gender difference could also be taken into account for the statistical comparison. A repeated measures two-way ANOVA was employed to compare FVCs under four conditions. The same statistic was applied to the Db and %BF comparisons at four different lung volumes. The Scheffe's post hoc test was employed only when the ANOVA for FVC measurement and Db estimation revealed a significant *F*-statistic. Because of the exploratory nature of this study, the α level was set at .05 for the *F*-test and Scheffe's post hoc test.

CHAPTER IV

RESULTS AND DISCUSSION

The purpose of this study was to see if one could reach the same TLC measured on land while submerged at chin level following breath-holding. The second purpose was to determine if the Db calculated when in the status of RV differed significantly from the Db calculated when in the status of TLC (TLCwet, TLCdry, and TLCwet with breath-holding). This chapter includes the statistical analyses of the data collected from FVC measurements under four conditions (in water and on land, with and without breath-holding), and also data from HW at different lung volumes (RV, TLCwet, TLCdry, and TLCwet with breath-holding). The physical characteristics of subjects are described in Chapter III, Table 1. To conclude the findings in this study, the possible factors which may influence the measurement of lung volumes and the experimental results are discussed in this chapter.

Pilot Study

Before actual data collection, five subjects (four males and one female) were tested for FVC and HW in order to observe the reliability of methods and testing procedures. For HW, the RV was obtained from on land FVC measurement converted by Wilmore's (1969) formula (RV = 0.24 x VC for males, and RV = 0.28 x VC for females). The TLCwet was then derived from RV plus FVC measured in water. The TLCwet with breath-holding was computed from RV plus FVCwet with prior breath-holding. The procedures for FVC measurement and HW are described in Chapter III. An intraclass correlation coefficient analysis was then used to determine the test-retest reliability coefficients relative to each FVC measurement under four conditions and relative to HW assessed with RV, TLCwet, and TLCwet breath-holding. The computation results are shown in Table 2 and Table 3. The correlation coefficients indicated that the methods and the procedures involved in this study were reliable.

Table 2.
Intraclass Correlation Coefficient For FVC Measurements Under Four Conditions

on land	on land, breath-holding	in water	in water, breath-holding
$R = .97$	$R = .97$	$R = .98$	$R = .97$

Table 3.
Intraclass Correlation Coefficient For HW at Three Lung Volumes

RV	TLCwet	TLCwet, breath-holding
$R = .99$	$R = .98$	$R = .98$

Lung Volume Measurements

The means and standard deviations (S.D.) of FVC (on land and in water, with and without breath-holding) for the 30 subjects are listed in Table 4. The mean FVC with prior breath-holding was slightly lower than without breath-holding in both males and females on land and in water. For females, the difference between breath-holding and non breath-holding in FVC was 40 ml both on land and in water. However for males, the difference was 60 ml on land and 90 ml in water. These differences in FVC between breath-holding and non breath-holding were statistically compared using Scheffe's post hoc test after the ANOVA *F*-test. There was no statistic significance between breath-holding and

Table 4.
Means and Standard Deviations of FVC Measurements

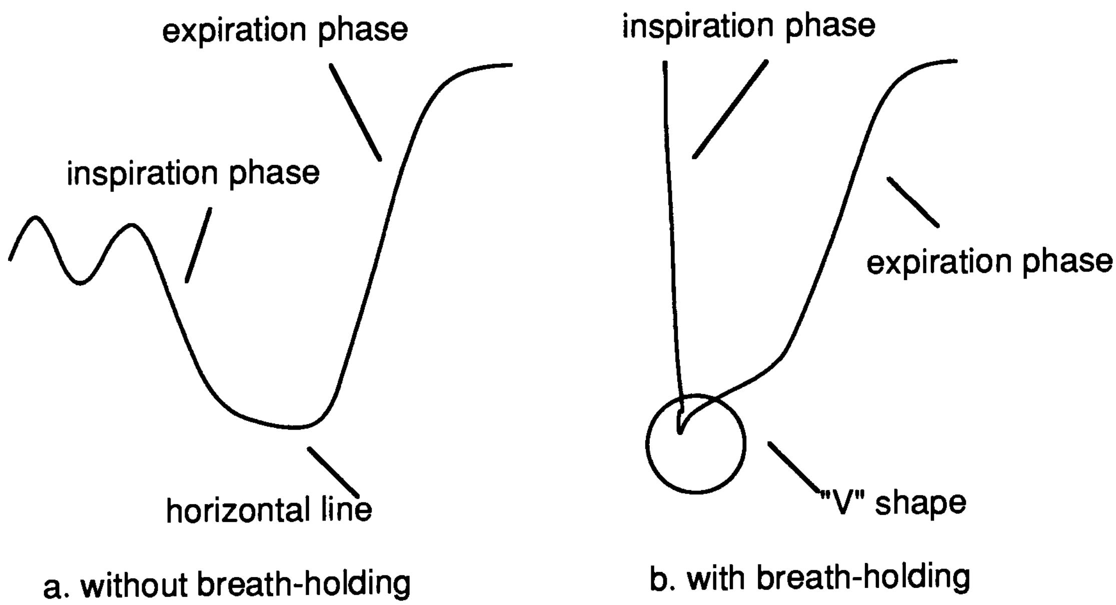
Gender	*n*	Test Condition	FVC (L., BTPS)/S.D.
F	15	on land	4.63 / 0.116
		on land, breath-holding	4.59 / 0.123
		in water	4.38 / 0.114
		in water, breath-holding	4.34 / 0.101
M	15	on land	5.75 / 0.275
		on land, breath-holding	5.69 / 0.264
		in water	5.56 / 0.232
		in water, breath-holding	5.47 / 0.238

non breath-holding in the reduction of FVC (p>.05, Table 6) both males and females.

The methodology may contribute to the difference in FVC between breath-holding and non breath-holding. Two typical FVC tracings displayed on the screen of the UCI spirometer system in the breath-holding and without breath-holding experiments are illustrated in Figure 1. On the FVC tracing with breath-holding, a very sharp descending slope at the beginning of the tracing represents the inhalation phase after breath-holding. Compared to this, the standard FVC maneuver (without breath-holding) shows a less steep slope on the

Figure 1.

The Simulated FVC Tracings From the Standard Maneuver and From the Maneuver With Prior Breath-holding



inhalation phase after tidal breathing. Notice that in the tracing of non breath-holding FVC, there is a nearly horizontal line following the maximal inhalation, indicating the achievement of maximum inspiration. The tracing illustrating the breath-holding maneuver does not have the same horizontal line. Indeed, there is a fairly sharp V-shaped curve consisting of maximal inhalation and the start of forceful exhalation. It was noticed in this experiment that no matter how long the subjects inhaled, in most cases there was no horizontal line between the inhalation phase and exhalation phase to indicate the achievement of maximal inhalation.

In this study, an Ohio 827 dry spirometer integrated with a UCI computer system (Vacumed Inc., Ventura, California) was used to measure FVC. As Dawson and Mohler (1982) pointed out, one of the possible problems dealing with the accuracy of using a microcomputer assisted spirometer is the resolution of the computer (number of the bit). A higher resolution will generate a more accurate computed FVC. It was apparent that the horizontal line was acceptable as a baseline for the computer to count the points of displacement from the lowest point to the highest. However, the very bottom part of the V-shaped displacement might not be completely taken into account by the computer. Therefore, due to the bit capacity of the computer the shape of the tracing from FVC with prior breath-holding tended to produce a smaller value than one without breath-holding.

From Table 4, a 250 ml reduction of FVC in water as opposed to on land was found in females both with and without prior breath-holding. This is equal to about a 5.4 - 5.5 % reduction in FVC while in water. In males, there was an average 190 ml reduction of non breath-holding FVC when submerged (3.4 % of FVC). With prior breath-holding, the loss in FVC was about 220 ml which is equivalent to about a 3.9 % reduction.

Table 5 shows results of ANOVA for FVC under different conditions, and Table 6 the results of Scheffe's post hoc F-statistics for multiple comparisons of the means of FVC.

As expected, the difference between genders was significant. In this study, male subjects were taller and heavier and therefore showed a larger surface area. Consequently, the FVC in males was expected to be greater than that of females. The differences among measurements (relative to different testing conditions) were also statistically significant (p=.0001). The multiple comparison post hoc analysis between measurements is showed in Table 6.

Table 5.
Two-Factor Repeated Measures ANOVA For FVC Measurements

Source	df	MS	F-ratio	p-value
Sex	1	38.556	17.426	.0003
Subjects w. Sex	28	2.213		
Measurements	3	0.551	28.292	.0001
Sex w. Measure	3	0.009	0.460	.7110
Measures x Subj. w. Sex	84	0.019		

α = .05

Table 6.
Scheffe's Post Hoc Test For Multiple Comparison Among FVC

Gender	Comparison	F-test	Significant*
F	Dry vs. Dry, holding	0.63	no
	Dry vs. Wet	24.67	yes
	Dry vs. Wet, holding	33.20	yes
	Dry, holding vs. Wet	17.41	yes
	Dry, holding vs. Wet, holding	24.67	yes
	Wet vs. Wet, holding	0.63	no
M	Dry vs. Dry, holding	1.42	no
	Dry vs. Wet	14.25	yes
	Dry vs. Wet, holding	30.95	yes
	Dry, holding vs. Wet	6.67	no
	Dry, holding vs. Wet, holding	48.36	yes
	Wet vs. Wet, holding	1.93	no

*$\alpha = .05$, $df = 3, 84$

As showed in Table 6, the differences in FVC between breath-holding and non breath-holding on land and in water was not statistically significant in either males or females. This small difference may be due to the methodology.

The difference in FVC performed on land as opposed to in water was compared. The difference was statistically significant ($p<.05$) when comparing mean FVC following breath-holding (dry vs wet) or when comparing mean FVC without breath-holing (dry vs wet) in both gender groups. The reduction of FVC due to submersion was 5.4 - 5.5 % in females and 3.4 -3.9 % in males. The amount of FVC reduction generally agrees with the 3.0 % - 9.0 % values reported in prior research (Agostoni et al., 1966; Carey et al., 1956; Craig & Ware, 1967; Hong et al., 1969; Morgan, 1983; Robertson, Engle, & Bradley, 1978).

The results of this study showed that women had a slightly greater reduction in mean FVC when submerged than men. For women the reduction was 250 ml when submerged under the conditions of prior breath-holding and without breath-holding. For men these reductions were 220 and 190 ml respectively. To establish whether or not these gender differences in reduction of FVC when submerged were statistically significant, a t-test for unrelated means was performed on difference scores. The difference proved to be not significant at $\alpha = .05$ (one-tailed, $df = 28$; $p = .36$, when 220 ml vs. 250 ml; $p = .26$, when 190 ml vs. 250 ml). However, it is also possible that due to some physical characteristic difference, males might be capable of overcoming the mechanical hydrostatic pressure effect on the chest wall when both groups' lung volumes are relatively large (the FVC average was 5.62 liters in males and 4.48 liters in female). The reduction in FVC between males and females in this study disagrees with recent findings. It was generally believed that males tend to reduce more in FVC when submerged due to relatively greater lung volumes (TLC). Weltman & Katch (1981) reported a greater reduction in %BF in women than in men at TLC. This would suggest a relative greater reduction in TLC in women during submersion. However, in their report they did not measure the FVC in water.

It has always been questioned whether the RV estimated can be used for HW instead of using the RV actually measured. Buskirk (1961), and Keys and Brozek (1953) estimated that by using an empirical average value of RV, errors in the estimation of body volume may cause an error of 500 ml for a given individual. Keys and Brozek (1953) also concluded that this could lead to an error of 4 % in the estimation of body fat in a hypothetical 70 kg man with 20 percent body fat.

Several studies suggest a very low correlation between RV and VC. Wilmore (1969) reported that the correlation between RV and VC was only $\underline{r}$ = .435 for the males and $\underline{r}$ = .165 for the females. In this study the RV was estimated via formula, FVCwet was measured in water, and TLCwet was obtained by adding together estimated RV and FVCwet values measured in water. Based upon the subject characteristics in this study, the difference in Db between RV calculated and TLCwet calculated was 0.014 gm/cc for males and 0.011 gm/cc for females (see Table 7).

This greater disagreement in Db between using estimated RV and TLCwet is believed to be due to the fact that RV was not measured but estimated. In this study, Wilmore's equation for estimation of Db was used. In his formula the ratio of RV and VC was 0.24 for males, and 0.28 for females. Based upon the data from other studies, the ratio of RV and VC was higher in a range from 0.28 to 0.34 (Hurtado & Boller, 1933; Weltman & Katch, 1981).

Another possibility for this wider disagreement is that the RV may actually increase when submerged due to the vascular engorgement of small airways, causing air-trapping (Carey, Scheafer, & Alvis, 1956; Girandola, et al., 1977).

The Breath-holding Maneuver When Immersed to the Neck

As discussed in a previous chapter, two major factors may be related to the changes of lung volumes during submersion: the hydrostatic force counteracting the action of the inspiratory muscles, and the shift of blood into the thorax (Agostoni, et al., 1966; Bondi, et al., 1976; Hong, et al., 1969). One of the hypotheses in this study was that an increased $PaCO_2$ due to breath-holding may stimulate the respiratory center to generate a more forceful inspiratory motion through the influence of the peripheral chemoreceptors and the Hering-Breuer reflex. The findings of this study disproved the hypothesis that prior breath-holding is capable of producing a sufficient force to compensate for the mechanical effects of hydrostatic pressure. It must be pointed out that the present study did not identify the $PaCO_2$ level after breath-holding.

Based upon observation during the experiment, the subjects held their breath an average of 14.3 seconds, with a range of seven seconds to 23.0 seconds. The level of $PaCO_2$ that can be generated by an average of 14.3 seconds of breath-holding following a full expiration is unknown. It is likely that such breath-holding may cause accumulation of CO_2 in arterial blood. However, from the findings of this study it would appear that the breath-holding failed to sufficiently stimulate the respiratory center to strengthen the action of the inspiratory muscles and overcome the hydrostatic force on the chest wall.

It is clear that an increased $PaCO_2$ motivates a greater tidal volume and a higher breathing frequency. Levitzky (1986) pointed out an elevated level of carbon dioxide is a very powerful stimulus to ventilation: Only voluntary hyperventilation and the hypercapnia of exercise can surpass the minute ventilations obtained with hypercapnia unaccompanied by exercise. This statement implies that in the conscious status, voluntary control may be more powerful than reflex hyperventilation via hypercapnia unaccompanied by exercise. When the subjects were asked to inhale maximally while submerged to chin level, they actually were voluntarily performing the breathing maneuver. The increased $PaCO_2$ via breath-holding was unable to generate a more powerful ventilation. It is also unlikely that the Hering-Breuer reflex plays an important role. Only when the lungs are deflated abnormally (as in pneumothorax) will the Hering-Breuer deflation reflex be responsible for increased ventilation (Levitzky, 1986).

Levitzky stated that the diaphragm is the primary muscle of inspiration and is responsible for about two-thirds of the air that enters the lungs during eupnea. It was observed that when submerged or during negative-pressure breathing, the inspiratory muscles (especially the diaphragm) are relaxed at the end of spontaneous expiration (Agostoni, et al., 1966; Johnson & Mead, 1963). Agostoni and Rahn (1960), and Agostoni and Torri (1962) reported that when the subjects expired maximally there was a strong contraction of the diaphragm. It was concluded by Agostoni et al. (1966) that the mechanism leading to this strong contraction is not elicited by a reflex originated by the collapse of the lung and air ways, or by the stretching of the diaphragm. In their experiment no significant higher transdiaphragmatic pressure was found at the end of expiration when one subject breathed for about one minute at - 40 cmH_2O. They believed that there is a contraction of the abdominal muscles simultaneously with that of the diaphragm which aids in the expiratory action.

Since the data from this study did not show an increase of FVC after breath-holding when submerged, it failed to verify the hypothesis that the increased PaCO2 may stimulate respiratory center and inspiratory muscles or the Hering-Breuer reflex may also play a role in increasing FVC. The probable increase of $PaCO_2$ may have virtually no influence on the Hering-Breuer reflex mechanism in initiating a deeper inspiration to reach a greater FVC in conscious subjects. The mechanism of respiration when immersed to chin level is complicated. It involves hemodynamic changes, the specific gravitational changes, hydrostatic dynamic changes, transthoracic and transdiaphragmatic changes, and respiratory muscle contractions.

Body Density and Body Fat Percentage

The raw data of subjects' body weight in water at the conditions of RV, TLCwet, and TLCwet with breath-holding were presented in Appendix C. Table 7 presents the means and standard deviations of Db and %BF. The RV was estimated empirically in the study. The TLCwet was calculated from RV and FVCwet. The TLCdry was obtained by adding together estimated RV and FVCdry. The TLCwet with breath-holding was derived from RV and FVCwet with breath-holding. These four variables (RV, TLCwet, TLCdry, and TLCwet with breath-holding) were used for calculating the dependent variables (Db and %BF). The ANOVA F-test suggested the statistical significance existed between measurements. Then, multiple comparison was carried out using Scheffe's post hoc test to detect the differences among methods of HW (using RV, TLCwet, TLCdry, and TLCwet with breath-holding).

As expected from Table 7, it can be seen that Db derived using RV has the lowest mean value (1.068 gm/cc in males and 1.051 gm/cc in females). Therefore mean %BF was the highest (13.71 % in males and 21.12 % in females) when calculated from Db derived from using RV. On the other hand, the highest mean Db (1.084 gm/cc in males and 1.067 gm/cc in females) and the lowest mean %BF (6.63 % in males and 14.06 % in females) were from the TLCdry method. The TLCwet and TLCwet with breath-holding methods produced values which were between those obtained using RV and TLCdry, and it seemed that TLCwet with breath-holding tended to produce a slightly lower Db than TLCwet in

Table 7.
Means and Standard Deviations (S.D.) For Db Estimation and %BF Estimation

Gender	@RV/S.D.	@TLCwet/S.D.	@TLCwet, holding/S.D.	@TLCdry/S.D.
		Body Density (gm/cc)		
F	1.051 / 0.016	1.062 / 0.012	1.062 / 0.016	1.067 / 0.013
M	1.068 / 0.012	1.082 / 0.013	1.080 / 0.012	1.084 / 0.013
		Body Fat Percent (%)		
F	21.12 / 7.17	16.07 / 5.07	16.22 / 6.85	14.06 / 5.52
M	13.71 / 5.24	7.38 / 5.53	8.35 / 5.21	6.63 / 5.39

both genders, resulting in a slightly higher %BF in both males and females.

The two-way repeated measures ANOVA (see Table 8) indicated that the differences in Db and %BF between genders were statistically significant (p<.05), and the differences in Db and %BF among methods (repeated measures) were also significant (p<.05). The calculations of Db and %BF were affected by the measurement of lung volumes.

A Scheffe's post hoc test was used for multiple comparisons among means of Db and of %BF assessed at different lung volumes (Table 9 and 10). It was found that the mean difference in outcomes between using TLCwet and TLCwet with breath-holding was not significant in either Db or %BF for either males or females. This was true also for the comparison of FVCs (TLCwet vs TLCwet with breath-holding) due to the fact that the computer integrated spirometer had a limited counting ability (Dawson & Molher, 1982) because of the shape of the tracing. A significant difference ($p<.05$) did exist in both Db and %BF for both genders when the measurements were made using RV as opposed to TLCwet. The difference in Db at RV as opposed to TLCwet was 0.014

Table 8.
Two-Factor Repeated Measures ANOVA For Db Estimation and %BF Estimation

Source	df	MS	F-ratio	p-value
		Db Estimation		
Sex	1	0.010	15.852	.0004
Subjects w. Sex	28	0.001		
Measurements	3	0.002	47.893	.0001
Sex w. Measures	3	1.485E-5	0.474	.7012
Measures x Subj. w. Sex	84	3.133E-5		
		%BF Estimation		
Sex	1	1848.510	15.879	.0004
Subjects w. Sex	28	116.410		
Measurements	3	286.953	48.160	.0001
Sex w. Measures	3	2.684	0.451	.7176
Measures x Subj. w. Sex	84	5.958		

$\alpha = .05$

gm/cc in males and 0.011 gm/cc in females. The difference in Db at RV as opposed to TLCwet with breath-holding was 0.012 gm/cc for males and was 0.011 gm/cc for females. In the comparison of %BF, the difference between using RV as opposed to TLCwet was 6.33 % for males and 5.05 % for females. And finally, the difference in %BF between using RV and TLCwet with breath-holding was 5.36 % in males and 4.90 % in females. As to the comparison of assessments at TLCwet versus TLCdry, there was no significant difference statistically (α= .05) in either Db or %BF both males and females (for males an average of

Table 9.
Scheffe's Post Hoc Test For Multiple Comparison Among Body Densities

Gender	Comparison	*F*-test	Significant*
F	RV vs TLCwet	28.96	yes
	RV vs TLCwet, breath-holding	28.96	yes
	RV vs TLCdry	61.28	yes
	TLCwet vs TLCwet, breath-holding	0.00	no
	TLCwet vs TLCdry	5.98	no
	TLCwet, breath-holding vs TLCdry	5.98	no
M	RV vs TLCwet	46.92	yes
	RV vs TLCwet, breath-holding	34.47	yes
	RV vs TLCdry	61.28	yes
	TLCwet vs TLCwet, breath-holding	0.96	no
	TLCwet vs TLCdry	0.96	no
	TLCwet, breath-holding vs TLCdry	3.83	no

* α = .05, *df* = 3, 84

0.002 gm/cc difference in Db and 0.75 % difference in %BF; or females, an average of 0.005 gm/cc difference in Db and 2.01% difference in %BF). The findings suggested that the difference between HW at TLC measured on land as opposed to TLC measured in water is fairly small, particularly in male collegiate students.

Weltman and Katch (1981) have reported in their study that the difference in Db between using RV and TLCdry was 0.0013 gm/cc for men and 0.0025 gm/cc for women. Consequently, the %BF difference

Table 10.
Scheffe's Post Hoc Test For Multiple Comparison Among % Body Fat

Gender	Comparison	*F*-test	Significant*
F	RV vs TLCwet	32.10	yes
	RV vs TLCwet, breath-holding	30.22	yes
	RV vs TLCdry	62.74	yes
	TLCwet vs TLCwet, breath-holding	0.03	no
	TLCwet vs TLCdry	5.09	no
	TLCwet, breath-holding vs TLCdry	5.87	no
M	RV vs TLCwet	50.44	yes
	RV vs TLCwet, breath-holding	36.16	yes
	RV vs TLCdry	63.10	yes
	TLCwet vs TLCwet, breath-holding	1.18	no
	TLCwet vs TLCdry	0.71	no
	TLCwet, breath-holding vs TLCdry	12.41	yes

* $\alpha = .05$, $df = 3, 84$

between using RV and TLCdry was 0.5 % for men and 0.9 % for women. The subjects in their study were middle aged. The RV they used was determined using a closed-circuit oxygen dilution technique. The FVCdry was measured on land. The TLCdry was determined by summing RV and FVCdry. Using similar methods but measuring FVC in water rather than on land, Timson and Coffman (1984) reported that the difference between using TLCdry and RV for both derivation of %BF was 2.0 % for men and 1.4 % for women. However, when using TLCwet as opposed to using RV the difference in computed %BF was 0.3 % for men and 0.1 % for women. Their subjects were volunteers with an average age of 31.0 years for males and 31.9 years for females. Comparing the present study to these previous findings, the difference between using RV and TLC in determining Db and %BF seemed greater. There was a greater than 0.01 gm/cc difference in Db and a greater than 5 % difference in %BF. McGarty (1982) measured RV using the open-circuit nitrogen washout technique and measured FVC in both the dry and wet condition. Comparing Db and %BF using RV and TLCwet as well as using RV and TLCdry, it was found the difference between RV an TLCdry to be 0.0111 gm/cc for males and 0.0075 gm/cc for females. The %BF difference was 4.37 % in males and 3.09 % in females. These findings were statistically significant ($\underline{p}$<.001). For the comparison between RV and TLCwet, the difference in Db and %BF was 0.003 gm/cc and 1.41 % for females with no signifcance ($\underline{p}$>.05); for males it was 0.006 gm/cc and 2.24 % and was statistically significant ($\underline{p}$<.01) The subjects in that study were collegiate students.

From the outcome of this study, it appears that the accuracy of RV determination may be more important in Db and %BF assessment by HW technique than making choice of using TLCdry or TLCwet. Burki, Barder, and Nicholson (1975) stated that there is a large range in closing volumes for individuals of approximately the same age. This may affect air trapping and RV. As discussed in Chapter II, the accuracy of determination of RV while submerged has not been well established. Obviously, there is a divergence of findings in the literature. It seemed possible that different populations may contribute to this disagreement. In the study of Weltman and Katch (1981), subjects were middle aged and had an average FVC of 4.70 liters in males and 3.52 liters in females. In Timson & Coffman's (1984) study, the average age of subjects was above 30 years, with an average FVC of 4.56 liters in males and 3.38 liters in females. McGarty used college students whose average age was between 20 years and 30 years, with an average FVC of 5.87 liters in males and 4.29 liters in females.

There is a tendency for the younger age groups to demonstrate larger variance between HW at RV as opposed to at TLC. This implies that persons who are younger and therefore may have greater lung volumes could be more subject to air being trapped in the lungs when submerged. This may be a partial explanation for the relative wider difference between HW at RV as opposed to TLC. These findings support the argument that RV may be increased while underwater. This increase is due to air trapped in the lungs while in the submersion condition at maximal expiration. The reason for this is the increased air-trapping when lung volume is relatively low while submerged. Consequently, pulmonary vascular engorgement induces a swelling of blood vessels, thereby closing small air ways (Dahlback & Lundgren, 1972). There is also lower compliance due to pulmonary vascular engorgement (Girandola, et al., 1977).

The wider differences between HW outcomes at RV as opposed to TLC is partly due to the fact that RV in this study was estimated rather than actually measured. As Wilmore (1969) concluded, RV varies considerably with such factors as age, sex, and the absence of or freedom from pulmonary disease. When Db, %BF, and lean body values are being used for research purposes; accuracy is essential. It is therefore necessary to directly measure the RV. The empirical estimation of RV may not be well suited to the college age population. The data from this study showed a smaller reduction with immersion of FVC in males than in females. However, the difference in Db and %BF between using RV and TLCwet was relatively greater in males than in females (6.33 % in %BF and 0.014 gm/cc in Db for males vs. 5.05 % in %BF and 0.011 gm/cc in Db for females). This may indicate that larger lungs could lead to reduced accuracy in the estimation of RV.

It is also possible that due to breath-holding, peripheral blood was redistributed to the thoracic cavity. After breath-holding, observation during the study indicated that subjects breathed in more rapidly. This rapid inspiratory motion may cause a rapid change of pressure gradient in the thoracic cavity. Moreover, the magnitude of change in lung volume from RV to TLC is greater than from functional residual capacity (FRC) to TLC. This could be another factor resulting in a relative rapid change of the transthoracic pressure gradient. Consequently, more peripheral blood may shift into the thorax. In male subjects with larger lung volumes, this blood shift may be facilitated. This may explain why in males the FVC after breath-holding was reduced more than without breath-holding (220 ml reduction with breath-holding vs 190 ml without breath-holding for males, and 250 ml reduction with or without breath-holding for females).

Interestingly, the difference in Db between using TLCwet and TLCwet with breath-holding was 0.002 gm/cc in males and no difference existed in females. Consequently, the %BF difference was 0.15 % in females and 0.97 % in males. This could be due to less change of transthoracic pressure gradient caused by breath-holding in females, comparing to males in this study.

As presented in Tables 9 and 10, the difference in Db and %BF between HW at TLCwet and TLCdry had no statistical significance in males (0.002 gm/cc in Db and 0.75 % in %BF, respectively). The difference between HW at TLCwet and TLCdry in %BF was 2.01 % and 0.005 gm/cc in Db for females. This latter difference was statistically significant. In contrast, the difference in Db between HW at RV and at TLCwet was 0.014 gm/cc for males and 0.011 gm/cc for females, with the difference of %BF being 6.33 % for males and 5.05 % for females. Based upon the data from this study, as well as previous research, it seemed that whether or not the FVC was measured in water may be unimportant in determining Db accurately, especially in males. The greater importance is the accurate determination of RV. An inaccurate estimation of RV may substantially increase the error of measurement of Db and %BF.

Weltman and Katch (1981) did not measure FVC submerged but since the increase in %BF in their female subjects was greater than in their males when derived from the RV condition, one might assume greater air-trapping when submerged in females. It is, then, recommended that for males, using TLC with FVCdry is acceptable for HW and has a limited measurement error. It appears that for females, due to relatively more air-trapping or greater reduction in FVC when submerged, using TLC with either FVCdry or FVCwet should be approached with caution.

Dahlback and Lundgren (1972), and Thomas and Etheridge (1980) suggested that use of large lung volumes may reduce or eliminate the problem of air trapping when submerged. From several research findings (McGarty, 1982; Timson & Coffman, 1984; Weltman & Katch, 1981) and from observations made during this study, the use of TLC for HW required fewer trials than for RV. Subjects seemed to prefer the method of TLC. From a practical point of view, use of TLC is an alternative way to assess of body composition by HW; however, it is more important to establish a reliable means to determine RV.

CHAPTER V

CONCLUSIONS

Summary

The purposes of this study were to determine: (a) If a person while submerged to chin level could reach the same FVC as measured on land employing a breath-holding maneuver prior to the full inspiration; and (b) If the Db calculated from HW at RV differed significantly from the Db calculated from HW at TLC. The FVC was measured on land and in water, both with breath-holding and without breath-holding. The RV was empirically estimated based upon land measured FVC. Subjects were hydrostatically weighed at RV, TLCwet and TLCwet with breath-holding. Four estimated Dbs and %BFs (at RV, TLCdry, TLCwet, and TLCwet with breath-holding) as well as four FVCs (on land and in water, with and without breath-holding) were statistically analyzed and compared to determine the significance of differences between HW methods and FVC measurements.

Conclusions

The results of this study lead to the following conclusions:

1. The outcome of this study failed to support the hypothesis that a possible increase in $PaCO_2$ as a result of breath-holding will

sufficiently stimulate inspiratory muscles via increased carbon dioxide respiratory drive to overcome the effects of hydrostatic pressure on the chest wall when submerged to chin level.

2. In the HW approach to assess body composition, a proper RV estimation or measurement is always the most important.

3. It is concluded that at the present the most accurate means of assessing Db and %BF via HW is to measure RV directly.

4. For males, while the reduction in FVC was statistically significant ($p<.05$, Table 6) between assessement on land as opposed to in water, the differences in HW outcomes (Db and %BF) were not significant ($p>.05$, Table 9 and 10). The smaller reduction of FVC in males is probably due to the fact that males have greater strength of respiratory muscles. A greater blood shift to the larger thorax may also contribute to the reduction of FVC in males.

Recommendations

For Practical Application

The following recommendations are based on the conclusions of this study and affect procedures for estimation of Db and %BF via HW:

1. An accurate RV measurement is essential in Db and %BF determination using the HW approach. For those who are younger adults and therefore have larger lungs than those older, there is a greater need to measure RV directly.

2. In cases where male subjects are reluctant to exhale underwater, TLC can be utilized in place of RV with FVC measured on land. This technique should be applied with caution to females. It must be pointed out that when using the TLC approach, the RV should still be measured directly.

3. When using TLC during HW, a slower inhalation to TLC in water is recommended to avoid a rapid gradient pressure change in the thorax. This procedure would perhaps reduce the influx of blood into the thorax and increase the accuracy of using submerged TLC.

<u>For Future Research</u>

The following recommendations are based on conclusions and are directed toward future research efforts:

1. The formula for prediction RV deserves further investigation in order to assure appropriateness, especially in the case of individuals with large TLC.

2. A further understanding of changes of RV when one is submerged would aid in a more accurate assessment of Db and %BF.

3. Future studies of the effectiveness of breath-holding on stimulating carbon dioxide respiratory drive should measure $PaCO_2$.

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APPENDICES

APPENDIX A. INFORMED CONSENT DOCUMENT

Project Title: The Effect of Prior Breath-Holding on Measurement of Lung Volumes for Hydrostatic Weighing

Principle Investigators: Dr. Christian Zauner (major advisor) / Gaoyong Zhu

1. The research will be carried out in the Human Performance Laboratory, Department of Exercise and Sport Science, Oregon State University. I understand that the purpose of this research is to compare the results of lung volume measurement under four different conditions (on land and in water, with and without breath-holding) and to evaluate the effect of breath maneuvers on hydrostatic weighing
2. I shall breathe through a spirometer with a mouth piece and a nose clip in order to have forced vital capacity measured. I shall need to inhale air as much and forcefully as I can, and from that point exhale as much of the air as possible, and as rapidly as possible. The prior breath-holding will require that I exhale air maximally and forcefully, then hold my breath as long as I can unless I extremely need to breathe in. After this breath-holding, I will inhale maximally and exhale forcefully again. Each lung volume measurement must be repeated three times. I shall be in a seated position to perform these breathing maneuvers.
3. On another day, I shall undergo the measurement of body fat through hydrostatic weighing with different lung volume performance. I will

abstain from food and drink for 3 hours before the test. Before underwater weighing, a body weight on land will be taken with only a swim suit. At least three repeated underwater weighings will be conducted at each lung volume. The hydrostatic weighing will require the head to be submerged in water, while in a sitting position. I shall be required to expel as much air as I can from the lungs, then hold my breath and immerse myself. For another set of trials, I shall be asked to inhale as much as I can, then hold my breath and submerge. In the final set of trials, I shall need to exhale first, and then hold my breath as long as I can. I will then inhale maximally when I feel an extreme need to, and then hold my breath again and submerge.

4. The test result of body density will be calculated and will be interpreted for me. Body fat percentage will be derived mathematically from body density. No one will be informed of the result except the tester and myself. My name will not appear in any reports of findings.
5. The benefit from my being hydrostatically weighed is that I am made aware of my body fat percentage. It is one aspect of my health profile and may indicate a need to maintain or to modify my life style. The procedure is not likely to be harmful. Some discomfort due to breath-holding while underwater may occur. Falling due to slipping on wet places is a possibility. All potential harmful situations will be minimized through close supervision and use of qualified personnel. The principle investigators have the responsibility to answer any questions concerning the procedures, risks or benefits.

6. I acknowledged that the requirement of the study is that I have no obstructive or restrictive lung disease or chest abnormality.

I have carefully read this document. Therefore, I fully understand the nature of the study, and the possible risks or discomfort associated with the procedures. I acknowledge that no representations, warranties, guarantees or assurances of any kind pertaining to the procedures have been made to me by Oregon State University, the officers, employees administration, or by any one acting on behalf of any of them. Also, there is no monetary compensation for my participation in this study.

I volunteer to take part in this study as a subject. I reserve the right to withdraw from the program at any time, and I will inform the investigators as to withdrawal. I am over 18 years old, and based upon my knowledge, I take full responsibility for my signature.

At anytime concerning the experiment, I understand that I can contact Chris Quinn at 737-2189, Gaoyong Zhu at 752-2837, or Dr. Christian Zauner at 737-2643.

Name of volunteer's (print)____________________phone__________

Address of volunteer's______________________________________

____

Volunteer's signature_____________________ Date______________

Witness' signature ______________________ Date______________

Subject code _______________

APPENDIX B. HYDROSTATIC WEIGHING AND % BODY FAT DATA SHEET

Subject Code: ______, Sex: ______, Age: ______, Date: __________

Ht (cm): ________, Wt (kg): _________, Hometown: __________

I. Lung Volume (FVC/liter, BTPS)

Temperature (^{o}C): ______, Barometric pressure (mmHg): ________

1) standard maneuver on land (FVCdry):

trial: 1)_____, 2)______, 3)______, mean: ______, RV: ______

2) with breath-holding on land:

trial: 1)_______, 2)_______, 3)_______, mean: ______
breath holding
time (sec.) 1)_______, 2)_______, 3)_______, mean: ______

3) standard maneuver in water (FVCwet):

trial: 1)_______, 2)_______, 3)_______, mean: ______

4) with breath-holding in water (FVCwet with breath-holding):

trial: 1)_______, 2)_______, 3)_______, mean: ______
breath holding
time (sec.) 1)______, 2)_______, 3)_______, mean: ______

II. HW (kg) Date: ____________

Water temperature (^{o}C): ________, Tare weight (kg): ________

1) RV
trial: 1)_____, 2)_____, 3)_____, 4)_____, 5)_____,

6)_____, 7)_____, 8)_____, 9)_____, 10)_____

mean: ______

2) TLCwet
trial: 1)_____, 2)_____, 3)_____, 4)_____, 5)_____,

6)_____, 7)_____, 8)_____, 9)_____, 10)_____

mean: ______

3) TLCwet with breath-holding
trial: 1)_____, 2)_____, 3)_____, 4)_____, 5)_____,

6)_____, 7)_____, 8)_____, 9)_____, 10)_____

mean: ______

breath holding
time (sec.) 1)_____, 2)_____, 3)_____, 4)_____, 5)_____,

6)_____, 7)_____, 8)_____, 9)_____, 10)_____

mean: ______

Note: The order performed for each measurement was randomized.

APPENDIX C. THE BODY WEIGHT IN WATER AT THREE LUNG VOLUMES
(unit = kg)

RV	TLCwet	TLCwet, holding	RV	TLCwet	TLCwet, holding
	male			female	
4.00	-1.00	-1.07	1.57	-2.20	-2.30
5.48	-0.30	-0.30	1.33	-2.98	-3.00
1.98	-1.57	-1.65	1.64	-2.13	-1.97
3.90	-0.60	-0.60	2.53	-1.21	-1.31
3.10	-1.13	-1.15	2.60	-1.52	-1.50
5.10	-0.43	-0.50	1.78	-0.97	-0.92
5.15	-0.57	-0.60	2.95	-1.05	-1.02
3.83	-1.55	-1.65	2.87	-1.53	-1.60
3.85	0.42	0.37	1.82	-1.38	-1.42
3.45	-0.43	-0.55	2.02	-2.00	-1.95
3.75	0.10	0.08	2.40	-1.13	-0.95
3.50	-0.60	-0.50	0.77	-2.75	-2.87
3.85	-0.90	-0.90	1.63	-1.87	-1.99
3.40	-2.40	-2.30	2.60	-0.93	-0.90
2.37	-1.58	-1.63	1.73	-1.57	-1.52
average					
3.78	-0.84	-0.86	2.02	-1.68	-1.68